BRUCELLOSIS IN MAN AND ANIMALS

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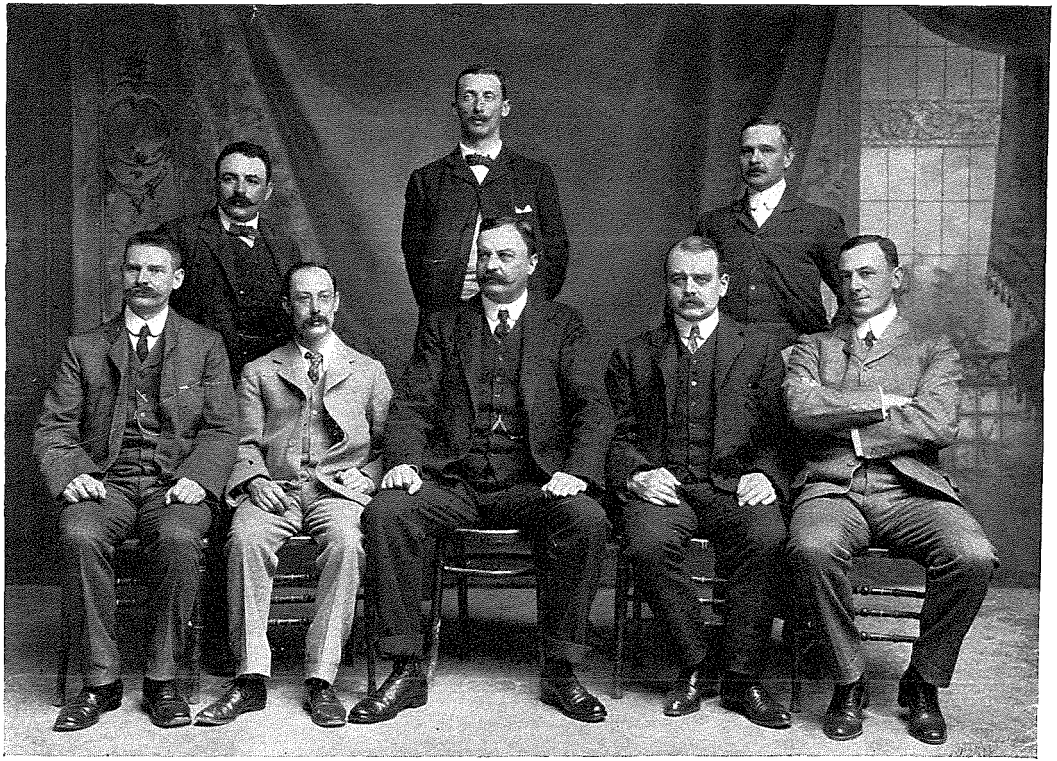

MEDITERRANEAN FEVER COMMISSION, 1904

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BRUCELLOSIS IN MAN AND ANIMALS

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Mediterranean (undulant) fever is in the course of evolution, and is tending to become chronic. It is a malady which, on account of its manifestation and chronicity, will become one of the most common and stubbornest diseases . . . Mediterranean fever is a disease of the future.

CHARLES NICOLLE

FOREWORD TO THE FIRST EDITION

FIVE years ago Doctor Huddleson published his *Brucella Infections in Animals and Man,* a treatise on methods of laboratory diagnosis. While the scope of this work was limited, it has met a need with measurable success and it has been received with favor both in our own laboratories and in those abroad. Forty years ago Hughes published his *Undulant Fever.* This work is a medical classic and may be fairly said to contain the available information on the subject at the time of its publication. But simultaneously with its appearance there was announced the discovery of Bang's bacillus and, with the turn of the century, knowledge of what soon came to be recognized as a related group of pathogens increased with bewildering rapidity.

The present volume by Huddleson not only replaces his previous one of limited scope by attempting to cover the same subject-matter in revised form but reaches out into the general clinical aspects of the disease, thus treating the subject with a degree of completeness destined to make it more attractive and useful to the medical practitioner as well as to the laboratory worker. It is not too much to hope that this treatise on the bigger subject of the brucelloses may serve as useful a purpose at this time as did Hughes' work four decades ago on the only known brucellosis of his day. No one appreciates more fully than the author of the revised book and his associates that this revision is not the *dernier mot* on the matters treated, but it is a successful attempt to offer in usable form the latest word on the *Brucella* infections. For over half the period that has elapsed since the appearance of the English classic on undulant fever, the author has devoted practically his entire time and energies to both intensive and extensive personal studies of *Brucella* and the brucelloses and to the direction of the work of others

as well as to consultations, correspondence, and travel that have contributed to his grasp of the fundamentals and appreciation of the implications of research in this field. We may expect this subject to be critically reviewed and appraised at more frequent intervals in days to come.

WARD GILTNER

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ILLUSTRATIONS

The figure in parentheses following the author's name refers to the publication, as listed in the Bibliography, in which the illustration first appeared.

PREFACE

THIS book, a second edition of *Brucellosis in Man and Animals* published in 1939, presents important changes that have been made in laboratory methods of diagnosis and new facts pertaining to the nature of the disease. It also contains a detailed discussion of the clinical manifestations and epidemiology of the disease in man. Data have been taken from several articles previously printed but no longer available and new information not heretofore published has been included.

Those who are familiar with the study of major diseases appreciate the fact that seldom, if ever, does one man have the opportunity to become sufficiently familiar with a disease to be able to write on all its aspects. The author has sought and obtained the cooperation of three individuals for the presentation of certain phases of brucellosis. Their training and experience have made them outstanding authorities on their particular subject. The three co-authors have made many contributions to the brucellosis problem. Dr. A. V. Hardy was the first to make a complete survey of brucellosis in man in the United States with special reference to the state of Iowa. Professor J. E. Debono of Malta has for many years devoted considerable time to the study of the *Brucella melitensis* type of the disease. The material included in his discussion is based on compiled data obtained from a study of a large number of cases occurring on the Island of Malta. Dr. Ward Giltner has interested himself for a number of years in the animal and human health problems evolving from the brucelloses. There is no one better able to present this phase of the problem.

Certain laboratory methods and data pertaining to the results of investigations in this field have been omitted from this book, not because they were considered less satisfactory than those described here, but with the thought that the inclusion of too many proce-

dures would only lead to confusion and would detract from the purpose for which the book was intended.

I welcome this opportunity to acknowledge my indebtedness to my chief, Dean Ward Giltner, whose interest and enthusiasm have been a source of inspiration to all engaged in the study of *Brucella* infections.

Thanks are also due to my present and past associates for the numerous contributions which they have made on the subject, and for the assistance and cooperation they have given me in the laboratory. I wish especially to express appreciation to Dr. A. V. Hardy for helpful suggestions relative to the preparation of the manuscript.

Without the financial assistance that has been given to the studies at the Central Brucella Station at Michigan State College, progress in the study of brucellosis would have been slow and the results uncertain. Organizations to which I wish to acknowledge my deep appreciation for their generous financial support are: the Abortion Committee of the National Research Council; the Bureau of Animal Industry of the United States Department of Agriculture; the Committee on Scientific Research of the American Medical Association; the Certified Milk Producers Association; and, in particular, the Horace H. and Mary A. Rackham Fund. To the Commonwealth Fund, which has also generously supported the research in brucellosis, I am further indebted for the publication of this book and for editorial assistance in the preparation of the manuscript.

Several investigators have also permitted the inclusion of important data, tables, and figures from their published papers without which the book would not be complete. The author wishes to express his appreciation to the investigators for their cooperation and to the publishers for their permission to quote and to reproduce photographs and charts.

I.F.H.

November, 1942

I

THE GENUS *BRUCELLA*

HISTORY OF THE THREE SPECIES

BRUCELLA MELITENSIS (Hughes), the first species of the genus *Brucella* to be identified, was isolated by Bruce (35) in 1887. It was isolated from the spleen of patients who had died of a disease named "Mediterranean or gastric fever" by Marston (295) as early as 1859. The causative organism was named *Streptococcus miletensis* by Hughes (222) in 1892 and *Micrococcus melitensis* by Bruce (36) in 1893. Early workers did not recognize the bacillary form of the organism. The chief host of *Br. melitensis* is the milch goat. This fact was discovered by Zammit (481), a member of the Mediterranean Fever Commission, in 1905. The organism appears to localize in the udder, spleen, and lymph nodes of the goat, giving rise to an interstitial mastitis and splenic lymphadenitis. It has also been recovered from the milk of infected cows in the United States, France, and Italy, and from aborted fetuses of sheep and goats in France, Italy, and Argentina.

Brucella abortus (Bang) was first isolated and described as a *Bacillus* by Bang (10), assisted by Stribolt, in 1897. They isolated the organism from the fetuses and fetal membranes of cows that had aborted and later established the fact that it was the cause of infectious abortion of cattle. The disease is now recognized as "Bang's abortion disease."

The udder of the cow as the reservoir for *Br. abortus* was discovered simultaneously by Smith and Fabyan (415) and by Schroeder and Cotton (400) through the examination of the milk of infected cows. The organism in the cow gives rise to an acute diffuse and to a chronic productive inflammation of the maternal and fetal placentae, fetal pneumonia, and chronic interstitial mastitis.

The organism may set up an inflammatory process in the uterus and Fallopian tubes of heifers and mature cows or in the testis of the bull.

Br. abortus has been found in animals in all parts of the world. It has been recovered from naturally infected horses, fowl, dogs, sheep, wild deer, wild buffalo, and human beings.

Brucella suis was first isolated by Traum (447) in 1914 from fetuses expelled prematurely from sows. Although this species of *Brucella* resembles *Br. abortus* culturally and antigenetically, it differs markedly in one respect, in that it does not require an increased CO_2 tension for primary isolation. The hog appears to be the chief host for *Br. suis*. *Br. abortus* has not been found to infect the hog naturally. In fact it is very difficult to infect the hog with this species artificially.

Up to the present time *Br. suis* has been isolated from hogs in the United States, Hungary, Denmark, Switzerland, Brazil, Argentina, and Japan. The Danish strains (436) differ from those isolated in the United States in that they produce little if any H_2S when grown on a suitable solid culture medium.

Br. suis has been isolated from the horse, the fowl, the cow, the dog, and human beings; all of these were naturally infected.

CLASSIFICATION OF THE THREE SPECIES

Bruce placed the organism which he discovered in the *Micrococcus* group, and others since have likewise maintained that *melitensis* is a *Micrococcus*. Bang, on the other hand, called the organism which he isolated and studied a *Bacillus*. Later it was classified as a diphtheroid by Preisz (367), and as a bipolar organism by Nowak (340). Traum classified the strain which he isolated from aborted pigs as a *Bacillus*. When *abortus* and *melitensis* were studied together for the first time by Evans (99), she saw very little difference in their microscopic appearance and suggested that both

be given the generic name *Bacterium*. The results of the studies of Evans were confirmed by Meyer and Shaw (302) on a much larger number of strains. They suggested a more fitting generic name *Brucella* to separate and distinguish them from the already overburdened genus *Bacterium*. *Brucella* is more than a fitting name for this group of organisms; it does honor to the name of one who stands alone as an investigator of tropical diseases. The generic name *Brucella* has now been approved and accepted by all investigators who are studying these organisms and the diseases for which they are responsible.

In addition to the question of morphological differences in *Br. melitensis* and *Br. abortus,* there remains the question whether they should be classified as varieties of one species or as separate species. Many have considered that the distinctions between the organisms were not sufficiently permanent to be of value in classifying them as separate species. This contention is based largely on their morphological appearance, staining properties, cultural characteristics, and behavior in agglutinin-absorption tests, and on the similarity of the diseases which they produce in animals and man. There are, however, distinct differences among the three organisms which one cannot continue to ignore: in world distribution or occurrence; in pathogenicity for animals; in nitrogen and glucose metabolism as pointed out by McAlpine and associates (276); in growth behavior toward anilin dyes in a suitable culture medium (203); in nitrate and nitrite reduction (484); and in chemical constituents (226, 351). These differences will be discussed more fully later.

MORPHOLOGY AND STAINING

Br. melitensis. The size and shape depend largely on the strain under examination, the age of the culture, and the medium upon which it is grown. Certain strains of *Br. melitensis* invariably show coccoid forms, while others show bacillary forms with a few scat-

tered coccoid forms. The length varies from 0.4 to 2.2 microns and the width from 0.4 to 0.8 micron. It is Gram-negative.

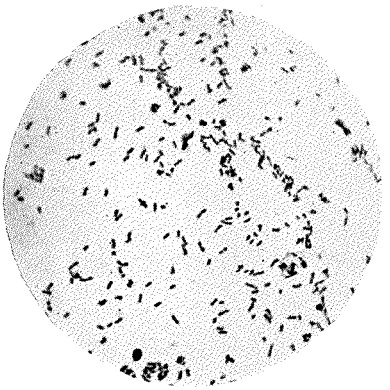
Br. abortus. The size and shape vary, but not to the same extent as the size and shape of *melitensis.* The organism is usually seen as a stumpy rod, but there may also be present coccoid forms. Occasionally in a stained preparation the coccoid forms may outnumber the rod forms. The length varies from 0.4 to 2.5 microns and the width from 0.4 to 0.6 micron. It is Gram-negative.

Br. suis. The organism appears as a rod form. The size varies from 0.6 to 3 microns in length and from 0.4 to 0.8 micron in width. It is Gram-negative. Smooth and intermediate forms of all three species are enclosed in a capsule which can be demonstrated by a suitable technic (207).

CULTURAL CHARACTERISTICS

Br. melitensis when first isolated by Bruce from cases of brucellosis was recognized as an aerobe, that is, no special atmospheric conditions were necessary to obtain primary growth. This fact has been substantiated by all who have isolated the organism. It may be said further that regardless of the host from which it is isolated it always grows out aerobically on suitable culture media.

Br. abortus when first isolated and grown by Bang failed to grow aerobically until it had been transplanted many times on artificial culture media. Bang considered an oxygen pressure above or below that of the atmosphere the factor chiefly responsible for growth. Nowak (340) later considered a lowered oxygen tension necessary for primary growth and believed that he obtained this condition by incubating primary cultures in a closed jar along with a medium inoculated with *Bacillus subtilis.* Nowak's method, as well as a sealed-tube method, was used by most workers until 1920. In that year the writer (201) presented the results of investigations which showed that a reduced oxygen tension had little, if anything, to



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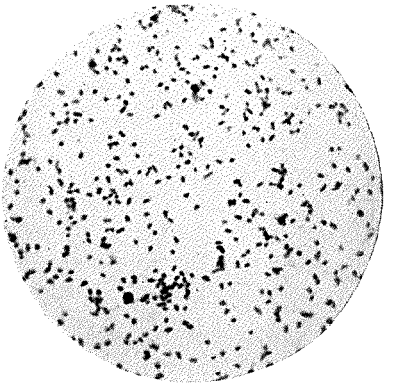

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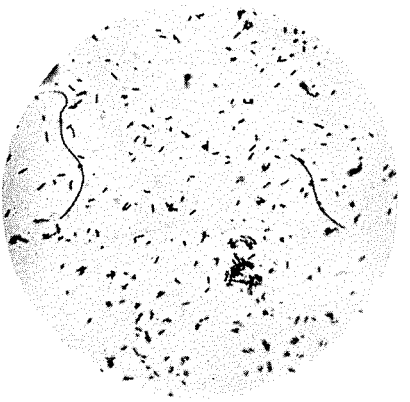


C

FIGURE 1. A. BR. ABORTUS, BACILLARY FORMS; B. BR. MELITENSIS, COCCOID FORMS; C. BR. MELITENSIS, ROUGH FORMS

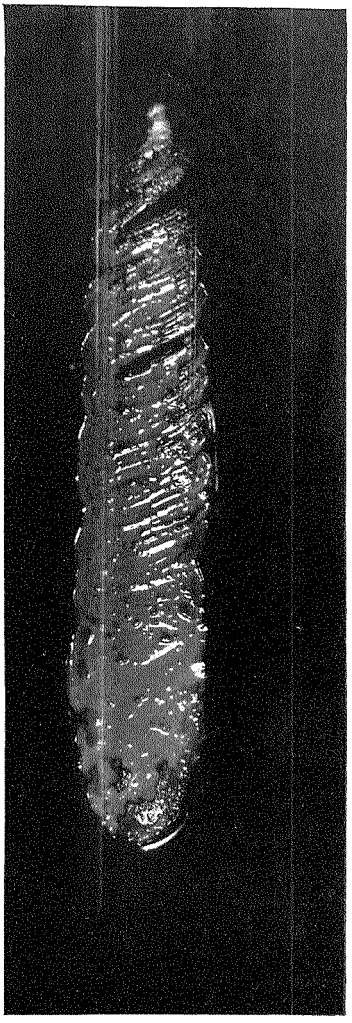


FIGURE 2. BR. ABORTUS ON BEEF LIVER INFUSION AGAR

do with the primary isolation and growth of *Br. abortus* and that an increased CO_2 tension was the important factor in the successful isolation of the organism. *Br. abortus* always requires an increased CO_2 tension of about 10 per cent by volume over that of the atmospheric air for primary isolation from milk, infected tissues and blood. Subsequent transfers of the primary growth from milk and all tissues require a 10 per cent increase in CO_2 tension until the culture has adapted itself to aerobic growth. An increased CO_2 tension is not always necessary for the primary isolation of a strain that has previously been adapted to grow under atmospheric conditions.

Br. suis is an aerobe. No special atmospheric conditions are required for its primary isolation from infected tissues. Growth from infected blood will take place much more rapidly if the culture taken in a liquid medium is incubated in CO_2 at a tension of about 10 per cent above that of atmospheric air.

On the surface of a solid medium, such as beef liver infusion agar, colonies of any of the three species vary in diameter from 2 to 7 mm. after three days of incubation at 37° C. They are distinctly spheroidal in shape, slightly opalescent in color, and translucent. Each of the species produces a heavy translucent growth when planted on the surface of liver agar slants. When viewed from the top, the growth on the surface of the slants has a moist, greasy appearance.

The growth of most strains of *Br. melitensis* and a few of *Br. abortus* on agar slants becomes brown with age. The pigment first appears in the growth on the surface and then gradually extends down into the medium. This browning also characterizes the growth on potato slants.

The optimum pH for the growth of each of the three species on solid media or in broth is 6.6 to 6.8.

In gelatin they grow moderately, producing grayish-white, filiform colonies. There is no liquefaction.

Glucose is utilized slightly by *Br. abortus* and to a greater degree by *Br. melitensis* and *Br. suis*. Litmus milk is turned slightly alkaline. According to Zobell and Meyer (484), both nitrates and nitrites are reduced in a semisolid medium. Nitrogen gas from a nitrate medium is produced by *Br. suis*. Ammonia is produced by *melitensis* to a greater degree than by the other two species. Hydrogen sulphide is produced in abundance by *Br. suis,* to a lesser extent by *Br. abortus,* and to a very slight degree, if any, by *Br. melitensis.*

In liver broth the growth of all three species is slow. During the first few days the broth appears uniformly cloudy. After ten days of incubation considerable sedimentation takes place and the sediment may become viscous.

DISSOCIATION

Nègre and Raynaud (329) while studying the suitability of strains of *Br. melitensis* noted that certain ones were agglutinated irregularly with specific serums (see Table XVII, page 220). They designated such strains as "para" forms of *melitensis*. Several years later, Burnet (44) noted that after prolonged incubation in broth *Br. melitensis* invariably forms a pellicle on the surface which settles to the bottom of the tube on slight agitation. When the organism grows as a pellicle, it undergoes a peculiar transformation in respect to its physical, antigenic, and pathogenic properties. Burnet found that "para" strains of the organism, when suspended in physiological salt solution, would agglutinate when subjected to a temperature of 80° C. for two hours in a water bath. As a rule "para" forms are not agglutinated readily by specific serums, but may agglutinate in the presence of negative serum.

The heat-agglutinable strains do not readily produce antibodies nor are they pathogenic for experimental animals.

Br. abortus and *Br. suis* also become dissociated when they pro-

FIGURE 3. COLONIES OF BR. ABORTUS FROM INFECTIVE MILK

duce a pellicle growth in broth. *Br. melitensis* very often undergoes spontaneous dissociation when grown on agar slants. Once a strain becomes unstable, it seldom, if ever, reverts completely to the normal form.

Henry (188) has made a very thorough and complete study of the dissociation of *Brucella* and the characteristics of the many dissociated colonial forms or types. A description of the dissociants can best be presented in his own words.

"S Type.—In most stock cultures and in all recently isolated cultures which have been examined, the majority of the organisms are of the S type.

"The colony of the S type is blue, greenish blue or gray-blue, moist, glistening and soft, and the surface is smooth. The organisms suspend readily in physiologic solution of sodium chloride and remain in suspension for several days. When the bacteria finally settle out, they form a compact central mass in the center of the bottom of the tube. Minor variations in the salt concentration of the suspending liquid do not cause spontaneous agglutination.

"In broth cultures, the S type produces considerable turbidity and soft, homogeneous sediment. No pellicle occurs on broth cultures until intermediate or R forms have arisen.

"This type is rather stable when optimum growth conditions are maintained, but even under these conditions an occasional intermediate or R form arises in most strains.

"Most strains of *Br. abortus* which contain organisms of the S type are virulent for guinea-pigs, rabbits and cattle, but apparently pathogenicity is not necessarily a characteristic of this type, as two strains which have been studied showed little or no invasive power even though they were predominately of typical S type.

"In all strains, regardless of relative pathogenicity, the S organisms are active antigens. This is in striking contrast with the S^R type, which is similar in colonial appearance but is non-antigenic.

"I Type.—While there are numerous reports in the literature tending to show that the transition from the S to the R type is sudden and complete, more extensive study has usually demonstrated at least one intermediate type. In many bacterial species, several such types have been described. The transient intermediate forms which occur in cultures of *Br. abortus* are so definitely gradations between the S and the R types that there seems little doubt that all changes from S to R type in this species are accomplished by passage through these intermediate types. This conclusion is borne out by the constant presence of these forms in cultures which are undergoing dissociation.

"Because there appear to be no relatively stable intermediate forms comparable with the O type as defined by Hadley, I has been used to designate all those forms intermediate between the S and the R types.

"In appearance, the I types of colonies of *Br. abortus* vary from those closely resembling S colonies to those which are almost indistinguishable from R colonies. All I types which have been encountered have been unstable, giving rise to R, S and I colonies on transfer. That the I form colony is not the product of the mixed growth of an R or an S organism is shown by the fact that frequently plates made from relatively new broth cultures will contain only I colonies or only I and S colonies.

"All I colonies of *Br. abortus* have one characteristic in common, namely, large size. As will be shown later, the relative sizes of the S and R colonies vary with the strains used, but in all cultures studied the I colonies have been as large or nearly as large as the type which grew most luxuriantly in a given strain.

"R Type.—Although the form designated as R in this study complies with requirements usually demanded for classification, it is with some hesitancy that this term is used. An R type of organism which contains only heat-stable R somatic antigen should be some-

what more stable and more uniform in the various strains of the species than is the R type of *Br. abortus*. However, no other form found has nearly the same claim to this designation as has the type described in subsequent paragraphs.

"In common with the R colonies found in other species, those of *Br. abortus* present an opaque, lusterless appearance. In consistency, they are dry and friable or may be somewhat mucoid, depending on the moisture content and acidity of the substratum. The colonies vary in color from white to buff and have a surface appearance similar to that of ground glass when viewed with oblique transmitted light. The edge of the colony is usually even, but when excessive moisture is present on the surface of the medium, the edge may become jagged as if small wedge-shaped sectors had chipped off and been washed away. The edges of the S colonies of the same plate show no change or may become somewhat indefinite and indistinct.

"The individual organisms may be somewhat longer than are those of the S colony from the same strain, but usually the bulk of the units are comparable in size with those of the S colony, with an occasional long, slender rod intermingled. . . .

"As compared with the S type obtained from the same parent strain, the R type is relatively nonvirulent. No macroscopic lesions, other than transient abscesses at the points of inoculations, have ever been produced by the injection of cultures of the 'pure' R type. . . .

"S^R Type.—This pseudo-S form is antigenically distinct from the true S, is self-agglutinating and nonvirulent. In its growth in broth, as well as in its colonial appearance, it is practically identical with the S type from the same strain. The rate of dissociation of the S^R form to the R, in broth cultures, is also similar to that of the S type.

"RB Type.—To classify this variant definitely and designate it properly is not possible at this time. . . .

"The use of 'mucoid' as a descriptive term in reference to this

form seems somewhat misleading, as the mucoid characteristic is not constant when this type is carried on a solid medium. . . . Continued transfers on this medium fail to cause permanent loss of this characteristic, which becomes apparent as soon as the culture is again placed on a medium with a higher pH. It seems probable that the reaction of the medium is the determining factor in the occurrence of the mucoid character in the RB type of *Br. abortus*.

"The RB type has been tentatively so designated because of its single stable characteristic, namely, the chestnut-brown color of its colonies. Other than by color the RB type of colony is distinguished from the R type by its smaller size and its occasional mucoid character.

"The RB type easily reverts to the R type in broth cultures, and on solid mediums it readily produces daughter colonies of the R type.

"Other Types.—In addition to the forms described, daughter colonies or papillae consisting of R or I type have occasionally been found developing from colonies of the S type on solid mediums. Except for the fact that these colonies were superimposed on the parent growth, they differed in no way from the typical R or I colonies.

"A sixth type has been encountered at intervals during the study of these variants, which deserves more thorough investigation. The variant has characteristics similar to Hadley's G type. This form occurs in cultures made from the exudate of uteri infected with *Br. abortus* and appears on the culture medium several days after the colonies of the S type develop. In appearance, the colonies are clear, smooth and slightly raised. The diameters of these colonies rarely exceed 0.5 mm., even after many days' incubation. On transfer, the S type of colonies may develop, but usually no growth occurs."

The colonial characteristics of the *Brucella* dissociants are controlled to some extent by the type of culture medium employed.

Henry employed a glycerin-dextrose-beef-infusion agar in his studies. The author's studies of dissociants have been made on Tryptose agar and the findings in general agree with those of Henry. There are, however, a few exceptions that need mentioning.

It has been observed that the presence of dextrose in a liquid or solid medium to the extent of one per cent hastens dissociation and affects the appearance of the R colonies. The ground-glass surface appearance of the R types is seen only when the amount of dextrose is increased in the culture medium. The author has observed two gradations of the I type based on the degree of opaqueness. These have been termed I_1 and I_2. They readily revert back to the S type. There are two distinct S types based on virulence and catalase activity. One is of high virulence and high catalase activity, while the other is low. Strain 19 now being used for immunizing calves against brucellosis is a good example of the latter.

The author has also observed in certain cultures a peculiar type of dissociated colony which will readily revert to the low virulent, low catalase activity S type. In surface appearance it resembles the R type to a slight extent. In all other characteristics, such as suspendability, agglutinability, and ability to stimulate agglutinins in animals, it acts like the S type. The type in question, designated as OW, appears to originate from the S type. When the colonies are forty-eight hours old, they are opaque. After another forty-eight hours of incubation, the opaque color is streaked with pale green wedges extending and widening from the center to the border. The green wedge pushes out from the border, giving the colony an irregular contour. It is almost impossible to obtain OW types of colonies without the presence of a small number of S types. If an OW type of colony is lifted from the surface of a plate, transferred to another plate and incubated in 10 per cent CO_2, 75 per cent of the colonies will appear as the S type. The OW type does not dissociate into the commonly recognized R, RB or I type.

The OW type colony suspends readily in physiological salt solution and is agglutinated by specific serum. It is not pathogenic for guinea pigs.

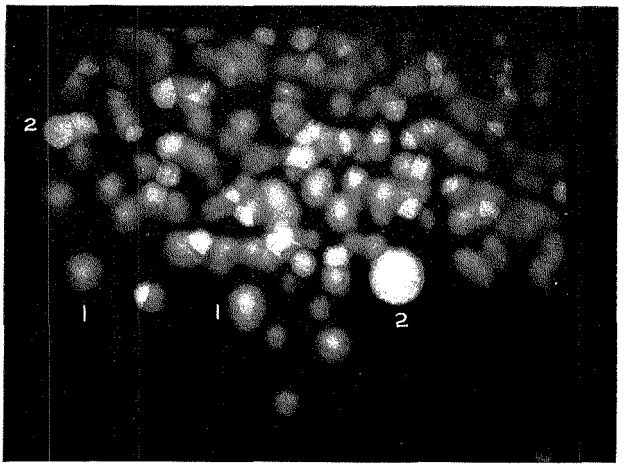
The author has not observed the dissociation of the R type to the pseudo-S type. The R type has the appearance of being non-revertible.

The type designated RB by Henry is seen in only small numbers among many other types in surface platings from a broth culture in which the growth appears distinctly stringy or mucoid in nature. This type is distinctly mucoid in texture. The entire colony may be easily lifted from the surface of a plate by means of a small needle. The author has always found that the RB type of colony is larger in size than other types and remains non-revertible. All of the dissociants observed by Henry and by the author have retained the species differential characteristics. The S and dissociant types of *Brucella* with the exceptions of the R and RB types show a distinct capsule when examined by the proper technic (207).

Just where the R form described by Mallmann and Gallo (291) and the W form described by Mingle and Manthei (313) fit into the *Brucella* dissociation picture is difficult to determine. The fact that they show no dye differential differences, stimulate only homologous agglutinins in high titer in experimental animals, are non-pathogenic, and possess a capsule, would place them outside the commonly known variants.

A representative diagram of the course of colonial dissociation as observed by the author is shown in Figure 4. Figure 5 illustrates colonial types of *Br. abortus*.

It was thought at one time that the partially dissociated forms of *Brucella* could be detected by means of the thermoagglutination test of Burnet (44). More recent studies (220), however, conducted on a large number of strains for a period of four years have revealed that such a test could not be relied upon as a constant



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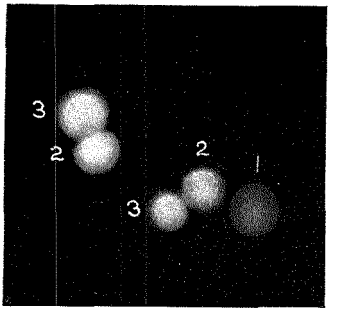
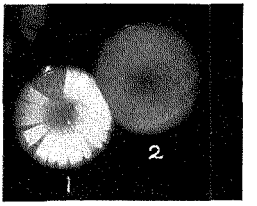




B C

FIGURE 5. SMOOTH AND DISSOCIATED FORMS OF BRUCELLA COLONIES. X 15

A. 1. smooth colony; 2. rough colony
B. 1. smooth colony; 2. rough colony
3. rough mucoid colony (RB type)
C. 1. opaque-wedge colony; 2. smooth colony

means for detecting dissociated forms. Very often a strain found heat agglutinable in one trial would pass through several successive trials before it again manifested this phenomenon.

Di Aichelburg (78) has proposed a unique and very simple test for the detection of antigenic variants of *Brucella.* The test is made by suspending cells from an agar slant growth in a 1:2,000 dilution of basic fuchsin in distilled water and incubating the suspension for two hours at 37° C. All unstable strains according to di Aichelburg are agglutinated in varying degrees at the end of two hours, while normal ones remain uniformly suspended. Many of

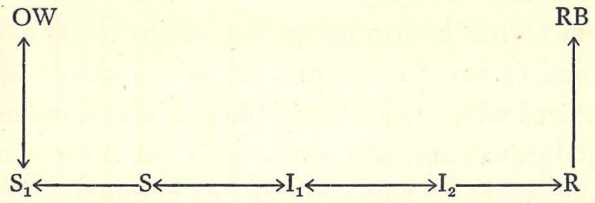


FIGURE 4. COURSE OF BRUCELLA COLONIAL DISSOCIATION

S. smooth type; I_1 and I_2. intermediate types; R. rough type; RB. rough mucoid type; S_1. smooth low-virulent type; OW. opaque-wedge type

the antigenic variants are agglutinated within five minutes after being suspended in the dye solution. While the basic fuchsin agglutination test is far superior to and much more delicate than the thermoagglutination test for detecting antigenic dissociants, it has also been found to give inconstant results.

Alessandrini and Sabatucci (2) and others have found that a 1:1,000 aqueous solution of trypaflavine is satisfactory for detecting the intermediate variants of *Brucella.* The growth of a forty-eight-hour culture is suspended in physiological salt solution to a turbidity of one by the McFarland nephelometer. Equal parts of the bacterial suspension and the 1:1,000 dilution of trypaflavine are mixed in a small test tube and incubated at 37° C. for six hours. If

the culture is a variant, agglutination will be observed. A normal or smooth culture is not agglutinated.

Ardrey, an assistant of the author, has found that the agglutinating action of trypaflavine is reversed for intermediate variants if the growth is suspended in distilled water instead of salt solution. The variant culture remains suspended after incubation, but a smooth one is completely agglutinated. Trypaflavine, therefore, may be used to detect both antigenic variant cultures and smooth ones.

A much more satisfactory and delicate method than those described has been developed for the detection of intermediate variant forms of *Brucella* (323). It involves the use of citrated whole blood from normal human beings in a phagocytic system. When a suspension (1 cm. Gates apparatus) of a partially dissociated strain is mixed with whole citrated blood of non-immune or non-infected individuals and incubated at 37° C. for thirty minutes, the neutrophiles ingest the bacteria in varying numbers. Bacteria from a smooth culture of *Brucella* are phagocytized only to a slight degree if at all by neutrophiles in normal blood.

In Table I are set forth the comparative results of three tests that are used to detect dissociated strains of *Brucella.*

The *Brucella* opsonic activity of the blood is determined by examining spreads under an oil immersion objective with a 12X ocular. A total of 25 polymorphonuclear cells is examined in different sections of the spread, and each cell is grouped in one of the 5 following groups: –, when no phagocytosis occurs; 1+, when 25 per cent of the cells show phagocytosis; 2+, when 50 per cent of the cells show phagocytosis; 3+, when 75 per cent of the cells show phagocytosis; 4+, when 100 per cent of the cells show phagocytosis (see pages 260–261).

VIABILITY

In a study of the viability of *Br. melitensis* under various conditions in Malta, Gilmour (147) and Horrocks (200) found that the

organism was alive after forty-three days in soil allowed to dry slowly, for sixty days in soil dried rapidly, for twenty-one days in damp soil, for forty-two days in sterilized tap water, for twenty-eight days in dry building dust, for fifteen days in sterilized sea water. Direct sun rays kill the organism in a few hours.

TABLE I

Comparison of thermoagglutination, dye agglutination, and phagocytosis of variant Brucella *cultures*

CULTURE		THERMO-AGGLUTINATION			DYE AGGLUTINATION			NORMAL BLOOD PHAGOCYTOSIS	
Number	*Type*	*First test*	*Second test*	*Third test*	*First test*	*Second test*	*Third test*	*First test*	*Third test*
S422	V	–	–	–	–	–	–	3+	1+
S429	V	2+	2+	4+	4+	4+	4+	3+	3+
S410	V	–	–	3+	–	–	3+	1+	3+
M623	V	–	–	2+	–	3+	2+	4+	3+
M350	V	–	–	2+	–	–	3+	4+	2+
M336	V	2+	–	4+	–	–	4+	2+	3+
A85	V	4+	4+	4+	4+	4+	4+	4+	4+
A36	V	4+	4+	4+	4+	4+	4+	4+	4+
A132	V	4+	4+	4+	4+	4+	4+	4+	4+
S400	N	–	–	–	–	–	–	–	–
M78	N	–	–	–	–	–	–	–	–
A154	N	–	–	–	–	–	–	–	–

After Munger and Huddleson (323)

+ = degree of agglutination or phagocytosis; – = no agglutination or phagocytosis; V = variant culture; N = normal culture; S = *suis;* M = *melitensis;* A = *abortus.*

Eyre and associates (110) prepared artificial gastric juice and then added it to milk naturally infected with *Br. melitensis.* The mixture was incubated at 37° C. The organism was recovered from the fluid in large numbers after two hours.

Huddleson and associates (216) held hog spleens naturally infected with *Br. suis* at a temperature of —10° F. to determine the effect of refrigeration on the viability of the organisms. *Br. suis* was cultured from the spleens after a period of thirty days. Infected

spleens were also kept in meat-curing brine and cultured at intervals. Positive cultures were obtained at the end of forty days.

When phenolized anti-hog cholera serum and blood virus are inoculated with *Br. suis* and stored in a cold room, positive cultures can be obtained from the former after two weeks and from the latter after five months (213). Bang (10) noted that *Br. abortus* was alive in infected exudate from the bovine uterus seven months after being stored in an ice chest. According to Bosworth (33) *Br. abortus* will remain alive in an infected bovine fetus for one hundred eighty-two days. When milk naturally infected with *Br. abortus* is stored in an ice box at 10° C., the organism is not viable after the tenth day (54).

Thompson (434) has found that when ice cream is made from milk naturally infected with *Br. abortus* and stored at 32° F., the organism remains viable for thirty days. When butter is inoculated with *Br. abortus* and stored at 8° C. the organism remains viable for one hundred forty-two days (54). *Br. abortus* can remain viable in Roquefort cheese for two months.

Boak and Carpenter (30) have found that *Br. melitensis* and *Br. abortus* are killed at 140° F. to 142° F. in fifteen minutes but not in ten minutes; that *Br. suis* to be completely destroyed requires twenty minutes at 140° F. or fifteen minutes at 142° F.; and that at 145° F. all three species are destroyed in ten minutes. Murray and associates (326) using a standard pasteurizing unit reported that a temperature of 143.6° to 145.4° F. applied three minutes was sufficient to kill both *Br. abortus* and *Br. suis* in milk.

Cameron (51) has made a very thorough and exhaustive study of the viability of *Br. abortus* under varied conditions. His findings are as follows.

"The organism was found to live for four and one-half hours when exposed to direct sunlight, for five days when dried in burlap sacking kept on a laboratory table, for thirty days when dried

in burlap sacking kept in an unheated cellar, for one hundred twenty-one days, the maximum interval covered, when dried in the presence of nutrient material; for seventy-two days when dried in the absence of nutrient material, less than four days in soil when dried quickly in Petri dishes placed in an unheated cellar during October; for twenty-seven days, the maximum interval covered, in soil treated in the same manner as in the preceding experiment but conducted during February; for thirty-seven days in soil that dried slowly in test tubes kept in a laboratory cupboard; for sixty-seven days, the maximum interval covered, after having been in wet soil stored in an unheated cellar; for four days in normal bovine urine at room temperature; for one hundred twenty days in bovine feces kept in a test tube in a laboratory cupboard and dried very slowly; for one hundred days in bovine feces which were kept in an unheated cellar and did not dry; for seventy-seven days, the maximum interval covered, in the presence of putrefaction; for seventy-seven days in tap water which was sterilized before the organism was introduced and kept at room temperature; for one hundred fourteen days in tap water kept at a temperature of approximately —40° C."

From all the carefully conducted studies relative to the viability of *Brucella* under varied conditions, one may conclude that it is not easily destroyed except by exposure to temperatures above 55° C. and to sunlight.

DETERMINATION OF VIRULENCE FROM CATALASE ACTIVITY

It has been found by the author that virulence or pathogenicity of cultures of each of the species of *Brucella* is directly associated with the activity of catalase, the enzyme contained in live cells. The activity of the enzyme is determined by measuring its ability to decompose a given amount of H_2O_2 under certain conditions during a fixed period of time.

Cultures of each species, regardless of whether they are smooth or dissociated, fall into three zones of activity. The *suis* species is the most active and contains the largest amount of catalase, and the *abortus* the least active, while *melitensis* falls between the two. The activity or amount of catalase in a given culture of each species decreases as the degree of dissociation increases and as the virulence of a smooth culture decreases. The titratable activity of cultures of each species, however, keeps within its own zone, except that of *melitensis*. Rough and low virulent cultures of *melitensis* often show about the same activity as *abortus* cultures of high virulence. In view of this finding it is obvious that one must first determine the species to which a given culture belongs before attempting to correlate virulence with the action of the enzyme.

The accuracy of the test depends upon the following factors: (1) the type of agar medium employed; (2) the period of growing the culture; (3) the degree of dispersion of the bacterial cells in a suspending liquid that will keep the cells viable for several hours; (4) the pH of the suspending liquid; (5) the method of standardizing the bacterial suspension; (6) the temperature and time interval at which the test is conducted; (7) the rate of shaking the suspension; (8) the amount of H_2O_2 added; and (9) the cleanliness of the glassware.

Keeping in mind the factors just mentioned, the test is conducted according to the procedure which follows:

A given culture is grown on a liver infusion agar slant for forty-eight hours at 37° C. A small amount of the growth is suspended in distilled water containing 0.05 per cent Tryptose peptone and 0.5 per cent NaCl (previously autoclaved and filtered through a fine fritted filter just before use) of pH 6.9 to 7.0. The pH of the solution rarely needs adjusting after autoclaving. Efficient shaking is needed to disperse the bacteria. The suspension is now standardized to a galvanometer scale reading of 28 on the Libby Photonreflec-

tometer.* The instrument should be standardized to a galvanometer reading of 15, before deflection readings of the suspension are taken. A 5 ml. portion of the standardized suspension is now placed in a clean 250 ml. Erlenmeyer flask. To this is added 15 ml. of cold (approximately 5° C.) 1.15 N H_2O_2 solution. (A sufficient amount of 30 per cent c.p. H_2O_2 is added to the Tryptose peptone-salt solution used for suspending the bacteria so that it will contain 19.5 gm. of H_2O_2 per liter of total solution.) The flask is placed immediately in a shaking apparatus and oscillated at a rate of 100 per minute. The flask and reagents are kept at a temperature of 25° C. At the proper time intervals, which may be five, fifteen, and thirty minutes if a time curve is desired, or at thirty minutes if only one determination is being made, a 5 ml. sample is withdrawn from the shaking flask and placed in an Erlenmeyer flask. At the same time 3 cc. of 1:4 H_2SO_4 is added to destroy the enzyme so that there will be no further action upon the H_2O_2 and to complete the chemical reaction. The sample is now diluted with distilled H_2O for titration against 0.1 N $KMnO_4$. The number of ml. of $KMnO_4$ required to decompose the H_2O_2 in the 5 ml. sample represents the equivalent amount of H_2O_2 remaining after the action of the enzyme. By subtracting the number of ml. of 0.1 $KMnO_4$ required at the time interval of titration from the number required to decompose the original amount of H_2O_2 available in the 5 ml. sample, one obtains a value expressed in ml. of 0.1 $KMnO_4$ which represents the amount of H_2O_2 in the sample decomposed by the bacterial enzyme. The titration values, therefore, are expressed in 0.1 N $KMnO_4$ rather than in mg. of H_2O_2 decomposed during a given time period.

It has been observed that when a culture of *Br. abortus* gives an equivalent value greater than 6 ml. of 0.1 $KMnO_4$, it is one of high virulence; that is, less than one hundred live organisms are required to produce sufficient infection in the guinea pig to cause

* Made by Dr. R. L. Libby, Pearl River, N.Y.

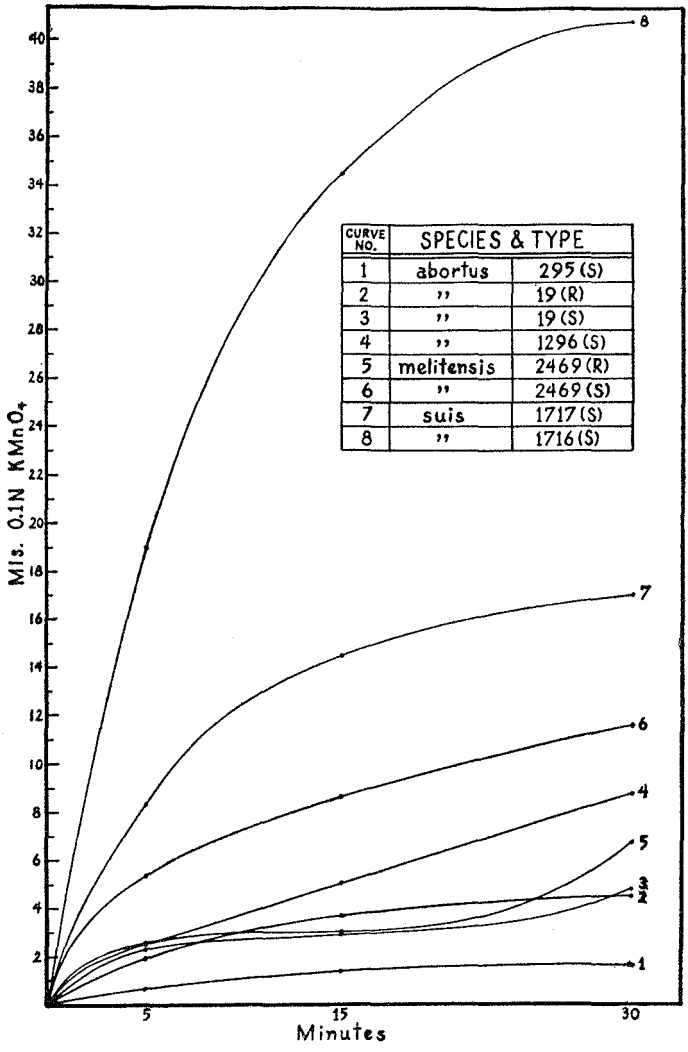


FIGURE 6. RATE OF DECOMPOSITION OF H_2O_2 BY CATALASE IN REPRESENTATIVE CULTURES OF BRUCELLA

macroscopic changes in the tissues. In the case of cultures such as the Bureau of Animal Industry strain 19, which exhibits a moderately low decomposition value, more than one million live organisms are required to infect the guinea pig. There are indications that some smooth cultures contain two or more types of cells and that the types contain different amounts of catalase and show different degrees of virulence.

The activity of a culture that is very rough, such as the RB type, cannot be accurately measured unless the cells are thoroughly dispersed when suspended in a liquid. The cells of such cultures tend to form small aggregates, and since the measured activity is an expression of the number of cells as well as the amount of enzyme contained in the cell, the value obtained is misleading.

One point of interest is that smooth cultures of *Brucella* regardless of their catalase activity or degree of virulence have the same colonial appearance on Tryptose agar, and all serve as good antigens for the agglutination test.

Certain cultures of *Br. suis* show a far greater catalase activity than those of the other two species. That this observation signifies a higher degree of virulence is borne out by the fact that less than 5 live organisms are required to produce extensive gross changes in the tissues of the guinea pig. It is the opinion of the writer that *Br. suis,* expressed in terms of the number of organisms required to produce infection, is much more pathogenic for man and most species of animals than the other *Brucella* species.

In Figure 6 there are presented time curves of the catalase activity of cultures representative of the three species and types within the species. The titration values which describe the curves are set forth in Table II.

From the slope of all curves, with the exception of 3 and 5, it is evident that the decomposition of H_2O_2 by *Brucella* catalase proceeds in the manner of a monomolecular reaction. This has been

proved by determining the velocity-constant K derived for each of the time intervals from the familiar "monomolecular" exponential equation.

The curves of the two exceptions appear to be made up of two reactions of slightly different velocities. This suggests that there are

TABLE II

Rate of decomposition of H_2O_2 by catalase in representative cultures of Brucella

CURVE NUMBER	SPECIES AND TYPE	H_2O_2 DECOMPOSED, EXPRESSED IN ML. 0.1 N $KMnO_4$ AFTER		
		5 min.	*15 min.*	*30 min.*
1	Abortus 295 (S)	0.602	1.350	1.470
2	Abortus 19 (R)	1.876	3.636	4.454
3	Abortus 19 (S)	2.243	2.849	4.692
4	Abortus 1296 (S)	2.441	5.184	8.694
5	Melitensis 2469 (R)	2.592	3.002	6.738
6	Melitensis 2469 (S)	5.298	8.603	11.592
7	Suis 1717 (S)	8.272	14.462	17.181
8	Suis 1716 (S)	18.889	34.523	40.758

S = smooth type; R = rough type.

present in the bacterial suspensions two types of bacteria, each possessing a different catalase activity.

A rapid *in vitro* method of determining the quantitative properties of cultures should have considerable practical application. It may be used to determine (1) change in virulence; (2) the virulence of a culture just before use in infection experiments; (3) the viability of cells from a culture when subjected to various physical and chemical treatments; (4) the changes in the state of the culture.

II

METHODS OF ISOLATING *BRUCELLA*

MEDIA

THE beef liver infusion agar medium described by Stafseth (421) has been successfully used in the laboratory at Michigan State College to isolate and cultivate *Brucella.* The medium is prepared as follows.

Liver Infusion Agar

Fresh beef liver, free from fat, is ground in a meat chopper to a plastic mass. One pound of the liver and 500 cc. of distilled water are placed in a container, mixed well, and then placed in a cold room for twenty-four hours. The container is now covered and placed in flowing steam for twenty minutes. The lid is then removed and the contents stirred with a glass rod so that all parts are reached by the heat. The heating is continued in flowing steam for an hour and a half. The mixture is removed and filtered through a wire screen. The infusion thus prepared is made into a solid medium or infusion broth. The infusion should not be sterilized and made into a culture medium later. Successive sterilizations reduce materially the growth-promoting factors in the infusion. The chief growth-promoting factors in liver infusion are heat decomposable sulfur compounds.

To prepare one liter of liver infusion agar, the following ingredients are measured out:

Washed agar	20 grams
Water, distilled	500 cc.
Liver infusion	500 cc.
Peptone (Bacto)	5 grams
Sodium chloride c.p.	5 grams

All ingredients are placed in a suitable container, covered, and placed in flowing steam for one hour. They are then removed and cooled to 60° C. The pH is adjusted at this time to 7.2. The pH of the medium during the process of sterilizing will usually drop to or approach closely 6.6 or 6.8, which is the optimum pH for growing *Brucella.* The container is placed in flowing steam again for one-half hour.

The contents are decanted and placed in sterile flasks or tubes and sterilized at 15 pounds pressure for thirty minutes. The final medium prepared according to the foregoing method will not be clear, but this will not interfere with its use. The final product may be freed from sediment and suspended particles if it is passed through a Sharples centrifuge before sterilization.

Bacto-Tryptose Agar

A highly satisfactory dehydrated medium, "Bacto-Tryptose Agar," that may be substituted for liver infusion agar for the isolation of *Brucella* has been developed by the Difco Laboratories. In its present state of development this new medium does not produce the quantity of growth that one obtains on liver infusion agar. It is prepared as follows:

Bacto-Tryptose Agar (dehydrated) ..	46 grams
Water, distilled	1,000 cc.

The mixture is boiled a few minutes to dissolve the agar, then sterilized at 15 pounds pressure (250° F.) for twenty minutes. The final pH should be 6.6 or 6.8.

Liver Infusion Broth

Liver infusion is prepared as described under *Liver Infusion Agar.* To prepare the broth, the following ingredients are measured out and mixed:

Liver infusion	500 cc.
Water, distilled	500 cc.
Peptone (Bacto)	5 grams
Sodium chloride c.p.	5 grams

The mixture is heated for twenty minutes in flowing steam to dissolve the ingredients. The pH is adjusted to 7.2 with N/1 NaOH. The mixture is then heated in flowing steam for fifteen minutes and filtered through coarse filter paper. The broth may be tubed or sterilized in bulk at 15 pounds pressure (250° F.) for thirty minutes. The final pH should be 6.6 or 6.8. Liver infusion broth should never be resterilized.

Bacto-Tryptose Broth

The Difco Laboratories have developed a peptone broth medium, "Bacto-Tryptose," that is excellent for initiating rapid growth of *Brucella*. The dehydrated medium will yield as much growth in forty-eight hours as liver broth will yield in ten days. Tryptose broth is prepared as follows:

Bacto-Tryptose	20 grams
Water, distilled	1,000 cc.
Sodium chloride c.p.	5 grams

The mixture is heated in flowing steam for ten minutes to dissolve the ingredients. The pH is adjusted to 7.2. The mixture is then filtered through coarse filter paper and sterilized at 15 pounds pressure (250° F.) for twenty minutes. The final pH should be 6.6 or 6.8.

If liver infusion from one-fourth pound of liver is added to the Tryptose broth, growth of the organism will continue for many weeks.

Potato Infusion Agar

Another medium that has proved satisfactory for growing *Brucella* is potato infusion agar. The method of preparation as recommended by the United States Bureau of Animal Industry, Animal Disease Station is as follows:

Sound, raw potatoes are washed and pared, and 250 grams are sliced thin into 1,000 cc. of distilled water with minimum exposure to air. The mixture is held over night in a covered container at approximately 60° C., then filtered through filter paper. The filtrate is made up to 1,000 cc. and the following ingredients added:

Sodium chloride, U.S.P.	5 parts
Bacto-peptone, or equivalent	10 parts
Beef extract	5 parts
Dextrose, U.S.P.	10 parts
Agar, U.S.P. (washed)	20 parts

The mixture is heated to dissolve the agar. Twenty cc. of glycerin, U.S.P., are added and the medium is adjusted to pH 7.4. This results, after final autoclaving, in a pH of 6.8. The hot solution is filtered in a Buchner funnel through a pad composed of two thin layers of absorbent cotton. Sterilization of the medium is effected by autoclaving at 120° C. for thirty minutes.

PREPARATION OF MEDIUM FOR CULTURE

When infective material is to be cultured for the presence of *Brucella,* a prepared 0.1 per cent aqueous solution of crystal violet (certified) is added to liquefied liver infusion agar or Bacto-Tryptose agar in a sufficient amount to give the original dye a final dilution of 1:1,000,000. The presence of the dye inhibits the growth of the majority of Gram-positive organisms, especially fast-growing ones, but in this dilution does not inhibit the growth of any of the three species of *Brucella.* Colonies of *Brucella* on this medium

have a very characteristic appearance and may easily be distinguished from others.

GUINEA-PIG INOCULATION METHOD

The guinea pig has been found to be the most satisfactory animal for determining the pathogenicity of strains of *Brucella* and for detecting the presence of *Brucella* in animal tissues, excretions, and secretions. Only normal animals from 300 to 600 gm. in weight should be used. Male pigs are preferable because of the characteristic lesions which develop in the testis and epididymis as a result of *Brucella* infection (see Figure 29). The blood of the pigs should be examined for *Brucella* agglutinins before inoculation. Normal stock should be housed in a separate room or at a considerable distance from inoculated pigs. Pigs inoculated with different materials should be housed in separate cages in order to prevent a spread of infection from one group to the other.

The pigs may be inoculated either subcutaneously or intraperitoneally, depending upon the nature of the inoculum. If the inoculum is fresh milk or fresh animal tissue, it may be injected intraperitoneally. If the inoculum is market milk or animal tissue that has undergone some decomposition, it should be injected subcutaneously in order to prevent death which may result from the presence of contaminating bacteria.

The period of time which should be allowed to elapse between inoculation and autopsy is about six weeks. This period allows sufficient time for the guinea pig to become extensively infected and for the development of *Brucella* agglutinins in the blood of the pig.

At autopsy, enlarged joints of the extremities and enlarged superficial lymph nodes are noted. The viscera and internal organs are exposed and all, including the lymph nodes external and internal, are examined for enlargement and evidence of macroscopic

changes so characteristic of the disease in guinea pigs (see Chapter V, pages 210–213). A blood sample is taken from the heart to determine the presence of agglutinins for *Brucella.* The lungs, liver, spleen, kidneys, testes, urine, and enlarged lymph nodes are cultured by removing pieces of each organ, clipping the cut surface in several places with a pair of sterile scissors, and rubbing the cut surface over the surface of crystal violet liver agar plates or Bacto-Tryptose agar plates.

SELECTION AND PREPARATION OF SPECIMENS

Uterus

If the non-gravid uterus of a cow or goat is to be examined for the presence of *Brucella,* a fold of the cervix is first grasped by a uterine forceps and retracted. A sterile metal catheter, 45 cm. by 6 mm., is then passed through the cervical canal. To the free end of the catheter is attached a piece of rubber tubing about 45 cm. in length which is in turn attached to a 250 cc. siphon flask containing 200 cc. of sterile physiological salt solution. The flask is inverted and held above the level of the uterus to allow the salt solution to flow in freely. A hand is now introduced into the rectum, and the uterus palpated and massaged gently for about half a minute. The flask is now lowered to permit the fluid in the uterus to flow back into the flask.

The uterine washing is taken to the laboratory and 0.05 or 0.1 cc. amounts spread over the surface of each of several crystal violet liver agar plates in order to determine quantitatively the number of *Brucella* present in the washing. The remaining fluid is centrifuged for two hours at 3,000 r.p.m., the sediment cultured as above and injected subcutaneously into a guinea pig.*

When a parturient uterus is examined for evidence of infection,

* As a routine procedure, when seeking *Brucella,* guinea pigs are not killed and examined until the end of six weeks, regardless of the nature of the material injected.

a sterile swab on the end of a 16-gauge wire enclosed in a glass tube, 45 cm. by 6 mm., is introduced deeply into the uterus, removed, and the swab washed in 5 cc. of sterile salt solution and the suspension cultured as previously described. The remainder is injected into a guinea pig.

Fetal Membranes

If the membranes are retained, two of the intact maternal-fetal placentae are removed at the peduncle and torn from the membranes. The excess liquid with which they are usually covered is removed by sterile gauze, and the chorionic and maternal villi separated. The exposed surfaces are then scraped with a sterile scalpel, and the exudate obtained is cultured and suspended in sterile salt solution to be injected into a guinea pig.

If the intact fetal membranes are obtained, they are laid open and spread out on a stone floor or table with the maternal side up. The examination of the placentae and interplacental areas for evidence of macroscopic changes is conducted according to a method described by Williams (471). The debris and excess of blood are washed out by a slow-flowing stream of water from a hose. The villi float in the running water and aid materially in the detection of evidence of disease. Placental areas and parts of areas showing evidences of necrosis are removed, washed in five changes of sterile salt solution, and ground in a mortar with sterile quartz sand and sufficient sterile salt solution to make an emulsion. The emulsion is then cultured and a 2 cc. portion injected subcutaneously into a guinea pig. This route of injection is used in order to avoid a possible peritonitis and subsequent death of the pig from contaminating organisms. If there are opaque, wrinkled, leather-like areas in the free intercotyledonary chorion, one of these should be removed, thoroughly washed in sterile salt solution, and cultured. Further, it should be ground to a paste in a mortar with sand and sterile

salt solution and a small amount injected subcutaneously into a guinea pig.

Fetus

In culturing the various organs of the fetus, about one square inch each of spleen, liver, kidney, and lung is removed with sterile instruments, the surfaces are incised in several places and smeared vigorously over the surface of several crystal violet liver agar plates. The stomach exudate and heart's blood are removed with a sterile pipette and placed in test tubes. Various amounts of these fluids are smeared over the surface of plates of the same medium. Meconium is removed from the large intestines or cecum with a sterile spatula and cultured in a similar manner. The small pieces of the various organs after culturing are ground with sterile quartz sand in a mortar. Sufficient sterile salt solution, 5 to 10 cc., is added for collecting the finely divided particles of tissue. The suspensions of the organs are injected into guinea pigs in 2 cc. amounts. Two cc. of the stomach exudate are likewise injected into a guinea pig.

Milk

In 1920 a simple, practical, and efficient method for isolating *Br. abortus* from cow's milk was described by the writer (202). Later, the technic of isolation was considerably improved so that its results agree very closely with the guinea-pig inoculation method.

The method offers a rapid, accurate, and quantitative means of detecting the presence of *Brucella* in milk drawn directly from the udder of the cow or goat. The milk should be collected at or near milking time. The teats should first be wiped with a clean damp cloth. The first two or three streams of milk from each quarter should be discarded. Samples of milk may now be taken for examination from the hindquarters in one sterile test tube and from the forequarters in another sterile test tube. About 15 cc. of milk

in each tube, or 7.5 cc. from each quarter, are sufficient for examination. The tubes of milk should be allowed to stand in a cold room for twenty-four hours to permit the cream to rise to the surface. The cream carries with it the majority of the organisms in the

TABLE III

The quantitative position of Br. abortus *in infective milk after standing twenty-four hours at ice-box temperature*

SAMPLE	NUMBER OF COLONIES IN 0.1 CC.		
	Cream	*Bottom milk**	*Whole milk*
1	520	6	40
2	450	2	34
3	140	2	20
4	90	1	14
5	100	2	30
6	280	3	35
7	140	6	30
8	89	2	15
9	160	3	20

* Guinea-pig inoculations with bottom milk were negative.

milk. The cream is pipetted off and cultured by placing 0.1 to 0.2 cc. on the surface of each of two liver agar or Tryptose agar plates containing crystal violet. The drops of cream are spread over the surface of the plate by means of a sterile glass rod, with a right-angle bend, by rotating the plate in a horizontal plane.

Urine

Brucella has been cultured but very few times from the urine of infected human beings and naturally infected cattle. In culturing *Brucella* from the urine of human beings, the method at Michigan State College is to centrifuge a 50 cc. sample of catheterized urine at 3,000 r.p.m. for one hour and spread the sediment over several plates of crystal violet liver agar.

Feces

Very few workers have succeeded in isolating *Brucella* from the feces of infected human beings or naturally infected animals. Amoss and Poston (4), by employing a unique method, have been more successful than others in obtaining positive cultures from human feces. The technic of their method follows.

"About 1 gm. of fresh feces is mixed in 50 cc. of sterile isotonic salt solution and shaken for a few minutes to insure thorough suspension. The suspension is filtered through four layers of hospital gauze to remove gross particles and centrifugalized at half speed for three minutes to throw down other particles and larger bacteria. To the supernatant suspension, a sufficient amount of immune serum is added to make the total dilution 1:100, and, after shaking, the mixture is placed in a 37° C. water bath for two hours. The suspension is centrifugalized at half speed for five minutes and the supernatant fluid discarded.

"The precipitate is resuspended in isotonic salt solution, stirred, and centrifugalized at the same speed again. The supernatant fluid is again discarded and the procedure repeated twice." The precipitate is spread with a bent glass rod over the surface of several liver agar plates or Bacto-Tryptose agar plates. The plates are incubated at 37° C. in an anaerobic jar containing 10 per cent carbon dioxide.

Blood

In the past the possibility of isolating *Brucella* from the blood of brucellosis patients has depended upon the species responsible for the infection and whether the culture was made during the pyrexial or the apyrexial period. If the infection is due to either *Br. melitensis* or *Br. suis,* little difficulty has been encountered in obtaining a positive culture during a pyrexial period. Failure to obtain a positive blood culture of *Brucella* in a large number of

acute and chronic cases of brucellosis has been a perplexing problem to investigators in North Europe and America. The failures may be attributed to the following factors: the absence of the organism in the blood; the use of unsuitable culture media; the absence of the proper gaseous tension in the blood culture.

The probability of isolating *Brucella* from human blood can be rendered more certain and the growth hastened by employing Bacto-Tryptose broth (Difco) and incubating the culture under an increased CO_2 tension.

The Bacto-Tryptose broth is prepared according to the method described on page 25 but with the addition of sodium citrate to one per cent to serve as a blood anti-coagulant. The medium is distributed in 50 cc. serum bottles in 20 cc. amounts. The bottles are closed with stoppers suitable for puncturing with a hypodermic needle. The bottled medium is autoclaved for twenty minutes at 15 pounds pressure.

The medium may be inoculated with 2 to 5 cc. of blood directly after collection from the patient by puncturing the stopper aseptically with the same needle used in collecting the blood sample. The bottle is shaken vigorously to prevent the clotting of the blood.

The proper gaseous tension found satisfactory for promoting the growth of the species of *Brucella* in a liquid medium in the presence of blood is approximately 10 per cent.*

The proper gaseous tension inside a culture bottle may be obtained by inserting a sterile 23-gauge needle through the diaphragm of the rubber stopper. The hub of the needle is connected to a short piece of glass tubing containing sterile absorbent cotton which is in turn connected to a two-way glass stopcock with a downward outlet. Partial vacuum is obtained inside the bottle through the stopcock and needle connection by means of a vacuum pump. The stopcock is turned in the opposite direction to make

* It is advisable to introduce the CO_2 into the bottles before inoculating with blood.

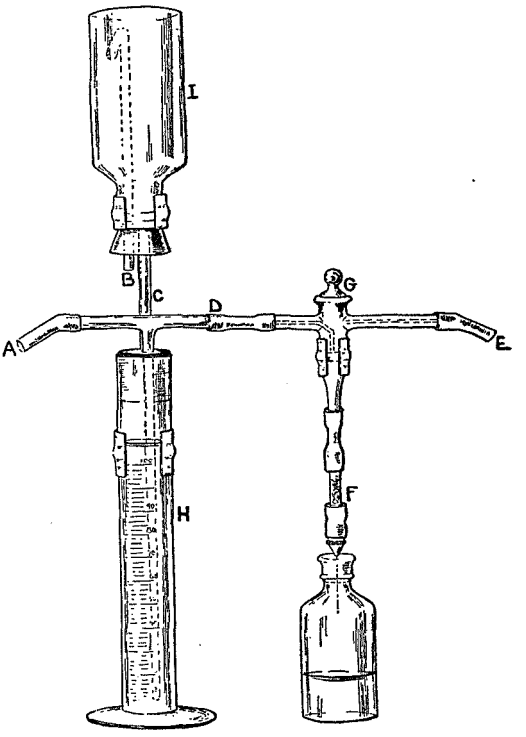


FIGURE 7. APPARATUS FOR DELIVERY OF CO_2 INTO BLOOD CULTURE BOTTLES

A. gas inlet from CO_2 source; B. outlet for displaced air in bottle I; C. tube through which water displaced by gas in cylinder H passes into bottle I; D. outlet through which measured gas passes from cylinder H by way of stopcock G and sterile needle holding tube F into culture bottle; E. outlet to vacuum pump used to establish negative pressure by way of the two-way stopcock in culture bottle

connection with a tube leading to the measured amount of gas collected over a column of water. The apparatus to be used in this procedure is illustrated in Figure 7.

At the end of each fourth day, the culture should be mixed by shaking, 0.5 cc. removed and added to a Bacto-Tryptose or liver agar Petri plate, and the plate incubated under 10 per cent CO_2 for

four days. If no growth is obtained from the blood culture within twenty days, it may be discarded. Suspicious colonies should be transferred to agar slants and their identity confirmed.

During the year 1937, the author had an opportunity to study the value of Bacto-Tryptose broth for the isolation of *Br. melitensis* from 55 cases of brucellosis on the Island of Malta. Of the total number of cases, 38 were febrile and 17 afebrile at the time the blood was cultured. Positive cultures were obtained in 32 cases of the former group and in 5 of the latter. Growth appeared within four days in 23 of the cultures. One culture required eighteen days of incubation before a positive subculture was obtained.

Other Tissues

The methods of isolating *Brucella* from the tissues of the naturally infected goat, sheep, hog, horse, fowl, and dog do not differ materially from those used in the examination of infected cows and human beings.

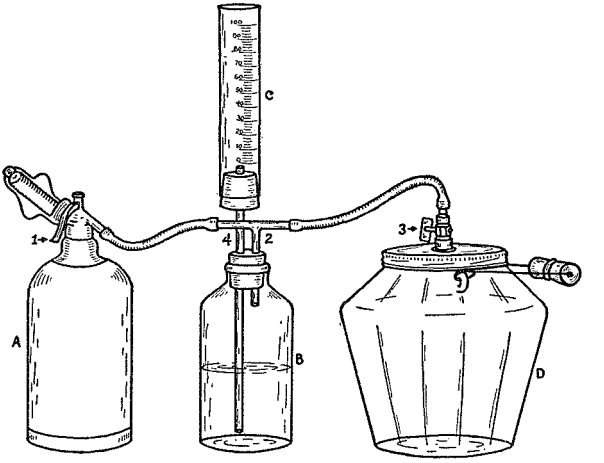
The location of *Brucella* in the tissues of different species of infected animals varies to a considerable degree and must be taken into consideration in a search for its presence. In the infected goat and sheep, *Br. melitensis* may be found in the spleen as often as in the udder and supramammary lymph nodes. *Br. suis* localizes in the spleen, kidneys, bone marrow, vertebrae, and lymph nodes of the infected hog. It may be recovered from the blood stream in the early stage of infection.

In infected horses, *Brucella* may be isolated from suppurative conditions involving the withers, poll, bony tissue, tendons, joints, and sternum.

The infected fowl appears to harbor *Brucella* in its tissues for only a short time after becoming infected. The liver, spleen, and ovary should be examined for the presence of the organism.

INCUBATION OF INOCULATED MEDIA AND IDENTIFICATION OF CULTURES

Plates or tubes inoculated with infective material are placed in a closed container in which 10 per cent of the atmosphere has been replaced by carbon dioxide, and incubated for five days at 37° C.



After Polding and Edwards (360)

FIGURE 8. APPARATUS FOR COLLECTING CO_2 AND CONTAINER FOR INCUBATING CULTURE UNDER CO_2

A. syphon bottle; 1. CO_2 gas capsule; B. bottle for collecting CO_2 over water; C. graduated cylinder for collecting displaced water from B and measuring amount of gas collected; 2. outlet for passage of collected gas to container; negative pressure should be established in jar D to pull in gas; D. container for incubating culture

Glass pickle or fruit jars similar to the one shown in Figure 8 make very satisfactory anaerobic containers. A metal cock is sealed into the top through which a partial vacuum is created in the jar by means of a vacuum pump. The partial vacuum is replaced by the required volume of CO_2 which is collected and measured over a column of water using the apparatus shown in Figure 8. Pressure cookers also make satisfactory anaerobic containers for a large number of Petri plates.

At the end of the five-day incubation period the plates or tubes are removed from the container and examined for colonies of *Brucella.* When present they have a clear light-blue violet appearance on media containing crystal violet. The size of the colonies varies from 2 to 7 mm. in diameter. The total number of colonies which appear on plates made from cream varies from 5,000 to 10,000 and depends upon the degree of infection in the quarter of the udder. Suspicious colonies should be transferred to agar slants or to another crystal violet liver agar plate if the inoculated plate contains colonies of other bacteria or molds. The suspicious culture should be properly identified and the species to which it belongs determined.

If aerobic types of *Brucella,* such as *suis* or *melitensis,* are present in the cultured material, their growth will not be inhibited by incubating the inoculated plates in an atmosphere of 10 per cent CO_2. If the first transfer of a primary culture grows aerobically, it is presumptive evidence that the species is not *abortus.* However, since it has been found that strains of *abortus,* which have become aerobic through artificial cultivation, may be recovered aerobically from animals following inoculation, it is evident that one must depend upon other methods to determine the species of a newly isolated aerobic strain of *Brucella.*

III

DIFFERENTIATION OF THE SPECIES OF THE GENUS *BRUCELLA*

THE discovery by Evans (99) in 1918 of the close relationship between *Br. melitensis* and *Br. abortus* was the starting point for numerous studies designed to throw further light upon these two organisms. Those who first studied *Br. suis* observed certain differences between it and *Br. abortus* (63, 412). It was observed that *Br. suis* grew aerobically on primary isolation, and that the gross changes which it produced in the tissues of inoculated guinea pigs were more numerous and more prominent than those produced by *Br. abortus*. For many years *Br. suis* was called the porcine type of *Br. abortus,* because there were no accurate methods available for differentiating one from the other. Today, there is no reason for calling the two organisms by the same name, since conclusive data have been obtained which show that they are not identical, though closely related, species.

AGGLUTININ ABSORPTION

The agglutinin-absorption test was the first method used to establish differences between the members of this genus. Numerous publications concerning its value have appeared. Feusier and Meyer (117) were the first to contribute data on this subject, dividing the strains which they studied into four types. Later, Khaled (248) identified three types, Evans (100) eight types, Burnet (43) two types, and Ross (389) four types. The study made by Evans was the most inclusive and embraced 68 strains of *Brucella* of animal and human origin, coming from all parts of the world. She established two main serological types and six subtypes distributed un-

der each of the two main types. Evans grouped the strains according to their agglutinin-absorption capacity into varieties, calling the species *melitensis*. All smooth strains of *Br. abortus* and *Br. suis* constitute one main type and all smooth strains of *Br. melitensis* constitute the other. Those strains forming subtypes are the antigenic variants or "para" forms. One may expect to establish as many different subtypes as there are different antigenic variants.

In typing strains of *Brucella* by the agglutinin-absorption method it must be kept in mind that it is not possible by this method to separate *Br. suis* from *Br. abortus*. Further, those strains of *Brucella* which possess antigenic variant characteristics cannot easily be classified as to species.

It has been pointed out by certain investigators that in conducting agglutinin-absorption tests one should employ more than one concentration or density of a given bacterium in determining its capacity to absorb agglutinins from a given serum. Wilson and Miles (475) have very clearly shown that the two main types or species of *Brucella* are distinguished not by qualitatively different antigens, but by a quantitative distribution of a common antigen. In conducting an absorption test it is important, therefore, that the dose of absorbing organisms be adjusted to the titer of the serum.

The Agglutinin-Absorption Test Applied to Unknown Cultures

Wilson and Miles (475) have obtained type-specific serums for typing strains by the employment of a sufficient quantity of the heterologous organism to absorb the minor agglutinin from a serum without affecting to a great degree the major agglutinin.

Miles (304) has characterized the homologous and heterologous surface antigens of *Brucella* as A and M, A being the major and M the minor antigen. The A:M ratios in *abortus* and in *melitensis* are assumed to be about 20:1 and 1:20 respectively. The description of a procedure for obtaining species-specific serums follows.

TABLE IV

World distribution of the three known species of Brucella *by host and by strain*

	NUMBER OF STRAINS									
	Man	*Cow*	*Hog*	*Goat*	*Sheep*	*Horse*	*Dog*	*Buffalo*	*Fowl*	*Rabbit*
United States										
Abortus	95	750	–	–	–	11	–	2	2	–
Suis	178	9	130	–	–	5	1	–	9	–
Melitensis	21	3	2	1	–	–	–	–	–	–
Not classifiable	7	3	–	–	–	–	–	–	–	–
Great Britain										
Abortus	2	17	–	–	–	–	–	–	–	–
Melitensis	2	–	–	–	–	–	–	–	–	–
France										
Abortus	29	112	–	–	2	11	–	–	–	–
Melitensis	552	71	–	114	46	–	–	–	–	2
Not classifiable	–	3	–	–	–	–	–	–	–	–
Italy										
Abortus	11	–	–	–	–	–	–	–	–	–
Melitensis	13	–	–	1	–	–	–	–	–	–
Germany										
Abortus	10	7	–	–	–	–	–	–	–	–
Melitensis	–	2	–	–	–	–	–	–	–	–

NOTE: Cultures received and identified at the Central Brucella Station, Michigan State College, from 1920 to 1942.

TABLE IV (*cont.*)

	NUMBER OF STRAINS									
	Man	*Cow*	*Hog*	*Goat*	*Sheep*	*Horse*	*Dog*	*Buffalo*	*Fowl*	*Rabbit*
Argentina										
Abortus	1	2	–	–	–	–	–	–	–	–
Suis	1	–	–	–	–	–	–	–	–	–
Melitensis	8	–	–	15	–	–	–	–	–	–
Switzerland										
Abortus	–	2	–	–	–	–	–	–	–	–
Melitensis	–	2	–	–	–	–	–	–	–	–
Tunis										
Abortus	–	1	–	–	–	–	–	–	–	–
Melitensis	36	–	–	14	–	–	–	–	–	–
Malta										
Melitensis	110	–	–	20	–	–	–	–	–	–
Mexico										
Abortus	–	5	–	–	–	–	–	–	–	–
Melitensis	5	–	–	–	–	–	–	–	–	–
Cape Colony										
Melitensis	1	–	–	–	–	–	–	–	–	–
Algeria										
Melitensis	4	–	–	–	–	–	–	–	–	–

TABLE IV (*cont.*)

	NUMBER OF STRAINS									
	Man	*Cow*	*Hog*	*Goat*	*Sheep*	*Horse*	*Dog*	*Buffalo*	*Fowl*	*Rabbit*
Chile										
Abortus	–	6	–	–	–	–	–	–	–	–
Melitensis	1	–	–	1	–	–	–	–	–	–
Uruguay										
Abortus	1	4	–	–	–	–	–	–	–	–
Melitensis	1	–	–	–	–	–	–	–	–	–
Denmark										
Abortus	1	1	–	–	–	–	–	–	–	–
Suis	–	–	6	–	–	–	–	–	–	–
Singapore										
Melitensis	1	–	–	–	–	–	–	–	–	–
Spain										
Melitensis	8	–	–	–	–	–	–	–	–	–
Greece										
Melitensis	4	–	–	–	–	–	–	–	–	–
Iraq										
Melitensis	2	–	–	–	–	–	–	–	–	–
Sweden										
Abortus	6	–	–	–	–	–	–	–	–	–

TABLE IV (*cont.*)

	NUMBER OF STRAINS									
	Man	*Cow*	*Hog*	*Goat*	*Sheep*	*Horse*	*Dog*	*Buffalo*	*Fowl*	*Rabbit*
Belgium										
Abortus	–	2	–	–	–	–	–	–	–	–
Holland										
Abortus	–	–	–	–	–	4	–	–	–	–
Rhodesia										
Abortus	6	–	–	–	–	–	–	–	–	–
China										
Abortus	–	1	–	–	–	–	–	–	–	–
Soviet Union										
Melitensis	2	–	–	1	–	–	–	–	–	–
Hungary										
Suis	–	–	7	–	–	–	–	–	–	–
Total										
Abortus	162	910	–	–	2	26	–	2	2	–
Suis	179	9	143	–	–	5	1	–	9	–
Melitensis	771	78	2	166	46	–	–	–	–	2
Not classifiable	7	6	–	–	–	–	–	–	–	–

NOTE: Cultures received and identified at the Central Brucella Station, Michigan State College, from 1920 to 1942.

PREPARATION OF SPECIES-SPECIFIC SERUMS. Agglutinating serums are prepared by the intravenous injection of normal rabbits with one cc. of one-tenth of the growth from a forty-eight-hour agar slant of known smooth strains of *Br. abortus* and *Br. melitensis*. The organisms should be alive. In the preparation of species-specific serums for agglutinin-absorption studies it is obvious that the rabbits should be examined for the presence of *Brucella* agglutinins before injection. No rabbit should be used for such studies if its serum contains agglutinins in a dilution of 1:5 or higher.

About fifteen days are required for the production of agglutinating serums of a titer of 1:1,000 or above. If in a preliminary test the serums of the rabbits show a titer of 1:500 or higher, the rabbits should be bled from the heart, the clear serum collected and, after a preservative has been added, filtered and vialed for future use. All serums should be adjusted by dilution to a maximum titer of 1:500 or 1:1,000 before use.

PREPARATION OF TYPE-SPECIFIC SERUMS. Wilson and Miles (475) state that "in the differentiation by the agglutinin-absorption technic of organisms having one or more qualitatively different antigens in addition to the common group antigen, the dosage of absorbing organisms in relation to the titer of the serum is not of major importance; when, however, as with members of the *Brucella* group, the two main types are distinguished merely by a difference in the quantitative distribution of the same antigens, it is of supreme importance to adjust the dose of absorbing organisms to the titer of the serum in such a way as to bring out this difference most clearly. The aim must be to absorb from one serum with a heterologous organism the minor agglutinin without affecting to any great extent the major agglutinin."

The previously prepared species-specific serums should be di-

luted to a maximum titer of 1:20 for the preparation of type-specific serums. The determination of the concentration of heterologous organisms that is necessary to remove completely only minor agglutinins may require several preliminary trials. This is accomplished by mixing the dilute serum with equal amounts of different concentrations of heterologous *Brucella* cells suspended in physiological salt solution preserved with 0.5 per cent phenol. The *abortus* agglutinating serum should be absorbed with varying suspensions of *Br. melitensis* and the *melitensis* serum with varying suspensions of *Br. abortus*.

The mixtures are now incubated for two hours at 37° C., being shaken well from time to time, and centrifuged at 3,500 r.p.m. for one hour. The supernatant liquid is removed and, together with unabsorbed control serum, titrated against the homologous and heterologous strains. The agglutination titrations are conducted in small agglutination tubes using suspensions adjusted to turbidity 1, McFarland standard, or 7 cm. Gates wire loop standard. Incubation is carried out for twenty-four hours at 37° C.

A concentration of antigen that is just sufficient to remove all minor ("heterologous") agglutinins while leaving most of the major ("homologous") agglutinins should be used when a large amount of type-specific serum for routine examination of unknown cultures is to be prepared. The serums after absorption may be filtered through a sterile Seitz filter, vialed, and stored until ready for use.

TYPING THE UNKNOWN CULTURE. When an unknown culture is being examined for species, it is always necessary to carry out agglutination tests with the two absorbed serums as well as with the unabsorbed diluted serums. The agar slant growth (forty-eight hours) of the culture to be identified should be suspended in physiologi-

cal salt solution with 0.5 per cent phenol added as a preservative. The turbidity of the suspension should approximate that of tube 1 of the McFarland standard or 7 cm. Gates standard. The absorbed and unabsorbed serums are added to one cubic centimeter amounts of the bacterial suspension in small agglutination tubes in sufficient quantities to obtain final dilutions of 1:20 and 1:40. The tubes are incubated for twenty-four hours at 37° C. The tubes of bacterial suspension to which the unabsorbed serums were added should be agglutinated to the maximum titer of the serums, while those to which type-specific serums were added should be agglutinated only by the homologous type.

The Agglutinin-Absorption Test Applied to Unknown Serum

The classification of strains by agglutinin absorption depends upon the following phenomenon: If a live antigen of sufficient density is used for the absorption, every strain belonging to the same group as the one used in the preparation of a given serum completely absorbs the agglutinins from that serum. The type of infecting organism may be determined by the agglutinin-absorption method in the absence of a positive blood culture, if the patient's serum shows an agglutinin titer of 1:160 or higher. A satisfactory method of applying the agglutinin-absorption test to the patient's serum is described by Evans (103).

"Since the *abortus* strains are not distinguishable from the *suis* strains by agglutinin absorption, the procedure to be described can only differentiate the *melitensis* type of infection. If the case in question is not of the *melitensis* type of infection, the *abortus* type cannot be differentiated from the *suis* type without the culture.

"A small quantity of the serum is absorbed with a dense suspension of *abortus* antigen, and an equal quantity of serum is absorbed with *melitensis* antigen of equal density. The remaining

agglutinins in each sample of absorbed serum are then titrated against both the *abortus* and the *melitensis* antigens. If the agglutinins have been completely removed by the *melitensis* antigen, and only partially removed by the *abortus* antigen, the infection can be assumed to be of the *melitensis* type. On the other hand, if the agglutinins have been completely removed by the *abortus* antigen and only partially removed by the *melitensis* antigen, the infection can be assumed to be of the *abortus* or *suis* type.

"In a typical absorption test, 0.5 cc. of serum is added to 2 cc. of antigen of a density of 60,000 p.p.m. (silica standard). The tube is incubated at 37° C. for four hours, then placed in a cold room until the following day, when the antigen is thrown down by centrifugation and the agglutinin content of the supernatant fluid is determined. If the agglutinin content of the unabsorbed serum is less than 1:1,000, it will be found that either the *abortus* or the *melitensis* antigen will have removed almost all agglutinins from the serum. If the serum is of a much higher titer, the procedure must be repeated with the partially absorbed samples of serum until the agglutinins have disappeared from one or the other sample.

"It was stated above that the agglutinins can be completely removed from a serum if a living antigen of sufficient density is used for the absorption. The risk of handling a living antigen in large quantities is too great a price to pay for perfect accuracy in these tests. Heat-killed antigen gives practically the same final results although it always leaves agglutinins in low titer in the serum, which cannot be removed by further absorption. Practically, however, this remnant of agglutinins does not interfere with the interpretation of results. For example, an *abortus* serum of a titer of 1:640, absorbed by an excess of heat-killed *abortus* antigen, will contain agglutinins specific for *abortus* in the 1:10 or 1:20 di-

lution, whereas the same serum absorbed by an excess of *melitensis* antigen will contain agglutinins specific for *abortus* in approximately the 1:160 dilution."

DIFFERENTIAL CULTURAL CHARACTERISTICS

Hydrogen Sulphide Metabolism

A preliminary report was made on this method in 1927 (208). It was stated that under aerobic conditions strains of both *Br. abortus* and *Br. suis* produce detectable H_2S, though they differ in their ability to do so, and *Br. melitensis* and "para"-*melitensis,* regardless of their origin, produce little or no H_2S. Differentiation of the species is accomplished by employing the following procedure.

A lead acetate solution is prepared by dissolving 10 grams of normal lead acetate (c.p.) in freshly boiled distilled water. A good grade of filter paper is immersed in the solution until it is thoroughly saturated. It is then removed, allowed to dry, and cut into small strips. The paper is stored in a stoppered bottle until ready for use. When H_2S determinations are to be made, liver agar slants, prepared from liver infusion which has previously been treated with one-half volume of ether at room temperature for three days and from which the ether has been removed in a separating funnel, are planted heavily from a forty-eight to seventy-two-hour growth. A strip of the lead acetate paper of sufficient length is placed inside the tube beside the cotton plug so that the paper will extend about one inch below it. One should make certain that the cotton plug is not moist and does not contain dried agar, thus preventing a partial anaerobic condition inside the tube.

The tubes prepared in this way are incubated at 37° C. for twenty-four hours. The paper is then removed, numbered, and preserved. A fresh piece of paper is placed in the tube in the same manner as the previous one, and the same procedure followed. This operation is continued for four successive days. One may

then determine, by comparing the extent of blackening or formation of lead sulphide on the four strips of paper, the group to which the strain in question belongs. The degrees of H_2S production plotted against time for the three groups are illustrated in Figure 9.

If a given strain continues to produce H_2S to a considerable degree for a period of four days, it belongs to the *suis* species. This does not hold true for those strains of *Br. suis* isolated from swine by Thomsen (436, 439) in Denmark. The *suis* strains which he isolated produce little if any H_2S during a four-day period. If the strain produces a considerable amount of H_2S for only two days, it belongs to the *abortus* species. When no H_2S, or only a trace, is produced during this period, the strain is *Br. melitensis.* (Note exception of Danish porcine strains.)

Bacteriostatic Action of Dyes

The dyes which have been found to give consistent results in the differentiation of the three species are thionin and basic fuchsin. They are certified dyes, made by the National Aniline and Chemical Company, Inc.

The medium used is prepared according to the formula already given for Tryptose agar.

The dyes are prepared in 0.1 per cent stock solution in sterile distilled water. The stock solutions should be freshly prepared every sixty days. The final dilution which should obtain for thionin and for basic fuchsin is 1:100,000. These dilutions are based on the actual amount of the original dye in the medium.

The dye suspensions should be heated in flowing steam for twenty minutes, shaken well, and while still hot added to the melted medium before it has time to cool. This procedure results in a more uniform mixture of the dye suspension and a more uniform distribution of the dyes in the medium. The medium and

dyes are thoroughly mixed and immediately poured into Petri plates. The plates are placed in a 37° C. incubator until the water of condensation disappears.

The plates may be divided into three or more sections to accommodate the growth of several strains of the organism. The seeding of the plates is accomplished with a loop of a heavy suspension of a forty-eight to seventy-two-hour agar slant growth. The suspension may be obtained by working up a portion of the growth in the water of condensation at the butt of the slant or by adding a small amount of sterile broth or sterile saline solution to the slant. It may be stated here that the dyes do not kill the organism, but merely inhibit its reproductive function; so if masses of the culture are streaked over the surface of the plates, slight growth is very apt to occur at those points where the seeded mass has not been evenly distributed.

The seeded plates are incubated aerobically at 37° C. for seventy-two hours, or in 10 per cent CO_2 when newly isolated bovine strains are used. At the end of the period of incubation one will find that strains of the *abortus* species have grown only on the medium containing basic fuchsin; those of the *suis* species have grown only on the medium containing thionin; and those of the *melitensis* species have grown on each of the media. The growth of the last species, however, is as a rule never as luxuriant as that of the other two species.

In recent years the author has studied 15 strains of *Brucella* of human, bovine, and equine origin which do not resemble the three known species of *Brucella* in their growth behavior toward dyes on culture media. The strains in question do not grow out on media containing dye either in the dilutions recommended or even in higher dilutions. According to the H_2S metabolism test and agglutinin-absorption test they belong to the *abortus* species. Members of these peculiar strains were isolated by Dr. Gilbert in New

York, Dr. Fitch in Minnesota, Dr. Taylor in France, and the author.

Glucose Utilization

The differentiation of the species of *Brucella* by their ability to utilize glucose was first applied by McAlpine and Slanetz (276). They classified their strains more on the basis of origin. Those strains of bovine origin were found to utilize less than 4 per cent of the available dextrose. Those of human and porcine origin as well as *melitensis* were found to utilize from 4 to 18 per cent of the sugar. In a later publication McAlpine and associates (275) stated that strains of *Brucella* of porcine origin consumed on the average 6 per cent of available dextrose. In a more recent report, Zobell and Meyer (485) state that they were unable to demonstrate any appreciable difference in the dextrose utilization of the three species of *Brucella.*

Plastridge (354) has described the following technic for differentiating the species of the genus *Brucella* by the glucose utilization method.

"The test strain is grown in liver infusion broth for several transfers. The strains to be tested are then grown on liver infusion agar. Usually two transfers on liver infusion agar are necessary to ensure luxuriant growth. The growth on forty-eight-hour liver agar slants is then suspended in about 2 cc. of physiological saline; 0.5 cc. of this suspension is then added to about 60 cc. of a one per cent glucose peptone water medium (pH 6.8 to 7.0) contained in a 100 cc. Florence flask. The inoculated flasks are then incubated at a temperature of 37° C. for a period of seven days. Two uninoculated flasks are also incubated. Any loss in weight during the period of incubation is made up by the addition of sterile water.

"Following incubation the flasks are analyzed for glucose in the following way: Twenty cc. of culture are added to each of two

flasks containing 60 cc. of distilled water, 10 cc. of a 10 per cent solution of sodium tungstate, and 10 cc. of H_2SO_4 (2/3N). Each flask is then shaken, allowed to stand for several hours, and then filtered. The filtrate is used for the sugar determination which is made in this way: Five grams of Na_2CO_3 are placed in a medium-sized casserole and 10 cc. of Benedict's reagent added. The mixture is brought to a boil over a low flame and a portion of the test filtrate, just sufficient to cause the blue color to disappear, is slowly added to the boiling mixture. The amount of filtrate required in each case is recorded. In the calculations, it is assumed that the uninoculated broth contained one per cent glucose. The per cent of the available glucose utilized is determined from the amounts of test filtrates required to decolorize 10 cc. of Benedict's solution."

Reduction of Nitrates and Nitrites

Zobell and Meyer (484) have found a sufficient difference in the nitrate and nitrite-reducing ability of the three species of *Brucella* to distinguish one from the other. The composition of the medium which they used and a description of their technic follow.

Medium:

Peptone	2.0 grams
Beef extract	1.0 gram
Sodium chloride	3.0 grams
Agar	2.0 grams
Distilled water	1,000.0 cc.

"The reaction was adjusted to pH 6.8. The semisolid medium insures the multiplication of the cultures and renders anaerobic growth and gas production more conspicuous. The mediums were uniformly inoculated with approximately the same number of cells. The presence of nitrites, disappearance of nitrates, and evolution of gas were observed as criteria of nitrate reduction. To detect nitrites the alpha-naphtholamine sulphanilic acid method was

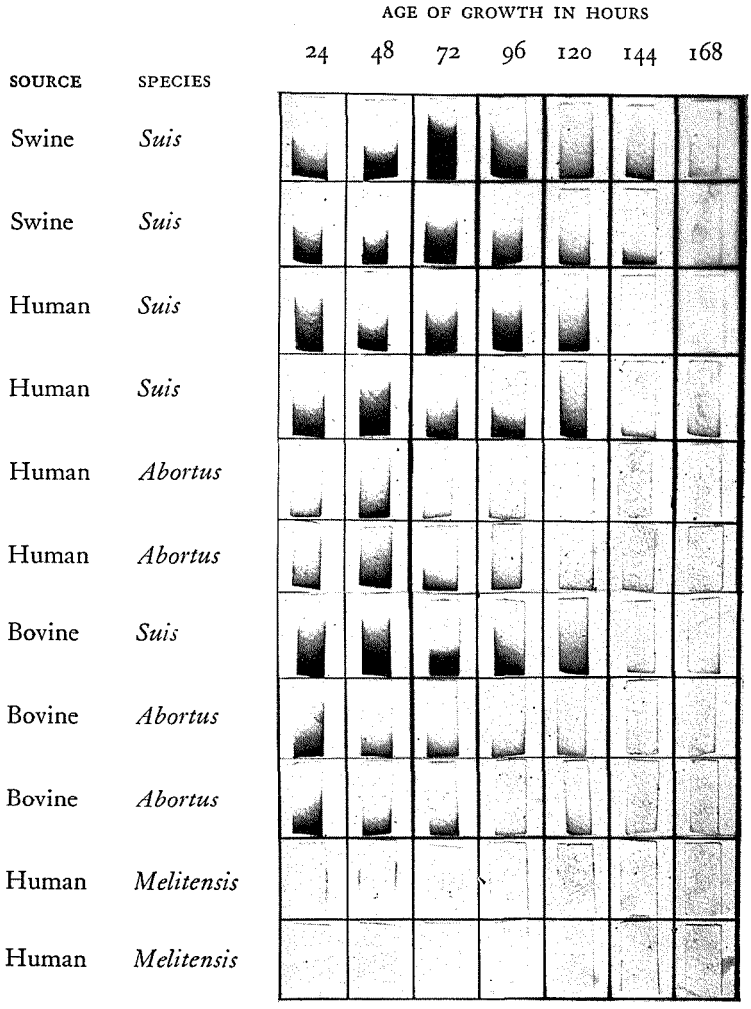


FIGURE 9. H_2S PRODUCTION BY SPECIES OF GENUS BRUCELLA

used. In the absence of nitrites, nitrates were tested for by adding about 20 mg. of zinc dust to the tube containing the nitrite reagents. The semisolid consistency proved useful because it suspended the reducing zinc dust throughout the medium, thus enlarging its sphere of action. Gas was observed in the viscous mediums as bubbles and froth. Smith fermentation tubes were used to collect the gas for analytic purposes.

"As previously mentioned, the representatives of *Brucella* usually grow in limited zones from 2 to 6 mm. below the surface of the medium. On the addition of 0.2 per cent potassium nitrate to the medium, the *abortus* and the *suis* types grow dispersed throughout the medium, demonstrating an appreciable pseudo-anaerobic growth, but the *melitensis* strains continue as before to localize a few millimeters from the surface.

"The *suis* strains destroy 0.05 per cent potassium nitrite in five days, while the *abortus* and *melitensis* types lack this ability. The *melitensis* varieties are in general more active reducers of nitrites than the *abortus*.

"In mediums containing 0.2 per cent each of potassium nitrate and potassium iodide, the *suis* types evolve an abundance of nitrogen gas with a rapid disappearance of nitrates and nitrites, while the *abortus* types very rarely liberate gas under identical conditions. The *melitensis* and the Danish porcine strains, which exhibit no pseudo-anaerobic growth, although capable of destroying 0.002 per cent potassium nitrite, fail to generate gas.

"When judiciously employed, these differences in the nitrate and nitrite metabolism may be used in the characterization of the representatives of *Brucella.*"

Measuring the Time Potential in an Oxidation-Reduction System

This method has been developed and studied by Tuttle and the author (450). Differentiation by this method requires considerable

experience and the use of delicate apparatus. It is not recommended as a routine procedure. A brief description of the method and results is given for the purpose of confirming other more simple differential methods and to show that it can be applied to the differentiation of the species of *Brucella.*

Briefly, the method consists in measuring the time potential of liver infusion broth containing either thionin or basic fuchsin, after inoculating with a dilute suspension of *Brucella.* The potential is measured daily for seven days by making use of a Leeds and Northrop student type potentiometer and a high-grade pointer galvanometer of the same make. A calomel cell with a long side tube leading into an intermediate vessel was used as a reference electrode. The inoculated half-cells and reference cell are held at a temperature of 37° C. in a constant temperature water bath. A special Acheson graphite electrode is used in the half-cell. The time-potential curves obtained with the three species of *Brucella* in broth containing thionin and in broth containing basic fuchsin are shown in Figure 10.

By measuring the time potential in an oxidation-reduction system it has been found that *Br. abortus* in the presence of thionin is unable to reduce the potential of the medium. *Br. suis* in the presence of basic fuchsin is unable to reduce the potential of the medium. The presence of either dye has little, if any, retarding effect upon the potential drift of the medium resulting from the growth of *Br. melitensis.*

Chemical Differences in the Brucella *Cells*

The results of several years of study of the chemical composition of the three recognizable species of *Brucella* at the Central Brucella Station at Michigan State College show clearly that there are distinct chemical differences in each species as well as known measurable metabolic differences (226).

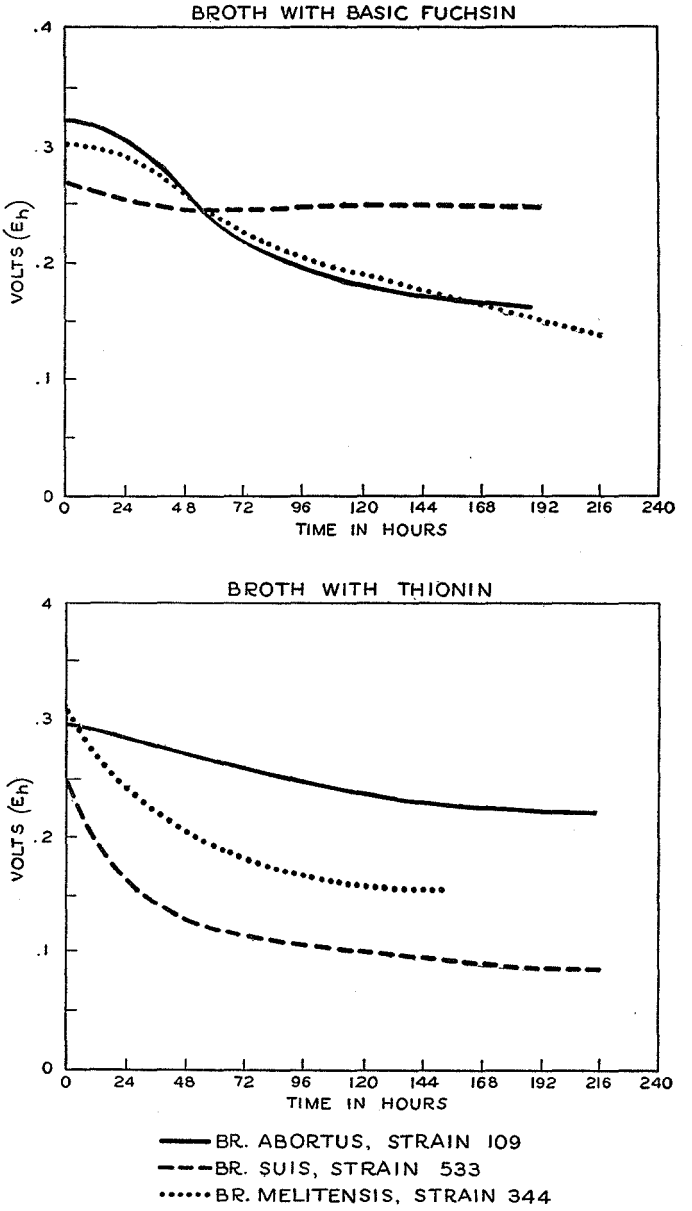


FIGURE 10. TIME-POTENTIAL CURVES OF LIVER INFUSION BROTH INOCULATED WITH THE THREE SPECIES OF BRUCELLA

The *Brucella* cells for chemical analysis were grown on beef liver infusion agar prepared from fat-extracted infusion to which glycerol (0.5 per cent) was added. The medium was placed in monel metal trays 6 inches wide, 10 inches long, and one inch deep. The trays were provided with closely fitting covers. The cultures were incubated at 37° C. for two days and a half. The growth was lifted off the surface of the medium with a celluloid spatula, washed once with distilled water, and dried *in vacuo* over sulphuric acid.

The yield of dry *Brucella* cells per culture pan varied from 0.68 to 1.30 grams.

The analytical determinations were carried out according to methods which others have used in the chemical analysis of bacterial cells.

The distribution of the important chemical fractions in the three species of *Brucella* derived from the analysis of more than 1,300 grams of dry bacteria is shown in Table V. The most notable chemical difference in three species was in the amount and physical character of the C_1 and C_2 polysaccharide fractions. The C_1 fraction found only in *suis* and *melitensis* was dialysable. The C_2 fraction found only in *suis* and *abortus* was non-dialysable. When freed from nitrogen-containing substances they were found to be biologically inactive.

One interesting fraction found to occur in water extracts of pulverized *melitensis* cells and in alkaline extracts from the albuminoid fraction of *abortus* cells was the S fraction. This fraction was found to contain 6 per cent of nitrogen and no reducing sugars on hydrolysis. Its specific rotation was +83.43. It was soluble in about 50 per cent of alcohol. This substance was found to contaminate the polysaccharide C_1 fraction from which it could be separated, after much effort.

The S substance in a 1:200 dilution elicits a non-specific skin reaction in either normal individuals or those sensitized to *Brucella*.

The reaction persists for about twelve hours. When a solution of the substance was diluted as high as 1:100,000, it precipitated antiserum prepared from any one of the species of *Brucella.*

It is the opinion of the author that the so-called specific-precipitating polysaccharide which Favilli and Biancalani (113), Topping (445), Higginbotham and Heathman (194), and Reiter (379), obtained from *Br. abortus* cells was contaminated with the bio-

TABLE V

Percentage distribution of chemical fractions in the three species of Brucella

	BR. ABORTUS		BR. SUIS		BR. MELITENSIS	
STRAIN	266	141	1,538	1,585	606	655
EXPERIMENT	B4	B8	B7	B12	B10	B13
Insoluble cell residue	64.40	68.00	70.60	70.60	61.80	66.00
Ether extract	0.03	–	0.11	0.07	0.08	0.09
Alcohol-ether extract	5.90	5.70	5.60	5.60	5.30	5.80
Acetone-soluble fat	0.90	1.00	2.40	2.80	4.80	5.60
Phosphatide	4.80	4.60	2.70	2.10	0.40	0.20
Chloroform extract	0.40	0.30	0.20	0.40	0.10	0.10
Nucleoprotein	15.00	15.00	15.00	15.00	13.60	13.60
Water-soluble albumin	0.90	0.70	0.80	0.70	0.70	0.70
S substance	–	–	–	–	1.40	1.50
C polysaccharides, crude	1.50	1.10	2.60	3.40	2.70	1.80
C_1 polysaccharide	–	–	0.60	0.70	1.50	1.00
C_2 polysaccharide	1.10	1.00	1.10	1.50	–	–
Alcohol-soluble fraction	8.00	6.70	7.50	10.30	10.50	8.10

– = none. Percentages are only approximate.

logically active S fraction. If the investigators just mentioned had carried their chemical separations further, they would have recovered a specific precipitable substance free from polysaccharide. One of the investigators, Biancalani Schapira (398), recently repeated previous studies, giving particular attention to the complete purification of the S substance. It was found to be non-polysaccharide in nature.

The distribution of the fat and phosphatide fractions constitutes

an important specific difference in the three species: the former increases in the order of *Br. abortus, Br. suis, Br. melitensis;* the latter in the inverse order.

The isolation of the protein nucleate fraction, which from the biological standpoint was the most important, is described in Chapter VI, Part Two, pages 244–246.

Topping (445) has studied the specificity of the protein nucleate fractions of the *Brucella* in a precipitin system and found no measurable differences.

In a recent study of the chemical constitution and biological properties of the large insoluble cell residue obtained after water extraction of *Brucella* cells, Pennell and Huddleson (351) have found it to consist chiefly of a highly antigenic and toxic fraction. It has been termed the endoantigen.

The method used in the chemical separation of the endoantigen from *Brucella* cells was a combination of the one used by Raistrick and Topley (376) and the one used by Boivin and Mesrobeanu (31) in the isolation of a toxic antigenic fraction from *S. aertrycke.*

The endoantigens obtained from the three species of *Brucella* are grossly similar. The fraction comprises roughly 25 per cent of the bacterial cell. While containing the same or similar constituents, however, the endoantigens from the three organisms have been shown to differ markedly in the distribution of some of these constituents.

Positive reactions are given to the Molisch test, the Biuret test, Millon's test, and Bial's test, and a very slight reaction to the Rosenheim test. The nitrogen content of the fraction varies from 6.08 to 12 per cent. Reducing sugars are absent before hydrolysis. Calculated as glucose after hydrolysis, reducing sugars represent from 4 to 12 per cent of the endoantigen. Amino-nitrogen, phosphorus, and sulfur are absent. In the determination of acetyl groups, distillable acid representing an average of 6 per cent of the endo-

antigen is obtained. This acid is presumably acetic acid although that product has not been isolated.

From the endoantigen there may be extracted by acetone and ether a compound having the properties of a di-ketone and an acetone-soluble, saturated, liquid, fatty acid. These two compounds represent from 10 to 15 per cent of the fraction.

The acetone-ether extracted product still reacts positively to the qualitative tests mentioned above. Tryptophane and tyrosine have been found to represent 18.92 per cent and 8.45 per cent, 11.46 per cent and no per cent, 7 per cent and 9.29 per cent of the extracted endoantigens of *Br. melitensis, Br. abortus,* and *Br. suis* respectively.

From the remaining 60 to 75 per cent of the original fraction there has been obtained an unidentified nitrogenous fraction and an optically inactive sugar acid. These are obtained in quantities such as to preclude the occurrence of any further compounds in a significant amount.

The endoantigen is shown to be relatively stable in the presence of dilute acid and dilute alkali, upon heating and upon long standing. Its activity is not completely destroyed by hydrolysis with dilute acids, but is destroyed by similar treatment with dilute alkali. The ability to precipitate specific serum is lessened by extraction with acetone and ether, but is enhanced by acetylation or by treatment with 25 per cent NH_4OH. The toxicity and antigenicity of the endoantigen are shown to be dependent upon proper dosage, an overdosage as well as an underdosage giving poor results. The toxicity and antigenicity are increased by lipide extraction. Acetylation causes a distinct decline in toxicity but a marked increase in antigenicity.

The endoantigen elicits specific skin reactions in sensitized animals, the lipide-extracted and the acetylated endoantigens showing some species-specificity in this reaction. The fraction from each

TABLE VI

Chemical analysis of the endoantigens and S substance of Brucella: *percentage distribution of chemical components (four experiments)*

CHEMICAL COMPONENT	EXPERIMENT 1 Br. melitensis *endoantigen*	EXPERIMENT 1 Br. melitensis *lipide-extracted endoantigen*	EXPERIMENT 2 Br. abortus *endoantigen*	EXPERIMENT 3 Br. suis *endoantigen*	EXPERIMENT 4 Br. melitensis *S substance*
Total N	9.40	12.00	6.08	8.86	5.86
Amino N	–	–	–	–	–
Reducing sugars before hydrolysis	–	–	–	–	–
Reducing sugars after hydrolysis	5.93	4.00	6.49	4.40	8.13
Sulfur	–	–	–	–	–
Phosphorus	–	–	–	–	–
Distillable acid	3.48	5.42	5.82	10.03	48.43
Acetone-soluble yellow oil	8.53	–	6.00	9.00	–
Fatty acid	4.87	–	9.00	5.57	–
Tryptophane N	2.13	2.46	1.26	0.77	–
Tyrosine N	0.51	0.60	–	0.52	–

– = none.

species, in dilutions of from 1:500,000 to 1:5,000,000, precipitates specific serums prepared in goats.

Injection of the endoantigen causes a hyperglycemia followed by a hypoglycemia in experimental animals. The basal metabolism of injected animals is at first stimulated and then depressed. A leucopenia occurs six hours after injection of the endoantigen into normal guinea pigs, chiefly due to the disappearance of neutrophiles from the peripheral blood.

The endoantigen may be produced from the previously described albuminoid fraction of the *Brucella,* thus accounting for the toxicity of that fraction and suggesting that this albuminoid is a combination of the endoantigen with a protein-like group. The endoantigen is shown to be similar to or possibly identical with the previously described S substance, the latter being probably a partially hydrolyzed endoantigen. The chemical structure of the endoantigen is summarized in Table VI.

Pop and associates (361, 362) and Vanghelovici and associates (457) have also made an exhaustive study of the chemical nature and biological properties of what they term the complete antigen of *Brucella.* The above-mentioned workers employed the methods described by Boivin and Mesrobeanu (31) for separating the complete antigen from *S. aertrycke.* The chemical characteristics of complete antigen (*Brucella*) were essentially the same as those of the endoantigen. The complete antigen was found toxic for mice, but not for guinea pigs.

Gwatkin (159) has made an extensive biological study of an antigenic agent extracted from *Br. abortus.* The reactions which it is capable of producing in experimental animals and its failure to immunize guinea pigs would characterize the substance as the endoantigen.

Miles and Pirie (305, 306, 307, 308, 309) have made a very comprehensive study of soluble fractions from *Br. melitensis* obtained

by extracting heavy suspensions with 2 per cent phenolchloroform water at 0° C. The extract contained a mixture of lipides, nucleic acid and nucleoprotein. They designated the fractions as: SSS, a complex of a phospholipid mixture with a substance giving the Sakaguchi reaction for arginine; AP, a highly aggregated formyl derivative of an amino-polyhydroxy compound; PLAPS, the whole complex or mixture. From antigenic studies they found the AP fraction to contain both M and A antigens; the PLAPS fraction to contain also antigenic determinants of rough forms. By hydrolyzing the AP fraction, formic acid, a reducing sugar and an amine were identified. In suitable doses the AP and PLAPS fractions were found to be toxic for mice, but not for guinea pigs.

IV

BRUCELLOSIS IN HUMAN BEINGS

Synonyms. Undulant fever, Mediterranean fever, gastric fever, Malta fever, rock fever, Gibraltar fever, melitococcie, goat fever, Texas fever, Rio Grande fever, *Brucella* fever, Bang's fever.

Definition. Brucellosis in man is a systemic or focal infection caused by *Brucella melitensis, Brucella abortus,* or *Brucella suis.* The disease is characterized by weakness, fever with morning remissions, occipital or frontal headache, muscular pains, profuse sweats, chills, constipation, secondary anemia, nervous disturbances, and metastatic involvement of the joints, the eyes, and the reproductive organs. The course is of indefinite duration, but may be marked by repeated relapses and may become chronic. The mortality is low.

PART ONE. HISTORICAL SURVEY

While there is reason to believe that the infection in human beings occurred many centuries ago on the plains of the Near East, it was not, however, differentiated and identified as a separate disease until 1861. In this year Marston (295), in his report on fever, gave a full and accurate description of the disease and designated it as Mediterranean or gastric intermittent fever. His description was in part as follows: "By this is meant a fever characterized by the following symptoms and course: a preliminary stage of subacute dyspepsia, anorexia, nausea, headache, feeling of weakness, lassitude, and inaptitude for exertion, mental or physical; chills, muscular pains, and lastly, a fever having a long course 3 to 5 or 10 weeks, marked by irregular exacerbations and remissions, great derangement of the assimilative organs, tenderness in the epigastric region, and splenic enlargement. It is prone to relapses, has a protracted convalescence, and is frequently marked by rheumatism."

The disease in human beings due to *Br. melitensis* was given the descriptive name "undulant fever" by Hughes (223) in 1897.

Wright and Semple (479) in 1897 made an important contribution to the diagnosis of brucellosis. They demonstrated that the organism which Bruce identified was agglutinated by the serum of those affected with the disease. This finding aided materially in confirming Bruce's original discovery.

Among the military and naval forces quartered on the Island of Malta "undulant fever" had been for years a major cause of disability. For this reason in 1904 the British Government established a Commission, headed by Bruce, to find if possible the source of the disease and effective measures for its prevention. Associated with him were Smith, Horrocks, Shaw, Weir, McNaught, Eyre, Zammit, Kennedy, Johnstone, McCullough, and Clayton. This group of workers labored exhaustively for the first two years with little apparent success. Having eliminated such possible sources as insects, air, sewerage, water, and dust, they had reached an impasse in their search. Needing a readily available supply of laboratory animals, Zammit decided to conduct experiments to determine whether milch goats might serve. These were, then as now, the chief source of the milk supply for the Island. Before attempting to infect goats, Zammit considered it advisable to examine their blood for specific agglutinins and to his great surprise several of the goats reacted to the agglutination test in a high titer. These observations were confirmed, and the source of the disease then seemed evident. Samples of milk were taken from the goats which had reacted to the agglutination test and were cultured. These were positive and the source of brucellosis stood revealed.

Zammit, in addition to discovering the source of brucellosis, made an important contribution to the foundations upon which the diagnosis of disease in animals and man was being built. He demonstrated that the milk from the udder of an infected goat contained specific agglutinins and that the detection of these was a reliable diagnostic measure.

The history of brucellosis in man in the western hemisphere probably began with the invasions of the Americas by Cortez and his legions. The studies (392) that have been made in recent years as to the prevalence of the disease in the Argentine serve to substantiate this view.

The first authentic case of brucellosis originating in the United States was reported by Craig (67) in 1905. The patient, a nurse in a hospital in Washington, D. C., had never used goat's milk. It is quite possible that the causative organism in this case was *Br. abortus* or *Br. suis*. About the time Craig reported his case another important chapter in the epidemiology of the disease in humans was being written. Mr. Thompson of the United States Bureau of Animal Industry was sent to Malta to purchase milch goats. On August 19, 1905, sixty-one female and four male goats were shipped on the S.S. *Joshua Nicholson*. At Antwerp they were transferred to the S.S. *St. Andrew* for the United States. *The milk of the goats was freely consumed by the crew on both ships.* Of twelve men on the *Joshua Nicholson,* eight became ill from eighteen to thirty-four days after leaving Malta. The other members of the crew drank little of the milk or boiled it. Positive serological observations confirmed the clinical diagnosis of brucellosis. After the goats were placed in quarantine at Athenia, New Jersey, a woman also drank milk from the goats and developed this same infection. All of these goats were slaughtered.

It was not until 1911 that the endemic occurrence of brucellosis in the United States was revealed. Ferenbaugh (116) and Gentry and Ferenbaugh (142) reported in that year that the disease was prevalent in southwestern Texas and came from infective goat's milk. Evidence was also obtained that the disease had been present in Texas for many years.

A major chapter in the history of brucellosis was written in 1918 when Evans (99) reported the results of her comparative study of

the organisms *Br. abortus* and *Br. melitensis*. Her conclusions are of such historical importance that they are here reproduced. She wrote:

"It is only with great difficulty that *Bact. melitensis* can be distinguished from *Bact. abortus*. They are alike morphologically, and no difference could be found in their biochemical reactions. The two organisms produced the same results when inoculated into pregnant guinea pigs. The only distinction between the two organisms in cultural characteristics was a more intense brown pigmentation by *Bact. melitensis*—an insignificant characteristic, which does not appear until after the cultures have been incubated for a week or more. This distinction can be made only when cultures of the two species which have been incubated for the same length of time can be compared. The agglutination reactions in *Bact. abortus* antiserum do not distinguish the two organisms; and the agglutination reactions in *Bact. melitensis* antiserum can distinguish *Bact. abortus* and *Bact. melitensis* only when the agglutinating strength of the serum for both species is known.

"The fact that *Bact. abortus* and *Bact. melitensis* are serologically so closely related explains Kennedy's (245) discovery that the milk and the blood serum of a considerable percentage of cows in London contained agglutinins for the Malta fever organism in high dilution. This author was unable to explain his findings, but suggested that agglutination of the Malta fever organism by cow's milk was not necessarily specific, or else that the reaction was indicative of infection with the organism in question—the latter alternative being an explanation too alarming to be acceptable, although he states that he has heard of two cases of undulant fever in people who have never been out of England, and he thinks it possible that there are other cases undiagnosed.

"The very close relationship between *Bact. abortus* and an organism pathogenic to human beings adds new interest to the subject

of the possible pathogenicity of *Bact. abortus* to human subjects. Considering the close relationship between the two organisms, and the reported frequency of virulent strains of *Bact. abortus* in cow's milk, it would seem remarkable that we do not have a disease resembling Malta fever prevalent in this country. A possible explanation can be offered. The data presented in the third paper of this series indicate that although there may be numerous *abortus*-like bacteria in the milk of cows which have aborted, the actual number of virulent bacteria which persist in the milk is not great, or in all probability it is negligible in many cases in which the milk and blood serum contain agglutinins. But the work of the British Commission indicates that *Bact. melitensis* is very abundant in the milk of infected goats, for those investigators were able by cultural methods to demonstrate the organism in the milk of 10 per cent of the goats of Malta. Since infection is dependent on the amount of infectious material, it may be that this difference in the number of bacteria in the milk of the two species of animals may account for our freedom from disease when cow's milk containing *Bact. abortus* is consumed. On the other hand, are we sure that cases of glandular disease, or cases of abortion, or possibly diseases of the respiratory tract, may not sometimes occur among human subjects in this country as a result of drinking raw cow's milk? It is certain that the agglutination tests, which have been relied upon for the diagnosis of Malta fever, have not proved *per se* whether the infections were due to *Bact. melitensis* or *Bact. abortus.*"

Several attempts were made prior to 1918 to connect *Br. abortus* with a disease in man, but it apparently was not clear to the investigators just what to look for. Mohler and Traum (314) as early as 1911, on examining the tonsils of children consuming raw milk, isolated *Br. abortus* in one instance. A few years later Larson and Sedgwick (262) examined the blood of 425 children that had received raw milk in their diet. Of these, 73, or 17 per cent, gave a

positive test. The clinical diagnosis in these cases was tuberculosis or rickets. In 1916 Cooledge (62) conducted an experiment in which 7 human subjects ingested for varying lengths of time milk containing *Br. abortus*. The subjects remained in good health during and after the experiment, but 5 showed an increase in blood serum antibodies. The increase in antibodies was thought to be due to absorption into the intestine of antibodies in ingested milk. In the light of our present knowledge of antibodies it is doubtful whether this explanation is tenable.

Duncan (92) in Rhodesia was the first to recognize and report cases of brucellosis due to *Br. abortus,* but the first case in the United States in which the infecting organism was shown to be due to a species of *Brucella* other than *melitensis* was reported by Keefer (243) in 1924. It was thought at the time that the infecting species was *Br. abortus*. Several years later the author had an opportunity to study this culture and it was then found to be *Br. suis*. This case did much to awaken a new interest in brucellosis in this country. Since it is so important in the history of this infection, the report is reproduced in full (see Case 20, Appendix, page 327).

Since Keefer's report, cases of brucellosis in human beings due to either *Br. abortus* or *Br. suis* have been found in all parts of the United States, as well as in Rhodesia, Germany, Denmark, France, Great Britain, Sweden, Argentina, and Brazil. Wherever *Br. abortus* or *Br. suis* is found in animals, there will also be found infection in humans. The world-wide distribution of brucellosis in both animals and man has recently been summarized and reported by Thomsen (440).

PART TWO. BRUCELLOSIS IN THE UNITED STATES

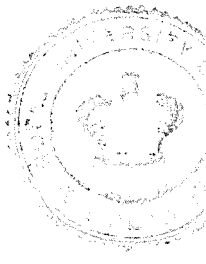
EPIDEMIOLOGY

Incidence

The present or past prevalence of brucellosis cannot be determined reliably. There is reason to believe that this clinical entity occurred in the United States and other countries for many years before it was finally commonly diagnosed. Undoubtedly the present completeness of its recognition and reporting varies markedly. However, it is known to be a widely distributed disease. No broad areas have been conclusively shown to be free of *Brucella* infection in animals and in man.

Variations in known incidence are in part explained by the differences in accuracy of diagnosis. In the United States the reported cases of brucellosis (undulant fever) have steadily increased from 24 in 1925 to 3,427 in 1941. There is reason to believe the true incidence during this period has decreased rather than increased, particularly in recent years. Within the country, however, persisting differences in case rates are evident. Those for the large cities of the east have been consistently low; in contrast, Iowa, Missouri, Kansas, and to a less extent Minnesota, have had rates in excess of those in other regions. The low incidence in urban areas is readily explained by the relative freedom from exposure to either infected livestock or raw dairy products. The high incidence in the midwestern states is not explained merely by an excess of rural population; the southern states have an even higher proportion of rural residents, though with relatively few cases of recognized brucellosis. Neither can these cases be attributed to any high incidence of *Brucella* infection in cattle. The examinations by the United States Department of Agriculture reveal a rather marked uniformity in the incidence of infection of these animals. Though the

NOTE: Part Two of this chapter has been contributed by A. V. Hardy.

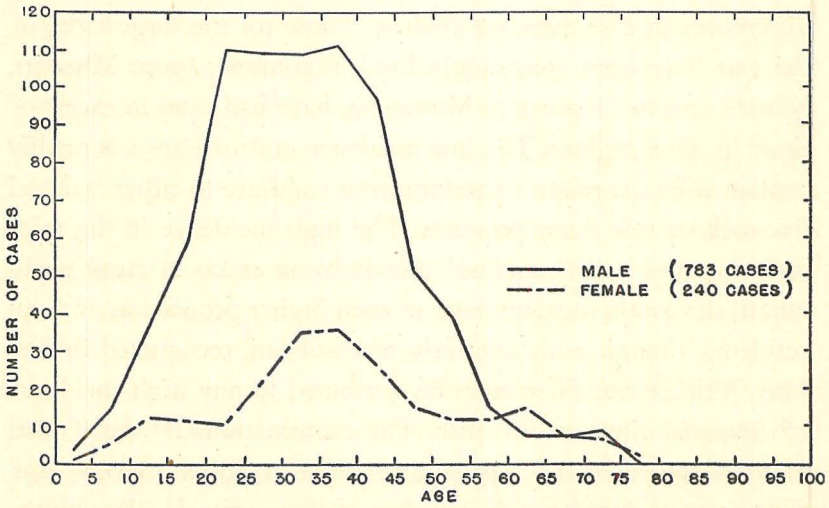


prevalence of contagious abortion in hogs is not accurately defined, it is still evident that in the United States the incidence of recognized brucellosis in man tends to vary directly with the extent of the hog-raising industry. In other lands brucellosis has for many years been a recognized public health problem in goat-raising areas—particularly in the Mediterranean countries. Apart from this, cases have been recognized sporadically and rather infrequently.

With the known wide distribution and high prevalence of *Brucella* infection of cattle, the low incidence of clinical brucellosis has been striking. It has been noted that there is a higher rate of human infections in areas raising large numbers of hogs and goats.

Age and Sex

Brucellosis in this country has involved most heavily the young adult males. The age and sex distribution of cases studied in Iowa



After Hardy and associates (174)

FIGURE 11. BRUCELLOSIS IN IOWA BY AGE AND SEX, 1927–35

is shown in Figure 11. The rarity of cases in the young and the relatively low incidence in females are striking. When what were presumably *Br. abortus* infections were considered, the variations were much less marked, a finding which agreed with those reported by other investigators concerned with similar cases.

Occupation

A wide variation in attack rates in certain occupational groups was also found in Iowa (see Table VII). The comparatively high rates for men on the farm and the excessive rates for packing-house workers strongly emphasize the risk in occupations involving direct contact with livestock and fresh meat.

TABLE VII

Relative incidence of brucellosis in Iowa, by occupational groups

OCCUPATION	POPULATION*	REPORTED CASES†	AVERAGE ANNUAL CASES	
			Number	*Per 100,000 population*
Packing-house employees ..	8,000	103	11.4	142.5
Workers on farms				
Male‡	324,000	422	46.9	14.5
Female	250,000	80	8.9	3.6
Other workers or students, ten years of age and over	1,375,000	371	41.2	3.0
Children under ten years ..	464,000	15	1.7	0.4
Total	2,471,000	991	110.1	4.5

After Hardy and associates (174)

* 1930 census. † 1927–1935. ‡ Includes farm laborers, estimated number 74,000.

Sources and Modes of Infection

Brucellosis is primarily a disease of cattle, hogs, and goats, but other domesticated animals or fowl may occasionally become involved, as, for example, horses, dogs, and chickens. Human infections arise from these animal sources, and rarely if ever from hu-

man cases or carriers. Bacteriologists working with *Brucella,* particularly the *melitensis* species, frequently acquire the disease.

The relative importance of these animal sources cannot be measured statistically. In this country cattle and hogs are chiefly concerned. The bacteriological evidence suggests a higher prevalence of *Br. suis* infection in the midwestern and southern states. The excess of *Br. suis* isolations can be partially accounted for by the greater ease of isolation of this organism from human blood cultures. These data and field epidemiological studies do indicate, however, that both infected hogs and cattle are important sources of the severer cases of brucellosis in certain areas of this country. It is equally evident that in other regions *Br. abortus* infection is found almost exclusively and that this has its origin in infected cattle.

It was previously assumed, as a result of the studies of the Mediterranean Fever Commission, that brucellosis was acquired through the ingestion of infected raw dairy products. It has since been established through experimental study and the interpretation of epidemiological observations (431) that the infection may readily be acquired through cutaneous contact with infective secretions, excretions, or tissues. This appears to explain the ease of infection of bacteriologists, who are generally able to avoid the ingestion of those organisms with which they work but can scarcely hope to prevent entirely the contamination of fingers and hands. The high incidence of infection in packing-plant employees is readily understood when it is known that *Brucella* may penetrate the normal or minutely abraded skin. Likewise the high rate of infection in men on the farm, as compared with the women, can be explained only as a result of the more common skin contamination by infective discharges of cattle or hogs. Taylor, Lisbonne, Vidal, and Hazemann (431) report a study of 466 cases of human brucellosis in France. They conclude that 37 became infected through

the ingestion of raw milk, 190 through direct contact with infective discharges of the animals, and 239 through one or the other of these channels. It is their opinion that the skin is a more common portal for infection than the digestive tract in cases occurring in that area. In our opinion it seems probable that in general the greater number of human infections are acquired through the ingestion of the organism.

Experimentally it has been shown that *Brucella* infection may be transmitted by mosquitoes and biting flies. There are as yet no epidemiological data available which indicate that insects are of any importance in the natural transmission of this disease.

INCUBATION PERIOD

Evidence relative to the incubation period in brucellosis has been gathered chiefly in the Mediterranean regions and in experimental laboratories in the United States. By observing the interval between arrival at Malta and the onset of symptoms, the shortest incubation periods were ascertained. There was little opportunity to ascertain either the average or maximum duration of the incubation period, but it was generally held that it varied from slightly under one week to about three months or possibly longer, the usual interval being about fourteen days. Rainsford (375) has measured this period in five cases, three with clear-cut evidence. In these latter the minimum lengths of the incubation period were twenty, thirty-nine, and forty-two days. These illnesses were also contracted in Malta and were presumably *Br. melitensis* infections. From the study of an epidemic of *melitensis* brucellosis involving 45 clinical cases, Huddleson and Munger (219) obtained data which indicate the incubation period may vary from ten days to two months.

Experimental inoculations of human volunteers with *Brucella* have been carried out by Morales-Otero (318). Exposures were made by feeding and by applications to the skin, both normal

and abraded. Of 40 so treated eight developed clinical evidence of brucellosis. The individuals concerned were hospitalized throughout the experiment. The incubation periods are measured not from the very earliest mild symptom but rather from the first which would ordinarily be given attention. Only one person fed *Br. abortus* became ill, and the incubation period was between twenty-eight and thirty-five days. Inoculated through the abraded skin, another had an incubation period of twenty days. Four became ill as a result of exposure to *Br. suis*. After ingestion the symptoms appeared in eleven to fifteen days in one; in seventeen to thirty-four days in another; and after exposure by the abraded skin, in eleven and in twenty-eight days in the third and fourth. Following ingestion of *Br. melitensis* the disease was manifest in ten days, and after abraded skin inoculation, in sixteen days.

An estimation of the incubation period in naturally acquired infection due to *Br. abortus* has been carried out in New York City by Hardy, Frant, and Kroll (173). The city milk supply is now 99.2 per cent pasteurized and the remainder is the high-priced certified raw product. Other commercial dairy foodstuffs are evidently not involved in the spread of brucellosis. Hence at home the people of this large urban area are essentially free from exposure. During almost a full decade only four unexplained cases appeared in persons who had not been absent from the city. There were 17 individuals who were absent from the city for one period only and remained continuously in the metropolitan area for several months both preceding and following this time. These served to give some indication of the probable duration of the incubation periods in brucellosis due to *Br. abortus*. Excluding an unusually short interval of 2.5 weeks and a very long one of 29.5 weeks, the incubation periods ranged from five to fifteen weeks with a median interval of ten weeks. While these observations are not presented as conclusive evidence, they do indicate that the incubation period

due to *Br. abortus* infection acquired by the ingestion of raw milk is both variable and quite prolonged.

PATHOLOGY

Although a considerable number of postmortem examinations on fatal cases of brucellosis have been recorded, few gross changes, with the exception of an enlarged spleen and superficial lymph nodes, were observed which could be attributed directly to invasion of *Brucella.* The anatomical and histopathological findings at necropsy have been reported in only a few instances. In general, it appears from these reports that the histological changes seen in the tissues of man are similar to those that occur in the tissues of the infected guinea pig, hog, and cow. In Iowa in one fatal case, a reticuloendothelial hyperplasia of lymph glands and centrolobular necrosis and degeneration of the liver were observed (see Case 14, Appendix, page 318). In another, the changes seen were chronic interstitial pancreatitis, chronic cholecystitis, fatty infiltration of liver, passive congestion of liver and fragmentary myocardial degeneration (see Case 15, Appendix, page 320). Figures 12 and 13 represent sections of the human brain removed at necropsy (see Case 18, Appendix, page 325). For further histological findings, the reader is referred to this case and to Case 19, Appendix, page 326.

The morbid anatomic changes in human brucellosis have been extensively reviewed by Sprunt and McBryde (420). They have also described the following anatomic changes in one fatal case of their own:

Brucella infection; extensive destruction of the blood with deposits of hemosiderin in the liver, spleen and kidneys; cloudy swelling of the hepatic cells and fatty change in the liver; necrosis of the convoluted tubules of the kidney; fibrinous and proliferative pleuritis; pleural effusion; edema of the glottis; laryngitis; tracheitis; tracheotomy wound; terminal staphylococcic infection, and acute mediastinitis.

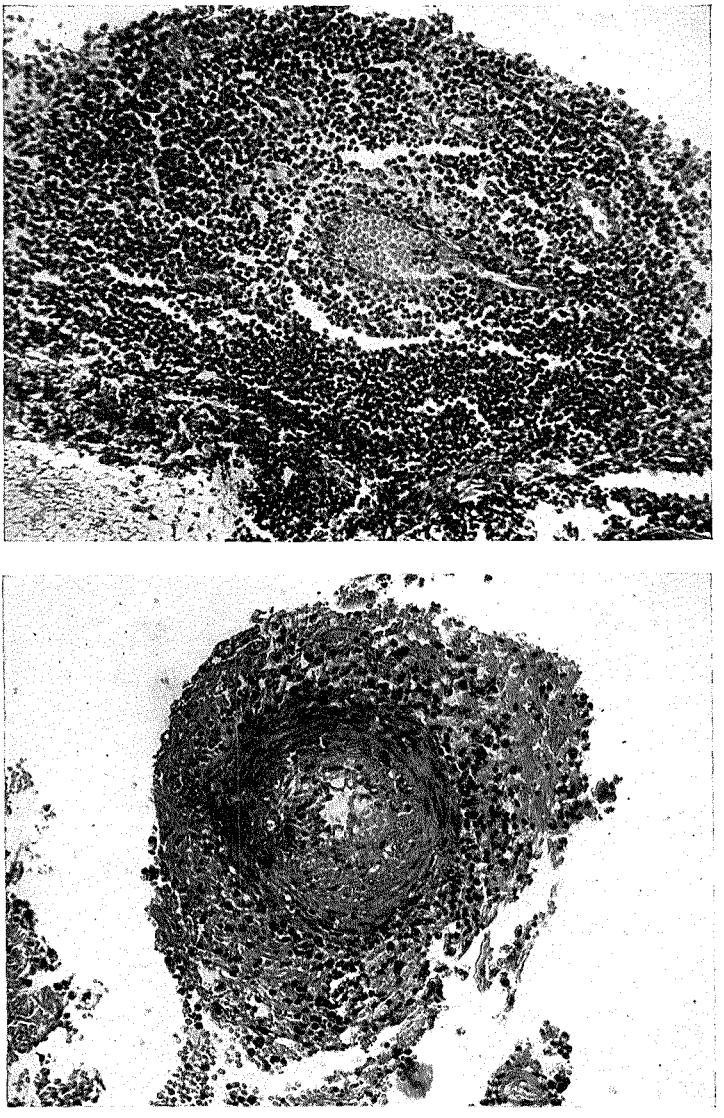
Bagley, Mueller, and Wells (9) report a fatal case of brucellosis due to *Br. abortus* in which death was caused by a pulmonary embolism. The anatomical findings at necropsy were as follows:

An antemortem clot 8 cm. in length was found plugging the left pulmonary artery, and several fragments of the same clot were present in the right pulmonary artery. In the left femoral vein, 4 cm. below Poupart's ligament, remains of a thrombus were found. Both iliac and femoral veins showed smooth, noninjected internal surfaces free from inflammatory changes. Microscopic sections of the embolus showed the characteristic architecture of an antemortem thrombus entirely free from unusual inflammatory elements.

There were evidences of an active toxic or inflammatory process widespread through the internal organs. The posterior portions of both lungs showed a moderate diffuse congestion with frequent scattered neutrophils in the interstitial tissues and many pigment-laden phagocytes in the alveoli. The 2,400 Gm. liver showed a moderate diffuse neutrophilic infiltration of its sinusoids and rather frequent small focal accumulations of neutrophils and monocytes, at times obliterating native tissues. The soft granular dark red pulp of the 375 Gm. spleen was severely congested with red blood cells and contained many pigment-laden phagocytes and occasional neutrophils. There was a mild swelling of the epithelial cells of the convoluted tubules of the kidneys, with finely granular debris in the lumen but no inflammatory cell infiltration of these tissues. No ulceration of the intestine was found, but the mesenteric lymph nodes were slightly enlarged and soft. Throughout the entire body, no localizing inflammatory processes could be demonstrated.

A fatal case of chronic brucellosis due to *Br. suis* has been reported by Menefee and Poston (297). The anatomical diagnosis at necropsy was:

Lymphadenitis, non-specific; cholecystectomy; brucellosis, with multiple small nodular lesions in lungs, liver, bone marrow, spleen, and bronchial, mediastinal, retroperitoneal, mesenteric, axillary, and inguinal lymph nodes; hepatomegaly; obstructive jaundice; splenomegaly with



After Hansmann and Schenken (171)

FIGURES 12 AND 13. SECTIONS OF HUMAN BRAIN SHOWING LESIONS DUE TO BRUCELLA. X 200

Figure 12. Dense collar of lymphocytes around a meningeal vessel
Figure 13. Infiltration of lymphocytes around a brain stem artery showing marked destruction of the media with connective tissue replacement

multiple necrosed lesions; cardiac dilatation, left, with hypertrophy; decubitus, emaciation, and dehydration.

CLINICAL TYPES

Marston (295), in his essay on fevers, pointed to the outstanding characteristic of this infection in these words: "There is no fever so irregular as this in its course and symptoms." Hughes (223), who has given us an excellent clinical description of brucellosis, introduced his chapter on symptomatology as follows: "So variable are the symptoms and so uncertain is the duration and course of this fever that it is impossible to give a description to which all cases can be referred." Craig (67) reiterated the same thought when he said, "It is extremely difficult to describe accurately all the forms which this truly protean disease may assume." A simple clinical description of the commonest type of case would therefore be misleading. In the following section, accepted clinical types of this disease are described; a clinical analysis of cases studied in Iowa is given in the Appendix, page 307, supplemented by data furnished through studies by Huddleson, Evans, Simpson, and others. The scope and method of the Iowa study are described in the following quotation from the report of the investigation (175).*

In Iowa an attempt has been made personally to investigate all reported cases of undulant fever. Blood specimens for diagnostic agglutination tests are sent by physicians located in all parts of the state to the laboratories of the State Department of Health, conducted in conjunction with the Department of Preventive Medicine of the State University of Iowa. Sera for specific examination for undulant fever are also sent here from hospitals and private laboratories. In this way we have obtained a clue to all suspected or established cases. Those patients

* With the permission of his co-authors, C. F. Jordan, I. H. Borts, Grace C. Hardy, and of the Director of the National Institute of Health, the author of this section (A.V.H.) has used freely the clinical observations published in the report of the Iowa study.

whose serum agglutinated *Brucella* in a dilution of 1:80 or higher were sooner or later visited. (The field studies were conducted by A. V. Hardy or C. F. Jordan.) Our clinical information was thus obtained directly by questioning and examining patients, and was supplemented by contributions from attending physicians and from clinical records so kindly placed at our disposal. The patient, other members of his family, and sometimes dairymen and veterinarians supplied us with data relative to sources and means of transmission of the infection. In the field we not infrequently inoculated culture media and guinea pigs with the patient's blood, or with milk from suspected cattle. Except during the early part of the study, responsibility for collecting blood or milk from animals, after we had obtained permission for their examination, was assumed by the State Department of Agriculture. A fairly complete study of many of our cases was made possible by this widely cooperative endeavor. It was soon apparent that *Brucella* infection of hogs as well as of cattle was widespread in the state. This situation gave us an unusual opportunity to study comparatively the *abortus* and *suis* species of *Brucella* infection in man.

Brucellosis was first classified by Hughes (223). On the basis of differences in temperature curves, he described three main types: the malignant, the undulant, and the intermittent. He recognized also an ambulatory form and mentioned the irregular, mixed, and chronic varieties. These same types have been observed in cases of infection with the more recently discovered species of *Brucella.* Though of minor importance in bedside diagnosis, this classification facilitates an adequate clinical description.

Intermittent Type

Most of the Iowa cases of human infection with *Brucella* were of this type. The onset was insidious, a sense of progressing afternoon weariness first oppressing the patient. General aching, some headache, a distaste for food, spells of chilliness in the early evening, and moderate insomnia follow in turn, and sometimes a suspicion of fever. Backache, stiffness or pain in the neck and joints,

constipation, and loss of weight were added to the accumulating signs and symptoms. There was, in some cases, a hacking cough which occasionally was persistent. Later, night sweats occurred, frequently drenching in character. Repeated rigors were sometimes distressing. It was usually a matter of weeks before these patients sought medical advice, most often in an office consultation. They often had difficulty in defining their ailments; or perhaps one of the above-mentioned symptoms was the chief complaint. Physical examination usually revealed no abnormalities except signs of anemia, weakness, and loss of weight, although sometimes the spleen was palpable or the abdomen tender. The patients usually felt much better when confined to bed either by the physician's advice or their own disabilities. With mild infections they might be up in the morning, but glad to rest in the afternoon. The most persistent symptoms were anorexia and weakness, or weakness alone. These symptoms, plus the fever, were in some cases the only manifestations of disease. The severity of these cases varied, so that while some were confined only for a few days, others suffered a prolonged infection which terminated fatally. Most of the infections lasted between six weeks and four months, with about one-third of this period spent in bed. Morning temperatures were found between normal and 100° F., and evening temperatures between 101° F. and 104° F. A few complete records revealed superimposed undulatory waves. The fever terminated by a slow lysis, but early in convalescence it readily recurred following overexertion. Cases 1 and 2 reported in the Appendix (pages 307 and 308) illustrate this type.

Ambulatory Type

An average of 25 per cent of the Iowa cases were ambulatory. Simpson (408) reported that one-fourth of his cases experienced a relatively short and mild illness and that 12 per cent remained at

work throughout. The onset in these cases was quite insidious, the one constant symptom, and occasionally the only one, being weakness or lack of endurance. All the symptoms already noted in the intermittent form occurred in some cases, though mild in degree. Physical examination usually revealed no abnormality. The spleen was palpable in a few patients. The temperature, normal in the forenoon, rarely reached 101° F. in the evening. The duration varied from two weeks to several months, but often it was more than one month and less than four. We have here a gradation from the mild intermittent form to subclinical infection. Illustrative cases are Nos. 3 and 4, Appendix, pages 308 and 309.

Undulant Type

The distinguishing characteristic of these cases was the occurrence of relapses. When intervening short periods of apyrexia occurred, the temperature records had a wave-like appearance. This feature was a frequent occurrence in the Mediterranean cases, but only occasionally has it been met in cases of infection with the *abortus* or *suis* species. Fifteen per cent of the Iowa cases, and also of Simpson's (408) Ohio cases, suffered relapses; but even in these typical undulations were rarely observed. Because the onset of these cases was accompanied by complaints of weakness, general aching, headache, and anorexia, it often suggested to the patient and his physician the presence of influenza or the so-called "intestinal flu" or "summer flu." Scarcely had the patient recovered from the first attack, when a second supervened in which the early symptoms were aggravated and headache, constipation, and insomnia were added. Night sweats sometimes occurred from the first, but often were not noted until later. Characteristically, the temperature increased day by day, in a step-like manner, until the maximum was reached. Morning remissions were not marked, and after a variable period the temperature decreased by a gradual ly-

sis. Occasionally such a train of events was repeated several times in the same patient. In other cases, the disease began as the usual intermittent type and was followed, at variable intervals, by one, two, or more relapses. These usually decreased progressively in intensity and duration. We have observed that the Iowa cases of the undulant type equalled in severity the milder and moderately severe intermittent forms. Without carefully following these cases, one cannot state with certainty their actual duration, but we have not observed an undue prolongation of symptoms. Selected cases are reported in the Appendix, Cases 5 and 6, pages 309 and 310.

Malignant Type

Infections of this nature due to *Br. abortus* or *suis* are rare. They are characterized by sudden onset, an acute course, and, according to the literature, usually a fatal termination. In most cases the temperature is high and sustained, with an extreme hyperpyrexia occurring before death. There is great prostration, severe headache and backache, marked anorexia, and usually true rigors, and constipation. Sooner or later delirium and coma appear. Perspiration is not profuse. The spleen is likely to be much enlarged. The duration of the two cases of this type which were observed in Iowa was about three weeks. These cases are described in the Appendix, Cases 7 and 17, pages 311 and 323.

Atypical Chronic Type

In the diagnosis of brucellosis, due consideration must be given to the occurrence of atypical forms. These infections may closely simulate other diseases, and an accurate diagnosis is then dependent upon laboratory findings. The chronic form of the disease may at times present clinical manifestations resembling the ambulatory form of typhoid fever, tuberculosis, bronchopneumonia, meningitis, cystitis, rheumatism, and various surgical conditions.

The infection may simulate other disease entities, especially during the period of onset, as is illustrated by Case 10 in the Appendix (page 312), in which the complication "orchitis" of brucellosis was first considered to be a gonorrheal epididymitis. Another case was particularly well disguised. A farmer had injured his right foot, but the wound healed after local treatment. Twelve days later, however, he returned to his physician complaining of a "stiffness" of his limbs, chiefly of the right leg. Because of difficulty in accounting for these symptoms on any other basis, they were regarded as the earliest indication of tetanus. Antitoxin was given, but brucellosis developed and during the course manifested the usual symptoms and signs.

There may occur no symptom or sign in chronic cases which even suggests a diagnosis of brucellosis. As a rule, the patient does not give a history of an acute attack accompanied by a high elevation of temperature, chills, and sweats. Both serological and cultural examinations may remain negative throughout the course of the illness. The only complaints during the first months of the disease may be exhaustion and occipital headache. Pains in the joints and back are also common. Many patients speak of vague gastric disturbances and pain in the right lower quadrant, the latter sufficiently marked to suggest chronic appendicitis. The temperature occasionally is found to be slightly elevated in the evening. According to Huddleson, the patient shows at times a subnormal temperature in the morning and a normal one in the afternoon, even weeks passing without any notable fever. A proportion of the patients gradually manifest neurological symptoms, such as mental depression, apprehensiveness, irritability of temper, shedding of tears without any occasion, sleeplessness, and even marked tremors. Occasionally there ensue temporary loss of memory, disturbances in speech, photophobia, and loss of sensation in the digits and extremities.

The diagnosis of such infections is obviously made with much difficulty.

The chronic type of disease may, however, be a purely focal infection. The focus may be located in the tonsils, at the root of one or more teeth, as noted by de Assis (76) in Brazil, in the gall bladder, bones, lymph glands, joints, or reproductive organs.

Evans (106) has recently reviewed the literature pertaining to chronic brucellosis in the United States and has emphasized the importance of conducting a more thorough study of this form of the disease. Of a total of 25 cases of the disease in Michigan during the summer of 1937, in which Huddleson cooperated with physicians in arriving at a diagnosis, 23 were of the chronic type. One typical example of those studied is illustrated by Case 11, Appendix, page 313.

A diagnosis of the chronic form in many instances is being made on the basis of the skin test alone. There is increasing evidence that immune individuals showing *Brucella* allergy are being confused with and mistaken for cases of chronic brucellosis (see Chapter VI, Part Two).

It is the opinion of the senior author (I.F.H.) that in the absence of a positive culture the only possible method of diagnosing the chronic form of the disease is the study of the combined results of a suitable allergic skin test, opsonic test, and agglutination test (see Chapter VI, pages 214–219).

Brucellosis in Children

Sander (396), discussing the symptoms noted in the study of 26 cases of brucellosis in children of ages varying from one year to ten, writes as follows:

> The symptomatology of these patients varied not at all from that described in the article already mentioned which dealt largely with brucellosis in adults. The constant symptoms were lassitude, fatigue,

weakness, and anorexia. The constant signs were intermittent fever, sweating, and loss of weight. One patient complained of dizziness, nausea, and vomiting at intervals. She also had severe headaches and generalized joint pains. The persistent diarrhea (watery pea-green stools) obtained for three weeks preceding treatment. One suffered chills and fever at such regular intervals that she was tentatively diagnosed malaria, admitted to the hospital, and the blood carefully studied for parasites which, of course, were not found. None of these patients was ever actually ill and even during the period of extreme high temperature did not impress clinically as being more than indisposed.

In general, the symptoms described by Sander are quite similar to those described for children in Malta by Debono.

Patterson and Hardwick (348) have studied 5 cases of brucellosis in children in England. The youngest was six years old. The beginning of symptoms was abrupt, with signs of an infection of the nasopharynx. Sweating was a prominent feature in two cases; marked fatigue in one. None complained of headache, joint pains or loss of appetite. The temperature ran an irregular course between 99° F. and 100.5° F. In one the fever recurred during a period of four months; in another, during one year. An enlarged spleen was noted in one. The agglutination titer was 1:500 in all. A positive blood culture was not obtained.

Fatalities

Data are inadequate at the present time for any description of the morbid anatomy of infections with the *abortus* or *suis* species of *Brucella*. This can be determined only from an accumulation of the information contained in the reports of fatal cases and necropsies. The symptoms, signs, and course of two fatal cases in Iowa, and the necropsy findings are presented in the Appendix, as Cases 14 and 15, pages 318 and 320 (see also Cases 16, 17, 18, and 19, pages 322, 323, 325, and 326). The following observations on ten fatal cases in Iowa are presented.

Death occurred in five cases without clinical evidence (in one case without pathological evidence) of any complication or localized infection; there was involvement of the cardiovascular system in three instances, revealing evidence of malignant endocarditis in two of these; in one a lung abscess occurred; in another the gastrointestinal system was mainly involved. The etiological relationship of *Brucella* to the production of these fatal complications was uncertain.

It is to be noted that some cases which began as infections of the intermittent or ambulatory type terminated fatally as well as cases which from the first were malignant in nature. Two additional fatalities occurred which may possibly be attributed to *Brucella* infection. One patient, with a past rheumatic history and a well-compensated mitral lesion, developed an auricular fibrillation early in his attack of brucellosis. Throughout his illness cardiac symptoms were prominent, and following subsidence of fever he failed to gain and died a few months later. The second case was that of a farmer who had prolonged clinical manifestations of brucellosis but whose serum agglutination was not above 1:40 dilution. Cultures were not taken. At his death the attending physician performed a necropsy but failed to find gross lesions which could account for the death. The tissues were not saved for section.

There are a few reports in the literature of fatal cases of *Br. abortus* infection. Baastrup (7) describes the six-month illness of a gardener forty-eight years old. The immediate cause of death was uremia, and this was attributed to an acute nephritis caused by *Brucella.* Such a complication is most unusual and the possibility of the nephritis having an unrelated etiology must be recognized. Scott and Saphir (401) report the isolation of *Br. abortus* from the blood stream of a patient whose illness of nine months was terminated by endocarditis with embolic phenomena. *Brucella* was also isolated from blood obtained at necropsy, and at no time was any

other organism cultured. The clinical history was that of a prolonged brucellosis, with at least one afebrile period of undetermined length. A leucocytosis was found, but only one count was reported. Clinically there was a mitral stenosis which was accounted for by a clear history of acute rheumatic fever. On both the mitral and aortic valves friable, grayish, or yellowish gray vegetations were found. The spleen was markedly enlarged. The authors are very guarded in their conclusions. We believe, however, that the terminal illness in this case may be largely explained as *Brucella* infection. Kristensen (257) mentions seven fatalities among 216 patients, but only two of these had been healthy immediately preceding the onset of brucellosis.

CLINICAL ANALYSIS

Brucellosis is a generalized infection. Occasionally definite evidence of localization appears, though the location is variable, so that all symptoms and signs must be included in a complete consideration of the disease. Three hundred of the Iowa cases provide adequate data for a detailed study. The findings on these cases have been confirmed by observations on more than 600 later infections. A composite presentation of the information contained in numerous reports in the literature, and our own study, ought to provide an adequate conception of infection due to *Br. abortus* and *Br. suis.*

Onset

The onset of brucellosis may be sudden or insidious. The physician may be called a few hours after appearance of acute symptoms, or, as in suspected tuberculosis cases, medical consultation may be sought after weeks of mild disability. The intervals from the appearance of first symptoms to the medical consultation, or to the time when the patient became bedfast, we have designated

in out-case records as the period of onset. Out of a total of 230 cases the onset duration in 27 cases (12 per cent) was less than one week, and in one-half of these the disease was ushered in abruptly. In 38 cases (17 per cent) the duration of the disease was one week; in 55 cases (24 per cent) ten days to two weeks; in 61 cases (26 per cent) three weeks to one month; in 19 cases (8 per cent) six weeks; in 30 cases (13 per cent) two months or longer.

During this period the symptomatology was extremely varied. In some cases clinical symptoms of an acute respiratory infection, including sinusitis, preceded the prolonged illness, and in others cystitis or pyelitis apparently first gave concern. Whether or not these local infections during the invasion were specific has not been determined. An acute onset following operative procedure has been noted by Kern (247). He further mentioned the case of Warren, Smith, and Linder (464), in which a sudden onset of illness followed a dose of typhoid vaccine. One of our cases was similar in nature. The patient suffered from chronic appendicitis and came into the hospital for operation with no immediate complaint. A slight elevation of temperature was manifest on the evening of admission, and after her operation the following day an acute febrile condition developed, which proved to be a *Brucella* infection. With these cases it seemed probable that a very mild subclinical or dormant infection had been provoked into acuteness by conditions which lowered the resistance.

Excepting two cases which began abruptly with rigors, all cases with rapid or with insidious onset were initiated by similar symptoms, though these differed markedly in intensity. Varying degrees of lassitude, weakness, lack of energy, or easy tiring were the initial symptoms in slightly more than one-half of our patients. Headaches gave the first indication of illness in 10 per cent, while in others spells of chilliness, anorexia, and general aching were noticed. Hence the patient sometimes stated that his illness

began with an attack of "la grippe," "flu," or "intestinal flu." Infrequently the first symptoms were night sweats, backache, stiffness of the neck or joints, arthralgia, abdominal pain, drowsiness, and dizziness.

On visiting a physician, patients complained of some or all of the above symptoms; constipation, or dizziness, either singly or variously combined, was the complaint emphasized. Others, though rare, were those related to the complication of brucellosis.

Symptoms

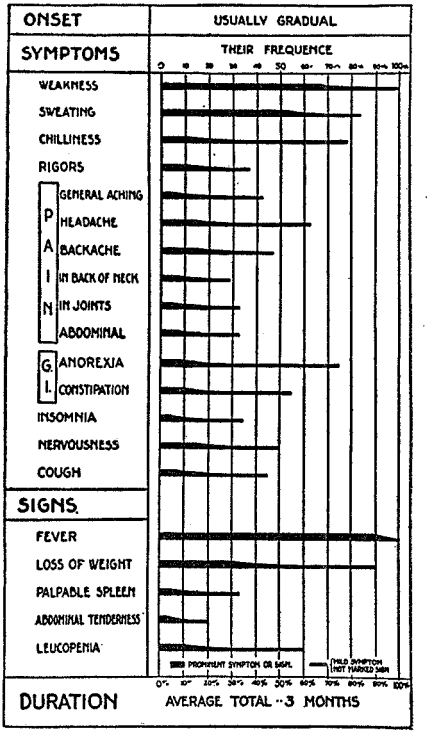
The common symptoms and signs and their relative frequency are shown in Figure 14. Such tabulations, in which severe symptoms and prominent physical findings were also indicated, were prepared from our clinical record forms. Not only the fact of their presence or absence but the degree of severity, the time of occurrence, and other characteristics of the symptoms were briefly noted at the time of taking the history. The following observations are based on our tabulations.

WEAKNESS. This was the one symptom assuredly present in all cases, although in mild infections it was often experienced only in the afternoon. Occasionally it constituted the only subjective manifestation of the disease. During the period of onset it was the most common symptom; during the fastigium in two-thirds of the cases, the most prominent or severe; and during convalescence, the most persistent.

SWEATING. The most distinctive feature of the disease was the sweating, which occurred in 84 per cent of our cases. Such patients experienced marked remissions of temperature and included most of those whose temperature curves were of the intermittent form. The very mild cases with a low-grade fever, and the malignant ones

with a high and sustained temperature, were those which experienced no sweating.

In 53 per cent of our cases, the sweating was profuse or moderately so. It usually occurred soon after midnight, and was of short duration. The patient ordinarily awakened bathed in perspiration, but again rested comfortably after a change of linen. The diaphoresis was sometimes, however, quite prolonged, necessitating several changes during a single night, or it occurred irregularly whenever the patient slept, even in the forenoon. This symptom still appeared at night in two ambulatory cases, who worked during night hours.



After Hardy and associates (175)

FIGURE 14. COMMON SYMPTOMS AND SIGNS SEEN IN BRUCELLOSIS

When true rigors did occur, sweating followed; but the rigor was not constantly related to any other symptom. Occasionally the nurse, attendant, or patient reported a very disagreeable odor associated with the perspiration. Sometimes a regional sweating was reported, but usually the diaphoresis was general.

CHILLS. Chilliness was a symptom of the period of invasion and usually occurred in association with the daily rise of temperature. Although experienced by 77 per cent of the cases, it usually gave

little discomfort. A farmer, for example, counteracted it by wearing a heavy sweater, even though others were complaining of the summer heat. An afflicted physician wore his topcoat, even in the warm operating room while giving an anesthetic. Relief was thus sought and ordinarily obtained by additional covers or external heat. Once the patients became bedfast the symptom usually disappeared.

True rigors were a feature of more than one-third of our cases, though in only 12 per cent did more than two occur. When these appeared early they frequently led to a diagnosis of pneumonia, and when they developed during the course, if regularly recurring, they suggested malaria. In an occasional case there was more than one in the twenty-four hours, one patient reporting two a day for several days in succession, and another stating that one day he had five. In the mild infections rigors were not noted; in the severe cases they were common.

Only in patients who experienced spells of chilliness did the true rigors occur, and occasionally from the history alone it was not clear whether the patients had only severe chilliness or a true rigor.

PAIN. In many cases the physician was much impressed by his patient's almost complete freedom from pain. There was usually no complaint in the morning and, if bedfast, the patient was generally ready to talk and joke. However, in the case of an infection so disseminated, one cannot be surprised that aches and pains do occur in association with almost every system.

General aching was complained of in less than one-half of the cases, but was prominent in but 5 per cent. In ambulatory patients it often persisted throughout the disease and was aggravated by exercise, but in patients who became bedfast it usually disappeared rapidly. Some individuals described this aching as resembling the muscular soreness which follows overexercise; some likened it to

the effects of a generalized trauma, while others said it was "just like the flu."

Headache, a common initial symptom, was ordinarily confined to the early stages of the disease. It was associated with fever and hence appeared in the afternoon and was most severe in the evening; at times it was accompanied by pain in the eyes. Usually it was bilateral and frontal, rarely occipital.

Early in the disease a mild pain in the lumbar region was often induced or aggravated by exercise. Sometimes it became quite persistent and difficult to control and in 15 per cent of our cases it became a prominent symptom. Pain in the back of the neck occurred in 29 per cent and was severe in one-quarter of these cases. A "stiff neck" (a muscular soreness with the pain aggravated by motion) was occasionally the first symptom of the disease. This was rarely so intense as to lead to a suspicion of meningitis.

In the Iowa series, arthralgia, frequently described as "stiffness," occurred in one-third of the patients, either during the height of the disease process or in convalescence. This was usually very mild, sometimes almost indistinguishable from the general aching. Several of the large joints were usually involved and the associated pain had been "shifting" in character. A hydrarthrosis occurred in only one case.

In 7 per cent of the Iowa series, abdominal pain was definite and severe, sometimes continuous and sometimes "cramplike." When mild, it was blended with the general aching, particularly since the localization was inconstant, appearing in the epigastrium, in the lower right quadrant, or in almost any other region. This symptom must be particularly borne in mind as it has led to erroneous diagnoses and needless, even harmful, surgical procedures.

GASTROINTESTINAL SYMPTOMS. Profound anorexia occurred in severe cases, but this symptom was entirely absent in the mild form.

It was found in three-fourths of the Iowa patients. It has varied with the degree of fever, so that patients have enjoyed a good breakfast and luncheon yet had no appetite for an evening meal. When normal appetite returned, even though the fever still continued, one could prophesy an early recovery.

Nausea and vomiting occurred in some of the moderately severe infections, but even in such cases were not persistent. Nausea alone was present in 8 per cent and in association with vomiting in 13 per cent.

Constipation was manifest in one-half to two-thirds of the cases and its degree paralleled the gravity of the infection. A specific diarrhea rarely if ever occurred.

Respiratory symptoms. We have stated above that acute upper respiratory symptoms occurred at the onset of the disease. There was little to suggest that these symptoms were due to specific infection, although possibly pulmonary involvement was. We have gradually become aware of the frequency of a hacking, non-productive cough. Rarely was it particularly troublesome, and in our earlier cases was attributed to unrelated pharyngeal irritation. More careful records in our later series of 175 cases (175) indicate that more than one-third of the patients had a cough, some with mucoid or mucopurulent sputum. Here we may make mention of two cases diagnosed by consultants as bronchopneumonia, of one case diagnosed as miliary tuberculosis, and of another in which a pulmonary abscess developed at the end of an infection in which the respiratory symptoms had been prominent throughout (see Appendix, Cases 8 and 9, pages 311 and 312). In infected guinea pigs definite areas of bronchopneumonia often occur and we believe a similar pathological process may be found in man. *Br. abortus* has been cultured from tonsils (314) and *Br. melitensis* from

the sputum (458)—findings which demand careful study of all respiratory symptoms and lesions in these infections.

NEUROPSYCHIATRIC SYMPTOMS. A quantitative measurement of symptoms so variable as those which result from a deranged functioning of the psychic or neurological processes has not been possible. Their importance is, however, constantly apparent. We therefore quote from the excellent description by Surgeon Major Veale (459) in 1879. While these symptoms apparently occur more frequently and in greater severity in *melitensis* infections, still all the symptoms described are observed as a result of both the *suis* and *abortus* invasions. Major Veale writes:

> It has been already stated that in the early stage of the disease there is marked depression of spirits with a tendency to drowsiness and stupor. In the secondary stage the enfeeblement of the nervous system becomes more conspicuous; there is frequently loss of memory, irritability of temper, a proneness to shed tears without any adequate occasion, and an unsteady, timorous, childish manner. The tremulousness is sometimes so great that the patient is unable to write or to carry a glass of water to his lips without spilling a portion of it. More rarely one may observe an unreadiness of speech, or aphonia, or a temporary loss of sensation or motion in the extremities; but hitherto I have seldom seen any defect of vision, hearing, smell, or taste. These deviations from the normal state exist in various degrees; sometimes one, sometimes another, condition assumes a special preeminence, and the medical attendant must be careful not to mistake the mental aberrations, the aphonia, anesthesia, hyperesthesia, etc., for symptoms indicative of permanent lesions of the brain or spinal cord.

In addition to the neurological manifestations noted above, which occur in the course of a general infection, the central nervous system is frequently the site of localized lesions. These complications will be described later.

UROGENITAL SYMPTOMS. A few patients in our series were first treated as cases of cystitis or pyelitis. Mild symptoms of a localized infection, such as burning, pain on micturition, or frequency, though transient in nature, have occurred in 11 per cent. Difficulty in urination or retention rarely occurred. There was in some cases a definite decrease in urinary output, due presumably to the excessive perspiration.

CARDIOVASCULAR SYMPTOMS. Palpitation and the symptoms of an irritable heart have occurred during the course of the disease. These same symptoms, through their long continuance, were in a few instances notable sequelae. Dizziness was at times a complaint early in the course or during the height of the disease. Other cardiovascular symptoms were related to the complications.

LOSS OF WEIGHT. A progressive loss of weight usually occurred. Emaciation was marked in the severe infections and in those of a prolonged, though mild, nature. Farmers, for instance, who continued to work throughout a two to four-month period of illness, became very much wasted. Bed rest and an adequate diet, both in severe and mild infections, largely prevented this. Among our patients who lost twenty pounds or more, one-third were ambulatory and one-third spent more than four weeks in bed. Among those who lost no weight, or less than ten pounds, one-third were ambulatory and one-third spent more than four weeks in bed. In 10 per cent of the cases there was no apparent loss of weight.

Sequelae

We have attempted by means of a questionnaire to follow the Iowa cases in order to obtain data in regard to persisting symptoms and sequelae. Eighty replies have been received. Since this follow-up letter was not sent at a regular period after apparent

convalescence, the replies are scarcely comparable. The striking feature, however, was the prolonged period of disability after the subsidence of the fever. This was much more than one would expect following an illness in which there were few acute symptoms. Its occurrence renders the incidence of the infection more serious. In one-half of the cases weakness or easy tiring was the longest persisting symptom. Other continuing symptoms which were mentioned more than once were fever, stiffness or pain of muscles or joints, headache, backache, general aching, anorexia, palpitation, and sweating.

Seventy patients replied that there had been no fever or other illness following the original infection. In one patient there was a persistent and possibly unrelated pyelitis. Two patients with the undulatory type, whom we considered well, had had further recurrences of fever. This was true of seven others who had previously had but one attack of fever. The following answers are taken from this latter group. "For about twelve months following my illness I had recurring spells of fever. These would last from one to three days, and following them I would be tired for several days. The spells were not serious and did not take me from my work." A packing-house employee, after apparent recovery, stated: "I had another illness which resembled in every way undulant fever. There were chills, fever, sweats, aching in joints and muscles, and general prostration." This last period of illness continued for eight days as compared with three months' duration of the first.

Fourteen patients reported that in convalescence there had occurred mild or moderately severe joint pains. This involved, in the order of their frequency, knees, shoulders, ankles, hips, and wrists. In one-half of the cases one joint only was affected. Swelling or redness was not mentioned in any instance. By some the discomfort was described as a stiffness. This symptom is known to have persisted for more than two months in only three patients. We

have also had reports of a few cases in which mental depression or nervous irritability was a serious and prolonged sequel.

Duration

Most patients have found it difficult to tell at just what date recovery from the infection took place. The onset was also insidious; hence one could not accurately determine the total duration of the disease process. However, we have been able to measure the period of 212 of our patients from the time the patient found difficulty in continuing his regular work until he was free from symptoms and able to resume it. The percentages by periods are as follows: one month or less, 19 per cent; one month to ten weeks, 27 per cent; three to four months, 34 per cent; five to six months, 11 per cent; more than six months, 9 per cent. The average total duration was therefore about three months.

Only the few patients who were acutely ill were ever strictly bedfast. Early in the disease and in convalescence patients were up and dressed, resting on a couch perhaps during the afternoon. Even during the fastigium most individuals got up for toilet purposes and in the morning many insisted on sitting in a chair or walking about for a short time. Records of "time in bed" therefore simply estimate the period during which the patient spent most of the time in bed. Those who got up, dressed, and went about, though being forced frequently to lie down to rest, are regarded as ambulatory. Making determinations in this way, we have found that 9 per cent spent more than ten weeks in bed; 24 per cent, one month to ten weeks; 33 per cent, two weeks to one month; 8 per cent, one to two weeks; and 26 per cent were in bed less than one week, or were entirely ambulatory.

Physical Observations

Our study of the signs of brucellosis has been somewhat un-

satisfactory. Usually we saw the patients once only, and often this was during convalescence. Although they recalled vividly their own symptoms, they knew little or nothing of the associated signs. Practitioners have generously placed at our disposal their observations, but these, owing to the many and urgent calls of practice, were often made hurriedly and seldom recorded. Our later data have, however, confirmed the observations already reported, and the findings in other series are in general agreement. We believe, therefore, that we have a fairly accurate knowledge of the physical findings of infection due to the *abortus* and *suis* species.

SIGNS DETECTED BY PHYSICAL EXAMINATION. There was a great variation in the general appearance of those ill with brucellosis. A majority of the patients seen in bed did not appear sick. They were fairly comfortable, mentally alert, and ready to talk. Pallor was frequently noted, and the patients often appeared quite tired. In contrast to these usual cases, some patients were obviously extremely ill, but even these were usually mentally clear and lacked the dulness so characteristic of typhoid fever.

The examination of the head rarely revealed anything significant. The tongue was usually somewhat coated, and a moderate congestion of the throat was not uncommon. About 10 per cent of our cases had the moist or dry râles indicative of bronchitis, while in two of the severe infections with recovery the findings justified the tentative diagnosis of bronchopneumonia. Any abnormality in the cardiovascular system was unusual, in uncomplicated infections. A low blood pressure, rarely of marked degree, has been found late in the disease.

Abdominal tenderness was commonly encountered (in 20 per cent of our cases) and was usually associated with abdominal pain. Occasionally the tenderness was diffuse, but frequently localized in the right upper or lower quadrant, less frequently localized in

the left upper quadrant. The spleen was palpated in one-third of the cases; marked enlargement was rare. It was quite firm and sometimes seemed tender. Occasionally the liver was definitely enlarged.

A localized hyperesthesia has been reported by a few patients. The lumbar and calf muscles were occasionally quite tender.

A skin eruption occurred in 11 per cent of Simpson's cases, and in the same proportion of Kern's series (247). A general eruption has been noted in only one of the Iowa cases, but physicians have frequently mentioned the observation of scattered maculae which somewhat simulated rose spots.

For many years veterinarians have noted the appearance of an erythema and multiple follicular eruptions on their arms following the manual removal of placentae from aborting cows. The cause of this condition was investigated by Huddleson and Johnson (212) in 1930. Two types of skin eruption were noted. In one, light red irregular blotches appeared on the arm, or the entire forearm became light red. From a distance the condition had the appearance of an erythema, but when viewed closely the blotches appeared to consist of adjoining raised points. The skin became discolored on firm pressure. There was an intense itching or burning sensation. The discoloration disappeared in four to eight hours. There was no exudation or desquamation. There does not appear to be any connection between this type of skin eruption and *Brucella* infection in the cow. It is due to a state of hypersensitiveness to a placental protein.

In the second type, the eruption appeared as small, discrete, elevated, reddish, widely separated follicular papulae from 2 to 5 mm. in diameter and 1 to 2 mm. in elevation. The lesions were accompanied by an intense itching and burning which at times was very severe. It persisted for as long as three to four weeks. Occasionally necrosis, sloughing, and scarring ensued. This skin eruption was

often accompanied by a systemic reaction, the symptoms of which resembled those observed in brucellosis. Many veterinarians who developed this manifestation also showed specific agglutinins in varying titers. A local and severe systemic reaction followed intracutaneous injections of *Brucella* allergens. The investigators concluded that this syndrome was due to a specific allergy which results from *Brucella* infection and which increases to a state of hypersensitiveness following repeated exposure to infective materials after an active immunity has developed. A typical case of skin eruption is illustrated in Figure 15.

Haxthausen and Thomsen (183) have made an extensive study of this condition in veterinarians in Denmark. Their findings coincide with the study just mentioned. They find that nearly one-third of all the veterinarians who graduated since 1915 have had one or several attacks of the skin eruption.

The other physical findings which have been noted were those associated with the complications. These and the findings related to them will be described later. There has not been found, therefore, any characteristic physical sign of brucellosis. Indeed, an outstanding feature of the disease has been the absence of physical abnormalities. Our questioning concerning the findings on examination usually called forth from the attending physician the answer, "I found absolutely nothing." Probably no one thing should so influence a physician to consider *Brucella* infection in differential diagnosis as a fever unexplained by positive physical signs.

TEMPERATURE. Representative curves illustrating the types of temperature in the different varieties of brucellosis are shown in Figure 16. For comparison a curve regarded by Hughes (223) as typical of brucellosis of the Mediterranean region is also included. In infections due to *Br. abortus* and *suis* such a fever must be very unusual, since as yet we have not encountered a single chart which

conformed closely to the type so frequently described. A few of our cases have shown definite undulations with periods of apyrexia, though all have had a rather low-grade fever. Complete temperature records have been available on only a small number of our cases, but, judging from the clinical histories and the available records, we found evidence of suggestive "undulatory pyrexial relapses" in less than 15 per cent, and in these the feature was rarely outstanding. Very few temperature curves of the malignant type have been described; only a small percentage had definite undulations. Not uncommonly an intermittent type was followed by one or two relapses, usually of short duration, which came after a few days or even after a period of months of pyrexia. The usual chart showed an intermittent fever, the temperature gradually increasing during the period of invasion and disappearing by a slow lysis. The height of the temperature was variable and was readily increased by overexertion in ambulatory cases. It was also noted that there were often peaks of fever in convalescence brought on by undue exercise.

There was frequently a wide discrepancy between the degree of fever as registered by the thermometer and the patient's sense of feverishness. It was not uncommon for a patient to apply to his physician without complaint of fever, yet to his own and the physician's surprise he would actually have a temperature of 102° F., 103° F., or even 104° F. Obviously fever occurring without, or with little, subjective feverishness might lead to clinical error. The following advice is as appropriate in America today as it was in the Mediterranean countries when the statement was made by Hughes more than forty years ago:

> It is always well to take the temperature of a case reporting sick with symptoms of dyspepsia, debility, etc., as a preliminary measure, and if there is any doubt, take it during the afternoon and evening. Fever is often overlooked for want of such precautions, and cases are treated for

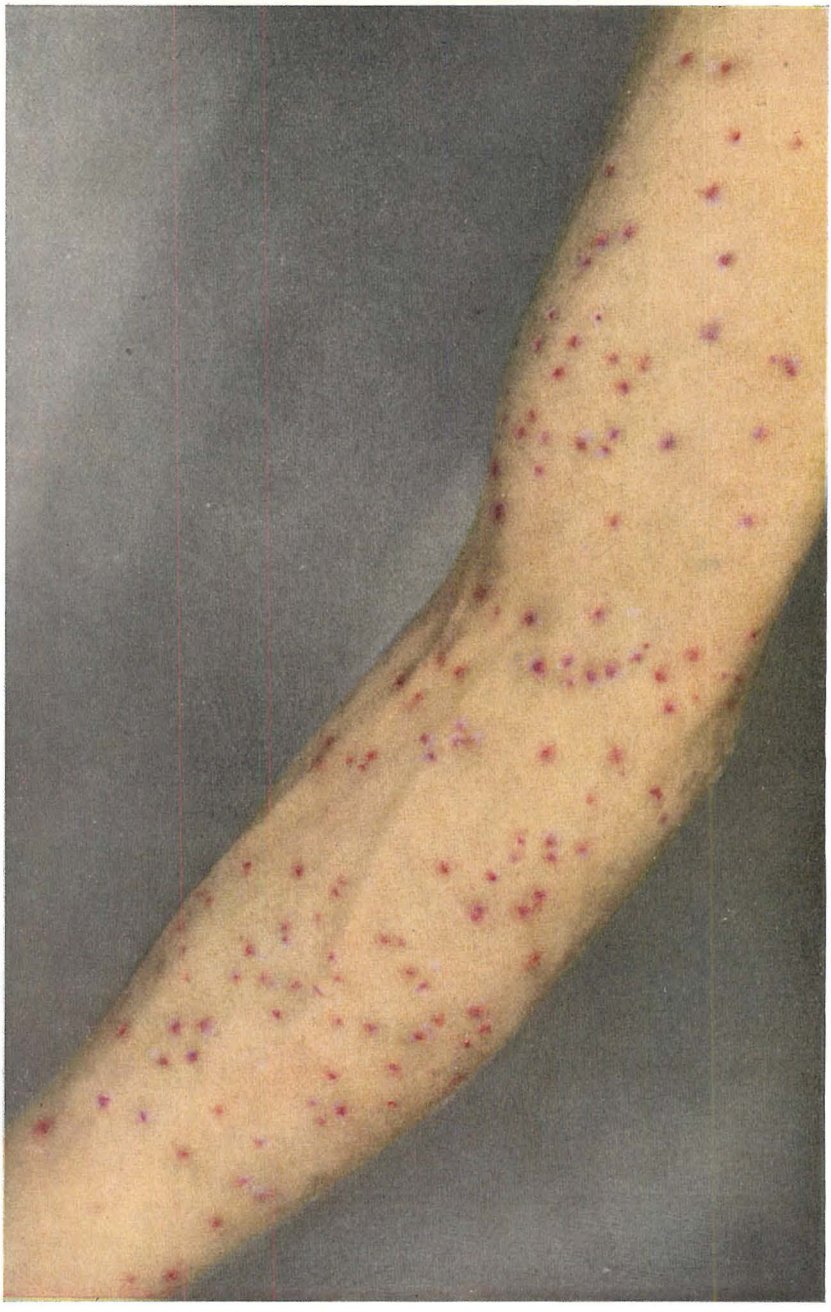
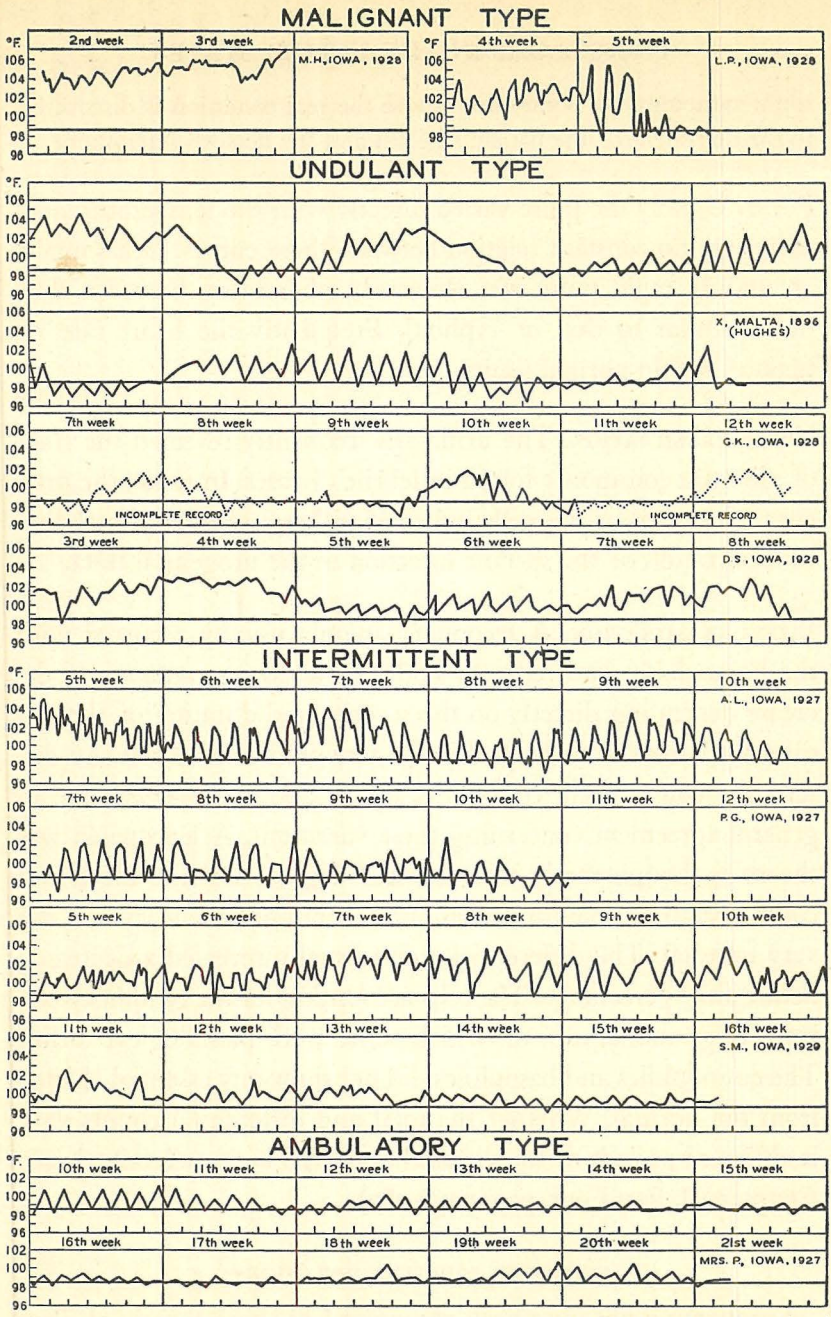


FIGURE 15. SKIN ERUPTIONS DUE TO BRUCELLA ALLERGY



After Hardy and associates (174)

FIGURE 16. TEMPERATURE CURVES BY WEEK OF ILLNESS IN THE DIFFERENT TYPES OF BRUCELLOSIS IN IOWA, WITH ONE CHART FROM HUGHES

slight symptoms for some time before the real condition is discovered, to the detriment of the patient's health and the doctor's reputation.

PULSE. Usually the pulse varied directly with the temperature, but there was no constant relation between these curves. Occasionally an unduly rapid pulse was observed; other cases showed a slow pulse similar to that of typhoid. Frequently the heart rate remained within normal limits.

URINE EXAMINATION. The urinalysis frequently revealed the trace of albumin commonly found in febrile diseases. In some, the presence of numerous pus cells indicated either a secondary infection or localization of the specific infection in the urogenital tract.

BLOOD EXAMINATION. A secondary anemia usually occurred with the hemoglobin and red cells both decreased, the amount of decrease depending directly on the severity and duration of the disease. Variations from normal in the total white blood cell and differential counts were commonly noted, and all observers are in general agreement concerning these variations. A leucopenia was the rule, though rarely of a marked degree; a white blood cell count within normal limits was not uncommon; a leucocytosis was very unusual. The differential count usually revealed a decreased neutrophile percentage. The cells accounting for the lymphocytosis were large monocytes, of which some were pathological forms. The eosinophiles and basophiles did not show any essential change from the normal. Whether the total and differential counts vary in different periods of the disease has not yet been ascertained (see Chapter VI, Part Four, pages 278–283).

LOCALIZED BRUCELLA INFECTIONS

Localization has long been recognized as characteristic of *Brucella* infections in animals. In guinea pigs, especially those inocu-

lated with *Br. suis,* we have repeatedly observed suppurative lesions, notably arthritis, osteomyelitis, spondylitis, meningitis, orchitis, and abscesses of the spleen, liver, lymph nodes, and other soft tissues. In cattle brucellosis is typically a localized infection, involving the udder, the pregnant uterus, the lymph nodes, and occasionally the joints. In hogs, according to the observations of Thomsen (439) in Denmark, and Feldman and Olson (115) in this country, focal lesions are not unusual. The pregnant uterus may be affected. Suppurative or non-suppurative epididymitis is relatively frequent. Occasionally the testis or seminal vesicles are involved. Destructive bone and joint lesions, meningitis, soft tissue abscesses, and tenosynovitis are also encountered. In horses, according to Fitch (122, 126), certain rather common suppurative lesions (poll evil and fistulous withers) may be due to *Brucella.*

With such lesions in animals, the recognition of similar conditions in human beings has not been unexpected. From inflammatory lesions in various sites *Brucella* has been isolated. In many instances the finding of no other organism than this tends to support its etiologic role; in others the causal relationship is still uncertain. In order that bacteriologic procedures effective for the isolation of *Brucella* may be employed more generally in the study of certain medical and surgical conditions, we shall enumerate the more common local lesions attributed to *Brucella* infection. Except as indicated, the conditions listed have been observed in Iowa. These localized lesions are as follows:

Skeletal System

Spondylitis. As observed and as reported, this has simulated Pott's disease. One proven case is cited (see Appendix, Case 12, page 316).

Arthritis. We have already pointed out that any detectable hydrarthrosis or swelling of the joints was unusual in our cases, oc-

curring in less than 2 per cent. Tenderness in the region of the joints was not unusual and pain on active motion was a rather frequent complaint. The specific nature of the hydrarthrosis has been established by the isolation of *Br. abortus* from the joint fluid of one reported case. Likewise from a destructive eroding arthritis in an Iowa case involving the wrist joint *Br. suis* has been cultured.

Osteomyelitis. Various long bones have also been involved both with and without an associated arthritis.

Cardiovascular System

Endocarditis and pericarditis. The occurrence of endocarditis associated with a *Brucella* bacteremia, which brought about a fatal termination of a clinical case of brucellosis, has already been indicated. The failure, after repeated attempts, to isolate organisms other than *Brucella* from the blood stream and the absence of corresponding findings of other infections strongly indicate that the endocarditis was due specifically to *Brucella*. This complication, associated in one case with pericarditis, occurred in one per cent of our cases.

Mycotic aneurysm. Involvement of the basilar artery was discovered in two cases at autopsy.

Digestive System. Cholecystitis

Amoss (3), likewise Gilbert and Coleman (143), have isolated *Brucella* from bile aspirated by the duodenal tube or obtained at operation. The organisms have also been obtained in cultures from subacutely and chronically inflamed gall bladders.

Respiratory Organs. Pleurisy with Effusion

In one case *Brucella* was isolated from the pleural fluid by guinea-pig inoculation.

Lymphatic System. Adenitis

One case of suppurative cervical adenitis suggesting a tuberculous condition yielded a pure culture of *Brucella.*

Wise and Poston (477) have succeeded in recovering *Brucella* from the affected lymph nodes of a great many cases of Hodgkin's disease. Most of the patients also had *Brucella* agglutinins in a diagnostic titer in their blood serums. They do not go so far as to claim that *Brucella* is the etiological agent in Hodgkin's disease. If this were true one would expect to find a large number of cases of the disease on the Island of Malta. The situation, however, is quite the contrary.

Urogenital System

URINARY TRACT INVOLVEMENT. Cases with initial symptoms of cystitis and renal tuberculosis have been diagnosed as brucellosis through isolation of the organism from the urine and positive agglutination test.

ORCHITIS. This has been noted in our series in 5 per cent of the males, but in one-third of these the symptoms were not severe or the findings marked. Orchitis has appeared during invasion, in the fastigium, and during convalescence. Its usual duration was two weeks, after which time it completely subsided. Whether the infection involves only the testis, or the epididymis as well, we have not been able to determine from reported observations.

Isaac (229) has reported a case in a man forty-four years of age who had at first a painful swelling of the left testicle and epididymis with only slight elevation of temperature. About five months later the patient developed acute symptoms and a painful swelling of the right testicle. The agglutination titer at this time was 1:640 for *Brucella.* The patient appeared to recover after three months of symptomatic treatment plus vaccine therapy.

Buckley (42) has reported a case of brucellosis in a boy nineteen years of age with apparent involvement of the left testicle. The onset was sudden. The symptoms were painful left testicle, nausea and vomiting, and elevation of temperature. The agglutination titer was 1:1,280. Recovery followed in 15 days with symptomatic treatment. Several months later the boy was returned to the hospital with symptoms of catarrhal jaundice, but no fever.

ENDOMETRITIS AND ABORTION. We have observed but one infection during pregnancy. The condition proceeded normally. However, there are reports in the literature of abortion associated with *Brucella* infection. Kristensen (257) isolated *Br. abortus* from the placenta in one case. Simpson (408) reported agglutination of *Brucella* antigen by the serum of five women who had no signs of syphilis, but who had repeatedly aborted. Four of these patients gave histories suggesting previous attacks of brucellosis. Prolonged observation will be necessary to determine the frequency of abortion as a complication or sequel of brucellosis.

Glandular System. Mastitis

In two of our cases (3 per cent of the adult females) a bilateral mastitis occurred as a symptom and sign of onset. Both patients were non-lactating, and the infections, which were of a mild degree, subsided spontaneously after ten days to two weeks.

Cutaneous and Subcutaneous Tissues

In several reported cases, chronic subcutaneous abscesses have yielded *Brucella* in pure culture.

Nervous System. Meningitis and Meningoencephalitis

These complications are less rare than has been supposed.

The occurrence of lesions of this general type needs to be more

widely appreciated. The variability in manifestation has yet to be adequately determined. In the reports from the Mediterranean area it is evident that varied clinical pictures may follow localized invasion of the central nervous system. Roger (386) and Roger and Poursines (387) mention "spasmodic progressive paralysis," "flaccid paraplegia," and various "meningeal reactions" including that which simulates tuberculous meningitis. Roger writes further:

> These nervous localizations have for the most part been misunderstood until the last few years as they are often late in appearing. The most characteristic reaction is, in our personal experience, meningitis. Sometimes this is cerebral and then it is at times associated with cranial neuritis and nearly always with cerebral vascular spasms. Sometimes it is medullary and associated with nerve root neuritis. This meningitis which is subacute in its course and fairly frequently shows no obvious clinical manifestation can be distinguished by the intensity of the reaction of albumin, and above all by the number of cells of the lymphocytic type which approach or pass 100 leucocytes.

A more detailed statement of nervous system involvements is to be found in the Appendix, Cases 13 and 18, pages 317 and 325.

Special Senses. Eye

In guinea pigs inoculated with *Br. suis* eye lesions are commonly noted. Lesions associated with *Br. melitensis* infection presumably of similar type have been described by Orloff (343). There was widespread evidence of a chronic inflammatory process with focal infiltration in varying areas. Concerning the infection in human beings Orloff is of the opinion that eye lesions must be rather frequent, but due to a general lack of knowledge concerning the disease such cases are erroneously diagnosed. In this country Rutherford (394) has studied the eye in brucellosis. He reports on the occurrence of papilledema. In his three cases in which this was clearly present there was clinical or pathological

evidence of localized involvement of the meninges. He also gives the sound suggestion that "undulant fever should be considered in the differential diagnosis in cases of obscure etiology in which the optic discs are edematous."

Lundsgaard (273) has further studied the optic manifestations and has described a case of iridocyclitis in a patient with brucellosis. The eye lesion made its appearance two weeks after onset of fever. It was unilateral, the cornea showing grayish, opaque streaks, superficial in some spots but in others extending to the membrane of Descemet. There was slight opacity of the lower cornea. In another case which came under his observation, ophthalmologic examination showed a slight diffuse opacity of the cornea with keratitis precipitates bilaterally. The iris was congested. There was a chorioretinitis of both eyes. Tuberculosis was eliminated as the cause in both cases.

Jones and Norris (238) believe that there is a definite relationship between brucellosis and widening of the normal shadows of the retinal tree or angioscotomas. Of 111 cases showing increased angioscotomas 75, or 67.5 per cent, showed a positive skin test indicating possible brucellosis. Since a large percentage of people show a positive *Brucella* skin test, one is not justified in making a diagnosis of the disease on this test alone.

DIAGNOSIS

Brucellosis is an infection having no pathognomonic symptoms or signs. Moreover, patients in the United States rarely appear dangerously ill; hence the taking of a detailed history and the performance of a complete physical examination are readily neglected. In addition, practitioners have not been familiar with the nature of this infection, nor are they accustomed to consider it in differential diagnosis. It is for these reasons, we believe, that ad-

mittedly erroneous diagnoses have been made in so many cases. It has been gratifying, however, to see the accuracy with which brucellosis was diagnosed when once the physician had seen cases or had familiarized himself with the clinical characteristics of the disease.

The importance of laboratory tests in diagnosis has been stated repeatedly. Stitt, for example, says: "Once there is a suspicion of undulant fever, one should try to confirm it by the more accurate method of agglutination tests or blood cultures, rather than from clinical observations." The laboratory procedures which are of value in diagnosis are the agglutination test, the intradermal test, cultural studies, white blood cell and differential counts, and opsonocytophagic tests. The two of the greatest value in differential diagnosis are the intradermal and the agglutination tests. They are usually readily available, often without cost to the patient or physician. It may be urged, therefore, that these should be more frequently used in the investigation of febrile illnesses. Agglutinins can usually be demonstrated when the patient first applies for medical advice in infections with an insidious onset, while in those with sudden onset they may not appear until the end of the second week or, according to Simpson (408), occasionally not until the fourth week. Apparently it is true of brucellosis as of typhoid that infrequently the serum of an infected individual may persistently fail to show any agglutinins. It must always be remembered also that a positive agglutination test may be related to the past or subclinical infection and not the present ailment of the patient. Blood, urine, and stool cultures are all valuable in the study of suspected cases of brucellosis, but these are practical in diagnosis only when the patient is within easy reach of a laboratory. Any cultural study in this infection will consume at least one week, and a negative report on a blood culture cannot be made reliable until the

end of the third or fourth week. Moreover, negative cultural findings on one examination can scarcely be given any weight. Cultural studies are, therefore, limited in their applicability as a diagnostic test.

The white blood cell findings in brucellosis are in no way peculiar to this infection; still a leucopenia with a relative lymphocytosis will serve to rule out all but a few conditions with which this disease may be confused. (For further information on laboratory diagnosis, see Chapter VI.)

DIFFERENTIAL DIAGNOSIS

We recorded in our case records the erroneous diagnoses, provisional diagnoses, or impressions of attending physicians. We found the three most frequent erroneous diagnoses to be typhoid fever, influenza, and tuberculosis. There were included also malaria, pyogenic septicemia, and various respiratory infections (bronchitis, sinusitis, and pneumonia). Appendicitis and cholecystitis accounted for 7 per cent of the erroneous impressions, the former being considered seriously twice as often as the latter. Disease of the cardiovascular system has been diagnosed, including subacute bacterial endocarditis, pericarditis, and hypotension. Infections of the urogenital system were also on the list, including cystitis, pyelitis, pyonephrosis, orchitis, and epididymitis. Other infrequent impressions were liver abscess, infantile paralysis, spastic colitis, carbon-monoxide poisoning (chronic), tetanus, and such conditions as "nervous breakdown," "liver trouble," and "eye trouble." In none of our cases was acute rheumatic fever or tularemia suspected, but these also may be considered in a differential diagnosis.

When the nature of brucellosis is not known and when it is not considered in differential diagnosis, or when immediate complaints or local conditions only are considered, one can readily under-

stand how the above-mentioned clinical impressions may be formed. When the physician, however, is armed with the facts, most of these possible diagnoses can be dismissed immediately. Some, however, may often present difficulty. The differential features of these may be briefly discussed.

Typhoid and Paratyphoid Fever

The more rapid onset, the dull, toxic appearance of the patient, the diarrhea and tympanites, the sustained temperature, and absence of sweats in typhoid fever usually lead to a correct opinion, while a positive Widal, or the isolation of *E. typhi* or *S. paratyphi,* establishes the diagnosis. It may here be urged that sporadic cases of typhoid fever are becoming more and more rare, and prolonged fevers, without localizing signs, which occur sporadically, demand a consideration of brucellosis.

Influenza

About 20 per cent of the cases erroneously diagnosed were called influenza. This is not because brucellosis has any similarity to the acute respiratory infection which occurs in pandemics or epidemics, but because the name is used as an accepted label for all indefinite fevers. We can but advocate a more careful and general consideration of brucellosis and less misuse of the name "influenza" as such, or its corrupted forms "flu," "intestinal flu," and "summer flu."

Tuberculosis

There may often be a real difficulty in the differential diagnosis of brucellosis and pulmonary tuberculosis. The insidious onset, weakness, night sweats, anorexia, and loss of weight are common to both, and cough also may be a prominent feature of brucellosis.

The chilliness or rigors, the general aching, headache, backache, or arthralgia, the constipation, and nervous irritability all point to brucellosis. Laboratory tests usually settle the diagnosis.

Malaria

The regularly repeated rigors which sometimes occur in brucellosis may suggest malaria. A careful history and a resort to the available laboratory tests will establish the diagnosis in either disease.

Pyogenic Septicemia

A leucopenia, or a normal white blood cell count associated with a relative lymphocytosis, which is ordinarily observed in brucellosis, usually accurately differentiates this disease from pyogenic infections. Cultural studies and agglutination tests may be necessary.

Subacute Bacterial Endocarditis

The course of this disease may simulate closely that of brucellosis. The weakness, remitting fever, loss of weight, and anemia are characteristics common to both. Sweating may occur in cases of subacute bacterial endocarditis. Moreover, in brucellosis there may also be an endocarditis, presumably caused by *Brucella.* When this does occur, the diagnosis may depend wholly upon laboratory studies, blood counts, cultures, and agglutination tests.

Acute Rheumatic Fever

We have encountered no case in which this diagnosis has been considered. The striking absence in our cases of any physical abnormality of the joints probably explains this. The arthralgia of brucellosis was often shifting in nature, but the definite swelling or hydrarthrosis, when it did occur, remained localized in the joint or joints attacked. The onset and course of acute rheumatic fever are in striking contrast to the insidious onset and the subacute course of brucellosis.

Tularemia

The clinical characteristics of the ulceroglandular, glandular, and oculoglandular types of tularemia are so striking that a clinical diagnosis of this infection is usually made with ease. However, in the typhoid type, which is of rare occurrence, there may be confusion with brucellosis. Moreover, in differentiating these infections the agglutination test may be misleading, owing to the phenomenon of cross-agglutination. *Brucella* antigens may be agglutinated in diagnostic titers by the serum of tularemia patients. Hence, if in that infection a test is performed for brucellosis only, agglutination of *Brucella* may lead to an erròneous diagnosis. If there has been any history of a possible exposure to *B. tularense,* agglutination tests for this as well as for brucellosis should be requested. The agglutination titer in tularemia is higher with *tularense* antigen than with *Brucella.* An intradermal test conducted with Brucellergen is positive in brucellosis, but negative in the case of tularemia.

Appendicitis and Cholecystitis

Fever, abdominal pain, and localized tenderness are the misleading features. When generalized infection is not considered, these clinical findings may seem to be best explained by a chronic or subacute appendicitis or cholecystitis. Simpson (408) has a record of twelve appendectomies and two cholecystectomies which were performed on cases of brucellosis. The pathological examination revealed no evidence of inflammatory process in the organs removed. In the case of this nature which we have observed, we have felt that appendectomy was too readily advised, and that a careful history with a complete physical examination, supplemented by blood counts, would have left no reason for surgical intervention. We have, however, seen one case with a retrocecal appendix, in which an appendicitis was allowed to continue until

the appendix was perforated, with a resulting peritonitis, because a weakly positive agglutination reported for brucellosis made the physician hesitate to operate. There were two of our cases in which the diagnosis of cholecystitis was seriously considered, but brucellosis when called to mind was accepted almost at once as a provisional diagnosis.

Infections of the Urogenital Tract

Frequent and painful micturition, and pus in the urine are not uncommon features of brucellosis. These may or may not be specifically related to the disease. Since these conditions have occurred late in the invasive period or during the fastigium, a careful history will usually lead to suspicion of a generalized infection with local manifestations. This is true also of orchitis when it is the major complaint.

PROGNOSIS

There has been a case fatality of 3 per cent in our cases. Deaths have occurred in infections beginning in the ambulatory type, as well as among those of the malignant variety. The duration of the infection has been variable and cannot be predicted. It is apparent, therefore, that prognosis must be somewhat guarded. This is particularly true in infections known or believed to be caused by *Br. suis;* on the other hand, we have found it safe to give a fair prognosis in cases which could be attributed to *Br. abortus.*

PART THREE. BRUCELLOSIS IN MALTA

EPIDEMIOLOGY

The prevalence of brucellosis varies from year to year and shows curious cyclical variations which are hard to explain (Figure 18). Attempts have been made to correlate the waxing and waning of the disease with the mean temperature and rainfall for the corresponding year (Hughes, 223), but the real cause seems to be a periodic "exaltation" of the virulence of the microorganism.

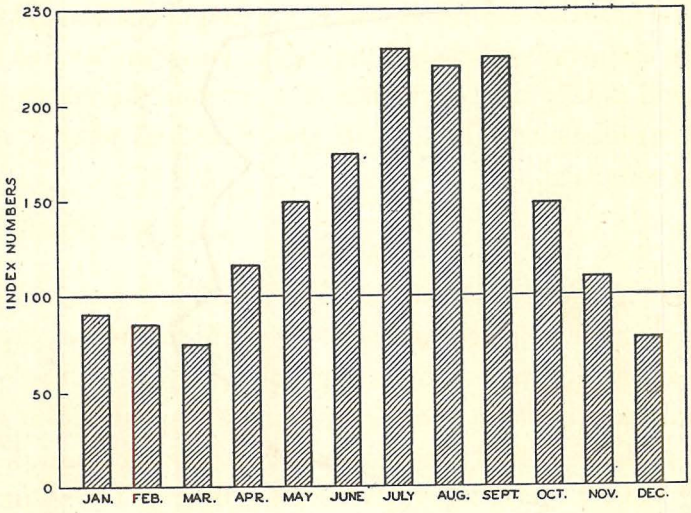
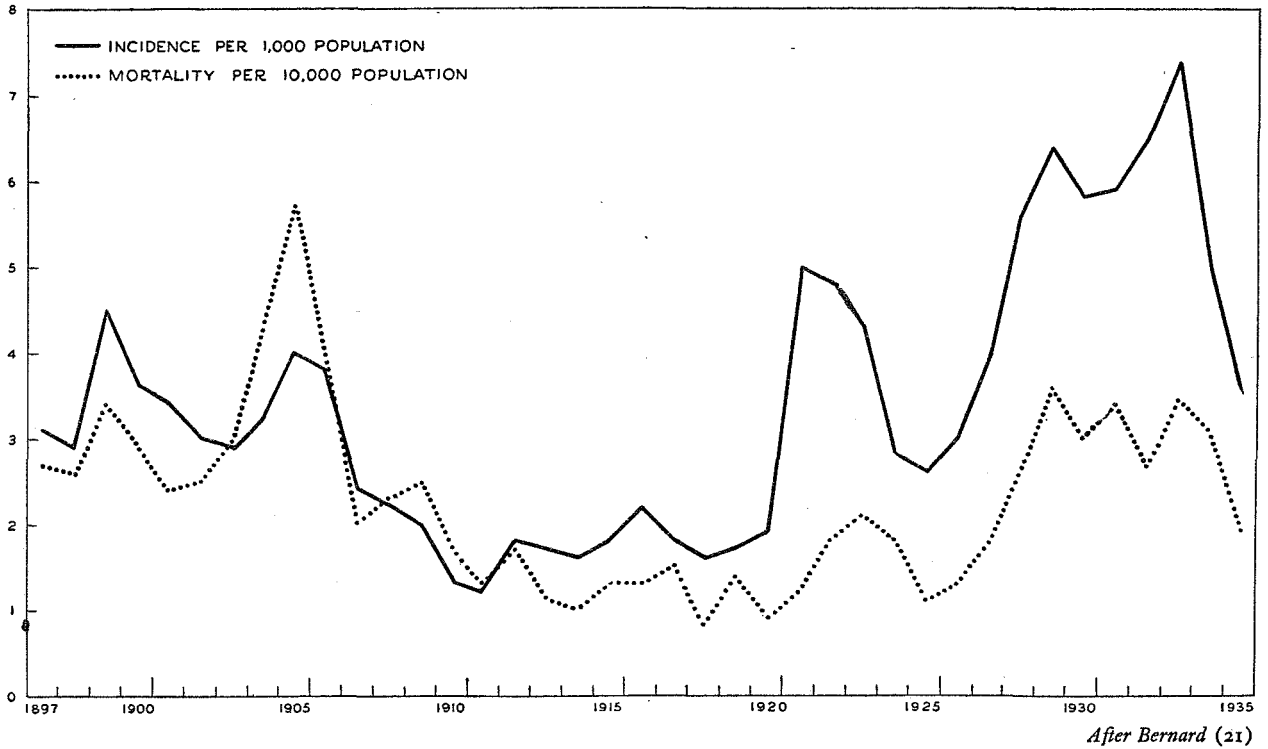


FIGURE 17. SEASONAL INCIDENCE OF BRUCELLOSIS IN MALTA BASED ON AVERAGE OF FIVE YEARS (1933–1937)

Cases of brucellosis occur all the year round. There is, however, a markedly increased incidence during the months of July, August, and September. In fact about three-fourths of the total number of cases are reported during the summer (Figure 17). Eyre (110) attributed this seasonal incidence to the fact that the princi-

NOTE: Part Three of this chapter has been contributed by J. E. Debono.



After Bernard (21)

FIGURE 18. REPORTED INCIDENCE AND MORTALITY RATES OF BRUCELLOSIS IN MALTA, 1897–1935

pal kidding season was in March and April, and to the more active excretion of the *Brucella* during the first months of lactation.

The influence of the temperature on the survival and the multiplication of the microorganism after milking is also important, and may explain the increase in the number of cases during hot summers.

Prevalence

Formerly brucellosis was more prevalent in the towns and in the suburban districts, but lately the rural districts seem to have suffered more in proportion to their population. This, at first glance, is surprising since country people drink less milk than town dwellers, but the discrepancy is explained by the greater opportunity for contact infection, and by the fact that country people *will not* boil their milk.

Occupation does not seem to have any particular influence on the chances of infection, except in the case of goatherds, who almost invariably have had the disease at some time or another. Cases of contact infection are difficult to prove, as careful interrogation almost always elicits the fact that the patient has drunk milk which might have been infected. The Undulant Fever Commission, in their 1906 report, describe two cases which were supposed to be due to contact with an infected pony. Infection through contaminated dust is a possibility to be reckoned with, especially in the case of barefooted people with abrasions, but the principal cause of the infection in Malta is undoubtedly the drinking of infected milk.

The disease is not usually contagious, and in spite of the great number of cases treated at the Central Civil Hospital, there is no record of any nurse having contracted the infection.

Laboratory infection is common among foreign workers who have to deal with the *Brucella,* and practically all the English

members of the Undulant Fever Commission contracted the disease. Curiously enough Maltese workers do not show the same susceptibility.

Sex

There is little difference between the two sexes as regards the incidence of the disease, but there is marked disparity with regard to the mortality, which is higher in the case of females.

Age

Contrary to what has been said by some authors, contrary also to local belief, brucellosis in Malta is commonest in children under five years. Unfortunately statistics showing the incidence of the disease according to age groups are not available except for 1936 (Table VIII). The official figures for the age group under five are

TABLE VIII

Age distribution of brucellosis in Malta, 1936

AGE	CASES		DEATHS	
	M	*F*	*M*	*F*
Under 5	69	56	3	1
5–10	38	41	–	–
10–15	34	28	1	–
15–20	48	35	2	–
20–25	52	51	1	2
25–45	145	125	7	14
45–65	66	66	8	11
65 and over	13	6	–	2
Total	465	408	22	30

After Bernard (21)

almost certainly an understatement of the real facts. Brucellosis in children is relatively mild, and often passes unnoticed, or is included under the generic heading of teething troubles. Routine examination of the blood frequently reveals infection in the most

unexpected cases, and in no other group of patients is Nicolle's dictum, *"Plus on la cherche, plus on la trouve,"* truer or more worth remembering. The frequency of brucellosis in young children is after all to be expected, considering that milk forms the principal item of their diet, among rich and poor alike. Fortunately the mortality is very low, and often a subclinical infection confers lasting immunity. These unrecognized infections in childhood may possibly explain the greater resistance of the Maltese as compared to the extraordinary susceptibility of English soldiers and sailors in the days before the source of the infection was recognized.

CLINICAL FEATURES

General Description

Brucellosis as it occurs in Malta is a disease of very variable severity, ranging from a mild and hardly recognizable form to a virulent and rapidly fatal infection. The average case, however, is moderately severe, comparable at its worst period to the average case of enteric fever.

The background of the infection is a highly irregular fever, rising and falling, remitting and recrudescing, forming a series of waves, but following no regular course and persisting for long periods. There are no characteristic symptoms by which the disease can be diagnosed with certainty, and the name "simple continued fever" by which the illness was formerly known adequately expresses the essence of the disease in the majority of cases. The Maltese name *deni irkik* or slow fever is equally descriptive.

The other cardinal symptoms are enlargement of the spleen, a tendency to profuse perspiration, and rheumatic manifestations toward the end of the infection. The disease tends to run a subacute or chronic course, lasting for weeks, months, or even years, but it is frequently broken by acute episodes and complications, such as bronchitis, bronchopneumonia, hepatitis, perisplenitis, and

orchitis. The patient is gradually reduced to a state of profound anemia and inanition, reminiscent in some cases of advanced tuberculosis or malignant disease. Eventually the fever burns itself out, although it is a long time before the patient is able to return to his work, as convalescence is often accompanied by marked physical weakness and mental inertia.

Incubation Period

There is no general agreement as to the length of the incubation period. In some cases infection has developed within four days of drinking infected milk, in others there has been a delay of almost a month. In the latter cases, however, it is difficult to fix the exact date of onset, as the first symptoms may have passed unnoticed. The average is from one to three weeks according to the size of the infecting dose. In cases of infection through the skin, as with laboratory workers, the disease may develop more quickly.

Onset

Although in the long run most cases of brucellosis come to resemble one another, the initial symptoms are protean. The onset may be acute or insidious. An acute onset is more frequent (60 per cent), although careful investigation will often reveal a preceding period during which the patient was not feeling quite well, especially a period of physical tiredness and mental lethargy. The usual symptoms are headache, general malaise, vague bodily pains, pain behind the eyes, and, of course, a raised temperature. The temperature may rise slowly or may be high (103° F. or 104° F.) from the first day. Pharyngitis and a slight cough are very common. Usually such cases are diagnosed as influenza in winter and as sand-fly fever in summer and, indeed, diagnosis in the first few days is almost impossible. Or the symptoms may be gastric, that is, anorexia, nausea, pain in the epigastrium, and occasionally intrac-

table vomiting, suggesting acute indigestion or enteric or paratyphoid fever, and rarely appendicitis or cholecystitis.

Not infrequently and particularly in children, a case may start as a pneumonia or bronchopneumonia, with typical pulmonary signs, and it is only when the expected resolution does not take place and the temperature begins to undulate that the correct diagnosis is made. At other times the first sign of infection is inflammation in one or more joints—the rheumatic type of onset—or else severe pain in the back, diagnosed as lumbago. Finally, in some cases the headache and the other nervous symptoms may be so intense that the suspicion of meningitis is aroused. It may be said that there is nothing characteristic about the onset of brucellosis, and that the disease is usually diagnosed as something else in the first weeks. It is only after this, when the spleen becomes palpable and the temperature curve begins to assume a characteristic shape, that the diagnosis is revised. It is usual in Malta to test the blood for *Brucella* infection in every fever lasting for more than a few days, no matter how suggestive of other disease the symptoms may be. This is the only safe method to adopt in a place where the disease is endemic, and the test is so simple that the routine might usefully be adopted in other places where brucellosis is less frequent and, therefore, more likely to be missed.

In another group of cases, almost equally important, the onset is more insidious, and it is often difficult to establish the definite beginning of the disease. The patient feels relatively well in the morning, but in the early afternoon he is overpowered by a mysterious bodily and mental weakness. Everything seems hard and difficult. Work is accomplished with a conscious effort. There may be slight headache and vague pains, and the patient diagnoses his own condition as being run down and has recourse to tonics or arranges to go for a holiday. Or else he attributes his symptoms to "intestinal intoxication" or to "septic teeth" according to the pre-

vailing fashion. At other times the slight cough and the night sweats make him think of tuberculosis. All along the patient rarely suspects that he is running a temperature and it may be months before a doctor is consulted. Eventually there may be a typical pyrexial wave forcing the patient to take to bed, or the enlargement of the spleen may be detected on examination. Some of these cases run a very mild course with a subfebrile temperature and are not confined to bed for more than a few days. The majority, however, develop into typical cases of brucellosis.

In others the onset is definitely rheumatic, with little if any rise of temperature. This is specially common in children in whom brucellosis may simulate very closely tuberculous disease of the hip or of the knee.

Clinical Varieties

Hughes (223) in his classical book on "undulant fever" described three main types: the malignant, the undulant, and the intermittent or irregular, and his classification is as good as any that has been suggested since.

Malignant or Septicemic Type

The malignant type of brucellosis is relatively rare. It accounts for 10 per cent of hospital admissions, but its frequency in relation to the total number of cases is less than this, as it is exactly this type of case which is remitted to hospitals while milder cases are treated outside. The disease may be malignant from the start or, beginning as an ordinary case, there may be a sudden change for the worse, frequently following attempts at vaccine treatment.

The characteristics of the malignant type are a high continued temperature, ranging between 104° F. and 105° F. and frequently reaching 106° F., delirium, acute toxemia with the development of a typhoidal state. Bronchopneumonia is very frequent, in fact

an almost constant accompaniment. The face is suffused and cyanotic, the tongue is dry and brown, the teeth covered by sordes, the liver is usually enlarged, vomiting is frequent, and the urine is scanty and loaded with albumin and may be suppressed in the final stages. The delirium is active at first, but soon gives place to apathy and bed picking. The pulse, which is over 120 per minute from the beginning, becomes thready and intermittent, tympanites develops, and death takes place either from cardiac or from renal failure in from ten to twenty-one days. All the symptoms point to a severe septicemia and some cases are difficult to differentiate from severe typhoid fever, more especially as diarrhea is a frequent complication.

The mortality is high, but the prognosis is not necessarily so fatal as the name malignant might indicate. With modern methods of treatment it is often possible to pull the patient through. In a series of 28 cases the mortality was 40 per cent. If the patient survives it is noticed that the fever does not last very long and that relapses, severe relapses at least, are not common. The rule, however, is not invariable, and the infection may continue for months, especially in those cases in which unsuccessful attempts at vaccine treatment have been made.

Undulant Type

This is certainly the most frequent type of case in Malta (60 per cent) and may be called the ordinary or typical brucellosis. The characteristic feature is a series of pyrexial waves, in which the temperature rises, attains a plateau, and gradually descends. The pyrexial waves have no definite shape or length and are rarely so regular and so staircase-like as the curve of typhoid fever. A sharp drop of one or more degrees somewhere on the curve is common, and is a useful differential feature. The waves may be as short as four days and as long as four weeks. The average is about a fort-

night. The number of waves varies from case to case, sometimes there is only one, sometimes as many as fourteen, but the average is three or four. The waves may be separated from one another by an interval of apyrexia lasting a few days or two or even three weeks. This apyrexia, however, is rarely complete, a low intermittent temperature, normal in the morning and 99° F. or 100° F. in the evening being more frequent. Sometimes the waves follow one another without interval and they may even overlap, giving rise to bizarre patterns. Each wave is usually accompanied by some symptom of localization. During one wave there may be bronchitis, during another perisplenitis, then perhaps vomiting or intense headache or neuritis or an attack of orchitis.

The duration of this type of case is very irregular. It may be as short as fifteen days and as long as eighteen months. The average, however, is two months with an undulating temperature and another month with an irregular intermittent fever. Toward the end of the infection the temperature tends to become markedly intermittent and oscillatory, being normal or subnormal in the morning and rising to 102° F. or more in the evening. This intermittence seems to be associated with the development of some kind of immunity, and cases with an intermittent type of temperature from the beginning as a rule run a mild course. Finally the temperature sinks to normal, but the possibility of a relapse is not yet over, especially if the patient gets up too soon or exposes himself to chills. The definite end is marked by a period of subnormal temperature. Should this last for more than five days, recovery can be assumed with a fair degree of confidence.

Intermittent and Irregular Types

These account for 30 per cent of cases. The fever is irregular, comparatively low, normal in the morning and rising to 99° F. or as high as 102° F. in the evening. Occasionally the temperature

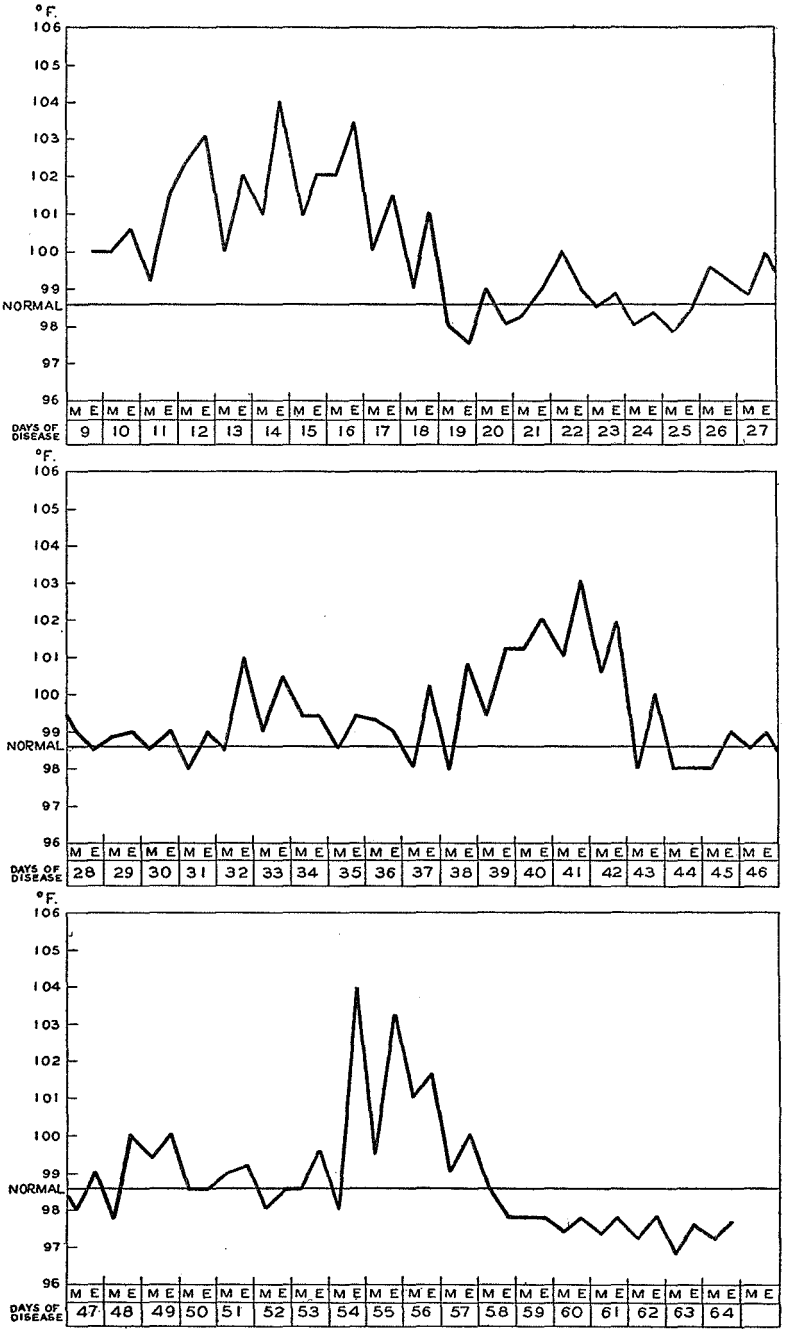


FIGURE 19. TEMPERATURE REACTION IN A MILD CASE OF BR. MELITENSIS BRUCELLOSIS

may shoot to 103° F. or 104° F. but it rarely stays at this level for more than a day or two. Sometimes the daily rise is very short-lived, from noon to six P.M., a fact to which Hughes drew attention in 1896.

Patients with this type of temperature can rarely be persuaded to stay in bed for any length of time, and many of these cases remain ambulant. In the long run, however, the infection begins to tell, manifesting itself as a general debility, anemia, and emaciation in all but the mildest of cases. The commonest symptom is rheumatism. This may be present from the beginning or appear later on. At other times cough and bronchial catarrh are the prominent symptoms and together with the night sweats and loss of weight produce a picture resembling pulmonary tuberculosis—a syndrome which has been described under the misleading title of Mediterranean phthisis.

The symptoms may be even more vague. Asthenia is usually very pronounced, so much so that at times the diagnosis of Addison's disease may be seriously considered.

The duration varies, some seem to recover fairly quickly, others drag on for months and months. Sometimes there are long periods of apyrexia and in a few cases the fever reappeared regularly for two or three summers.

SYMPTOMATOLOGY

General Appearance

There is little that is noticeable in the facies or the general appearance of the ordinary undulant fever patient. As a rule the patient feels and looks remarkably well in spite of a temperature of 102° F. or over. This is one of the most characteristic features of brucellosis.

In the malignant cases the aspect is definitely typhoidal, and

tremor, especially of the hands and tongue, is common in these cases.

When the fever has been going on for a long time, there is pronounced muscular atrophy, which makes the joints stick out like the knots on a bamboo rod. This peculiar appearance, together with dirty pallor of the face, the sunken temples, the prominent cheekbones, and the thin hair, make up a picture which once seen is not easily forgotten.

Skin

There are no specific rashes in brucellosis, the so-called Mediterranean exanthematic fever having no connection whatever with this disease. In very severe cases one may see petechiae and even extensive ecchymoses. Scarlatiniform rashes have been described but they are very rare and may be due to the administration of such drugs as quinine, hexamine, and barbituric acid derivatives. Sudamina are common especially in summer, and boils may appear during convalescence.

Sweating is a prominent feature of brucellosis, so much so that at one time the disease was called febris sudoralis. It may be present from the onset, in which case it should give rise to the suspicion of brucellosis at an early stage. Unfortunately the almost routine administration of antipyretics confuses the issue, as it is impossible to determine whether the perspiration is due to the infection or to the drugs.

More commonly the sweats appear with the decline of each pyrexial wave, and especially when the temperature is markedly intermittent. The sweating occurs in the earlier part of the night, earlier than in pulmonary tuberculosis. The perspiration may be very profuse, obliging the patient to change, sometimes more than once, and soaking through the bedclothes. Although the tempera-

ture falls, the patient feels very exhausted and much worse than when the temperature was up. Altogether these sweats are one of the most unpleasant features of brucellosis.

In cases where the perspiration has been marked, the skin of the palms, fingers, and forehead presents a curious sodden appearance.

During convalescence the skin is often dry and harsh and desquamation, branny or in largish flakes, is frequent. The hair usually falls out in the more prolonged cases.

Circulatory System

The heart is not interfered with to any extent in ordinary cases. The pulse remains relatively steady and of good volume, and a rate of 80 to 90 is quite common with a temperature of 103° F. to 104° F., when there is no toxemia. In the malignant type of case, on the other hand, the heart suffers severely. The pulse is well over 120 per minute and soon becomes thready and hardly palpable. Hyperpyrexia and bronchopneumonia put a severe strain on the heart, and in the aged and in individuals with damaged organs may easily lead to a fatal issue. In long-standing cases there may be tachycardia which persists even when the temperature is normal. The so-called effort syndrome—acceleration of the pulse with palpitations, easy fatigue, and perspiration on exertion—is common in convalescence and in ambulant cases.

Endocarditis of the aortic valves has been described elsewhere, but it must be exceedingly rare. In spite of the frequency of brucellosis in Malta, cases of valvular heart disease are not more common than in other countries, and such cases as occur are accounted for by the usual causes. Murmurs are frequent during the height of the fever and in convalescence, but they disappear eventually. Pericardial effusion may occur in severe malignant cases but is usually overshadowed by the importance of the general phenomena, and

its discovery is of little prognostic or therapeutic importance as such cases invariably die.

The blood pressure shows little change beyond a slight fall in protracted cases and, of course, a decided fall in the circulatory failure of the septicemic cases.

Thrombophlebitis, usually of the internal saphenous vein, is not very frequent, occurring in about 2 per cent of cases. It may affect one or both legs, and as a rule appears late, when convalescence is almost established. There is little constitutional disturbance and the thrombosis would seem to be due more to the anemia and the stagnation than to any localization of the microorganism.

Respiratory System

Redness of the throat is common at the onset and may lead to the diagnosis of tonsillitis. A dry tickling cough is very frequent and examination of the chest generally reveals scattered ronchi. Attacks of bronchitis, pulmonary congestion, and definite bronchopneumonia may occur during one or another of the pyrexial waves, especially in children and in old people. Pulmonary congestion is a constant feature in the malignant cases. Occasionally there may be lobar consolidation and patients are at times remitted to hospital with the diagnosis of pneumonia. The resemblance may be very close indeed, even to the fall of the temperature by crisis. That the agglutination reaction in these cases is not a coincidence is proved by the isolation of *Br. melitensis* from the blood in a number of instances, and by the subsequent course of the illness.

These pulmonary complications seem to be due to secondary invasion, as it has been impossible to recover *Br. melitensis* from the sputum in spite of repeated cultures.

Mediterranean phthisis has already been referred to. There may be dulness at one or both apices, together with râles and ronchi.

The physical signs, however, are not so "fixed" as in tuberculosis, but shift from time to time. Examination of the sputum is of course negative, and x-rays show very little alteration.

Fleeting attacks of dry pleurisy are common especially following chills and exposure, but effusion is rare.

Epistaxis may occur, usually late in the disease, and is of little importance.

Alimentary System

Gastric disturbances are more frequent than is usually supposed, and it is worth remembering that the disease was sometimes called gastrobiliary fever. At the onset and during the climax of the pyrexial waves there is marked anorexia; at other times the appetite may be surprisingly good in spite of the raised temperature. The tongue is furred but clears when the temperature becomes subfebrile. Epigastric tenderness is common and vomiting is often troublesome. Persistent vomiting is a bad prognostic sign. Constipation is the rule, but diarrhea may occur in the more severe cases and may give rise to confusion with typhoid fever.

Liver

The liver is affected to a greater or lesser extent in a large percentage of cases, and the anorexia, nausea, and epigastric tenderness may be due to hepatitis. Not infrequently it is enlarged and tender, sometimes to such an extent as to give rise to the suspicion of hepatic abscess or cholangitis, especially when the temperature is intermittent. A subicteric tinge is common, and in all the severe cases there is urobilinuria. Toxic hepatitis may complicate malignant cases and may prove fatal.

Sometimes there is marked and apparently persistent enlargement of the liver and spleen, which together with the secondary

anemia and the leucopenia of brucellosis produces a syndrome exactly similar to Banti's disease. Ascites, however, did not occur in the cases so far observed.

Spleen

Enlargement of the spleen is one of the cardinal signs of brucellosis. Sometimes the spleen is so soft that palpation is difficult and a certain experience is necessary to detect the enlargement. Percussion shows a dulness extending to the seventh rib in the left hypochondrium.

The splenomegaly usually persists throughout the course of the infection, but the spleen may exhibit alterations in size, diminishing and growing with the waves of pyrexia.

The enlargement is usually moderate, about two fingers' breadth below the costal arch; in chronic cases, however, the enlargement may be more pronounced.

Perisplenitis is common, giving rise to pain and tenderness in the left hypochondrium, accompanied by a rise of temperature. Occasionally a rub may be heard.

Lymphatic Glands

Although little attention is devoted to the state of the glands, enlargement of the glands in the groin, neck, and axilla is frequent. The glands are small and separate and may be slightly tender. In the case of cervical adenitis there may be some confusion with tuberculosis. Glandular fever may also be mistaken for brucellosis or vice versa, but more on account of the resemblance in the type of temperature than on account of the state of the glands.

Blood

Secondary anemia is common in the later stages and the red cell count may fall to three million with 40 per cent hemoglobin. In

protracted cases the hydremia may lead to edema of the legs when the patient first gets out of bed.

Urogenital System

FEBRILE ALBUMINURIA is very frequent when the temperature is above 104° F. In the septicemic type there may be marked diminution and even suppression of the urine, and death from renal failure is common.

ORCHITIS occurs in about 4 per cent of cases, usually late in the disease and when the patient has been up and about. As a rule it is unilateral but the second testis may be affected subsequently. There is acute pain in the beginning and the testis swells and becomes hard and intensely tender. There may be a sharp rise of temperature. The acute symptoms last for two or three days and then gradually subside. The swelling may persist for weeks, but eventually disappears altogether. Sometimes the orchitis may be the first symptom to attract attention. No cases of sterility following orchitis have been recorded.

MENSTRUATION. In cases of slight or moderate severity the menses are not interfered with. In the septicemic type there may be severe menorrhagia. In protracted cases and in convalescence amenorrhea is frequent. In a case under observation, brucellosis was followed by dystrophia adiposogenitalis. Oöphoritis—the counterpart of orchitis in males—is said to occur, but this is not confirmed by local experience.

ABORTION. There is no tendency to abortion in ordinary cases of brucellosis, and pregnancy may be carried to term and successful lactation undertaken in spite of the fever. A continued temperature of 104°F., however, usually leads to abortion, and the prog-

nosis in these cases is exceedingly bad. This is perhaps the reason for the preponderant female mortality in the age group 20 to 45 years. Brucellosis in females is not followed by sterility.

Nervous System

Hughes (223) in his book on "undulant fever" drew attention to the "irritative" effect of the *Brucella* toxins on the central nervous system, and instanced as an example of this the exaggeration of the knee and ankle reflexes.

Headache is the commonest of all symptoms at the onset, and recurs frequently during the course of the disease. Sometimes it may be so severe as to suggest meningitis. Symptoms of "meningism" are fairly frequent in children. Examination of the cerebrospinal fluid in these cases reveals no alteration except increased pressure.

Giddiness, a "dazed feeling," and an inclination to sleep are common, while at other times there may be intractable insomnia. Partial deafness is not rare; sometimes it is due to the use of quinine as a febrifuge, but it may occur independently of this.

Delirium is frequent whenever there is hyperpyrexia. In children it may occur with lower temperatures. It is a prominent feature in malignant cases. Ataxiadynamia, manifested by tremors on any movement, may be observed in toxic cases and indicates a very bad prognosis.

Encephalitis has not been described, although in fatal cases the brain and meninges are often congested, and there may be small punctiform hemorrhages. It is probable that many of the meningitic symptoms are due to some degree of meningoencephalitis. Convulsions, however, are rare except in infants and there are no permanent aftereffects.

Polyneuritis may be met with especially in severe protracted cases. Usually it affects the lower limbs. There is pain, loss of

power, tingling and numbness, and loss of muscular sensation giving rise to ataxia. The condition may persist for months, but complete recovery is the rule. There is often accompanying edema of the ankles, and the complication may be due more to a dietetic deficiency than to the *Brucella* toxin.

The depression and the mental inertia which accompany brucellosis have already been referred to. As a rule the patient feels better when the temperature is up. During convalescence there may be marked mental asthenia, with difficulty in concentration, lack of initiative, and impairment of memory and of will power. Definite psychosis is rare, but there may be a marked alteration in character, the patient becoming depressed and irritable, and it may be months before he returns to his normal self.

Rheumatism

Rheumatism in one form or another is so common in brucellosis that it must be regarded as an integral part of the disease rather than as a complication. The rheumatic manifestations may vary from a slight fleeting pain in one or more joints to a severe and persistent spondylitis. As a rule the rheumatic manifestations appear in the later subacute stages of the disease, when a certain degree of immunity has been developed. There is some evidence that they may be allergic in nature.

The onset of rheumatism is usually regarded as the prelude to convalescence, and it is exceptional to observe a severe relapse after rheumatism has become established. In some cases—the so-called rheumatic type of brucellosis—arthritis may be the first and frequently the only symptom. Such cases usually run a mild subacute course and are a confirmation of the general rule that rheumatism implies a fair degree of immunity or resistance.

The rheumatic manifestations may be classified as fibrositis, neuritis, synovitis, arthritis, and spondylitis. Attacks of fibrositis

are common, affecting mainly the neck, shoulder, lumbar region, and the tensor fasciae femoris. As a rule they are of short duration and of little importance. Neuritis is also very frequent, the favorite nerves being the sciatic, the intercostal, and the circumflex. Sciatica is not an uncommon sequela of brucellosis and may be due either to an interstitial neuritis or to sacroiliac arthritis.

Synovitis and Arthritis

It is difficult to establish in individual cases whether the patient is suffering from synovitis or from arthritis. Radiological examination does not reveal any bone changes and opportunities for pathological examination do not occur. When there is marked effusion, the condition may be regarded as predominantly synovial; when there is little or no swelling, arthritis may be considered as the cause.

Synovitis is usually sudden and acute in its onset, occurring without warning and without apparent cause. The patient awakes with intense pain, and the joint swells rapidly, becoming tense and exceedingly tender but never red. The acute stage does not last very long, rarely for more than twenty-four to forty-eight hours, and by the end of the week the joint has usually resumed its normal appearance. As a rule there is elevation of temperature. The affection may be monoarticular, but more frequently several joints are affected in succession. The whole process may last for weeks with intermittences and recrudescences. The liquid obtained from the joint cavity is sterile and contains an excess of lymphocytes.

The joints most frequently affected are the knee, the ankle, the elbow, the wrist, and the fingers.

Arthritis is more frequent than synovitis. It is usually subacute and lasts longer. Sometimes it is monoarticular and there is a peculiar form in children which simulates tuberculous disease of the hip. At other times more than one joint may be affected simul-

taneously. In a series of one hundred cases the different joints were attacked in the following proportion:

Joint	*Per cent*
Ankle	40
Knee	33
Hip	25
Sacroiliac	25
Shoulder	20
Fingers	15
Elbow	10
Sternoclavicular	2

The rheumatic phase may last from fifteen days to as much as three months, the average being four weeks.

Spondylitis occurs in 4 per cent of cases. It affects chiefly the lumbar and lumbosacral regions of the spine and is accompanied by radiculitis. The pain is most severe and radiates along the nerves, running down the thighs and encircling the abdomen. The pain is aggravated by straining, coughing, or sneezing and the patient is unable to move or to sleep. At times the pain may be so excruciating that repeated injections of morphine must be given. The condition may persist for as long as six weeks. Although the rheumatic manifestations may be very severe while they last, complete resolution is the rule, and there are no aftereffects except occasional stiffness.

Affection of Bones

Very occasionally (in less than one per cent of cases) brucellosis may be complicated by the development of subperiosteal abscesses, the usual site being on one of the ribs. The condition resembles a tuberculous cold abscess in many respects. On incising, thin yellowish pus is found, which on culture may yield numerous colonies of *Br. melitensis*. These abscesses may develop weeks and

months after the complete cessation of the fever and seem to indicate a high degree of sensitization to *Brucella.*

DIAGNOSIS

When the disease has been running for weeks and a temperature chart is available, the diagnosis is easy if the possibility of brucellosis is kept in mind. It is in the first weeks that difficulties arise. A list of the diseases for which brucellosis has been mistaken would include practically all the affections accompanied by a rise of temperature. The clinical symptoms, although suggestive, can never be conclusive, and as already stated the only safe rule is to suspect brucellosis and to have the blood examined for *Brucella* agglutinins in all cases with a temperature lasting for more than ten days. Enlargement of the spleen should be diligently looked for and the occurrence of profuse perspiration noted.

The conditions which are most frequently confused with brucellosis in Malta are tuberculosis, enteric fever, pyelitis, hepatic abscess, cholecystitis, sand-fly fever, bronchitis and bronchopneumonia, hyperthyroidism, and, in children, kala azar.

The easiest and the most reliable method of diagnosis is the serum-agglutination test. A reaction of 1:80 is regarded as diagnostic; in some cases the reaction does not rise above 1:40, and if this titer persists a diagnosis can be made with confidence. Cases with a positive hemoculture and a negative serum reaction do occur, but they are exceedingly rare, and it is extremely dangerous to diagnose brucellosis in the face of a persistently negative serum reaction. Such cases usually turn out to be something quite different.

Huddleson's rapid agglutination method used with whole blood has given consistently accurate results over a period of seven years, and the test can be carried out in two minutes in the office or at the bedside. In early cases, and in cases where the serum reaction is negative in spite of suggestive symptoms, hemoculture is indi-

cated, and with modern technic a positive result can be expected in about 75 per cent of cases with *Brucella* infection.

The intradermal test does not give very reliable information as it may be positive in cases that have been exposed to infection and have acquired an allergy to skin test agents.

The opsonocytophagic index has not been used extensively enough for an opinion to be offered.

TREATMENT

The course of brucellosis is so erratic and its duration so indefinite that it is difficult to assess the value of any particular line of treatment. Reports based on single cases or on a small series are definitely misleading, and the number of remedies that have been described as specific cures is an eloquent testimony to their inefficiency. Treatment may be divided into symptomatic and specific.

Symptomatic Treatment

Symptomatic treatment follows general medical lines and need not be detailed here. The patient should be kept in bed throughout the course of the disease. There is incontestable evidence that if the patient is up and about the disease will run a more severe and prolonged course, and such complications as rheumatism and orchitis are more likely to occur. When the temperature is not very high and the patient's appetite is good, a liberal diet may be allowed, but the patient must not be fed too frequently and his digestive powers must not be overtaxed, as vomiting and diarrhea may follow overeating.

Septicemic cases are best treated by external and internal hydrotherapy, especially by the intravenous injection of glucose and saline to replace the liquid and salt lost in the profuse perspiration. Cardiac and circulatory stimulants are given as required. Specific treatment by vaccines or otherwise is contraindicated.

In the milder cases a change of air, especially to a hilly and inland locality, often acts very beneficially, and is considered as the best treatment by many experienced local practitioners.

Specific Treatment

The only methods which have given satisfactory results are the intravenous injection of trypaflavine and the intradermal injection of Brucellin.* At the moment it is difficult to define the exact indications of each, and the choice of treatment is very much a matter of personal clinical experience.

Trypaflavine seems to give the best results in relatively early cases, during the first pyrexial wave when the temperature is still fairly high, provided that there is no renal or hepatic impairment. The dose employed is 10 to 15 cc. of a 2 per cent solution every second or third day for three or four doses. The effect in suitable cases is sometimes dramatic, the temperature falling with the first or second injection and remaining at a subnormal level. But the results are not always so favorable. Sometimes no impression at all is made, or else the temperature rises again. The treatment appears to have been successful in about 25 per cent of the 40 cases in which it has been used, and the best results were obtained in patients of relatively small size in whom a higher concentration of the drug in the blood was probably obtained. In one case, toxic jaundice followed treatment by trypaflavine.

Brucellin has given very encouraging results, and it appears to be safer than trypaflavine. But here again it has been difficult to establish the exact indications—whether it acts better in the early or in the late cases, whether it should be given when the temperature is rising or when it is falling. The percentage of success has been about equal in the early and in the late cases. The criteria on which I have acted are: (a) not to give Brucellin with a tempera-

* See Treatment with Brucellin, page 149.

ture above 103° F. or when the fever is rising and (b) to gauge the dose by means of a preliminary intradermal injection of 0.2 cc. If the local reaction is very severe and there is no immediate fall in temperature, the treatment is abandoned as too dangerous. If there is no reaction at all, as happens in a number of early cases, the treatment is postponed until an allergic response has developed.

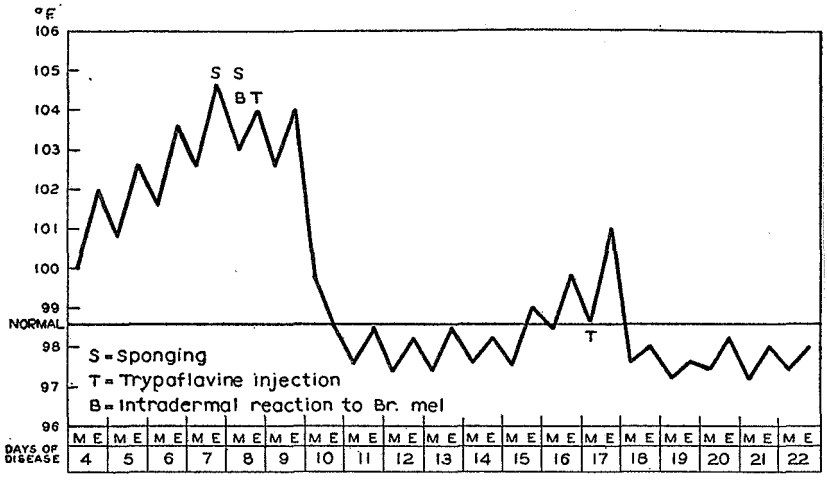


FIGURE 20. TEMPERATURE REACTION IN A PATIENT IN MALTA DURING TREATMENT WITH TRYPAFLAVINE

If the reaction is moderate, a dose of between 0.5 and 1 cc. is injected intramuscularly; if after this the temperature rises to above 102° F. an equal or a slightly smaller dose is injected in three days' time; if the temperature does not rise above this level the second dose is increased to 1 to 1.5 cc. It is essential to obtain a sharp reaction as soon as possible; to start with small doses and to climb up gradually is fatal to the success of the treatment, as the patient seems to become desensitized and the fever tends to persist indefinitely. If a decided improvement is not obtained by the fifth injection it is useless to persist. When some improvement has been obtained, one may continue until five or six injections have been

given, especially in the subacute type of case with rheumatic manifestations.

In all, 150 cases have been treated with Brucellin. The results are difficult to tabulate as the cases do not adapt themselves to any possible classification and the results obtained varied from case to case. The following table is an attempt to analyze the results obtained.

Results	*Cases treated*
Very good (immediate cessation of fever) ..	36
Good (considerable shortening of course) ..	48
Moderate (fever at lower level)	40
Failures (no reaction)	20
Bad (severe reactions or fever prolonged) ...	6
	150

PROGNOSIS

Duration

It is difficult to determine the exact duration of a case of brucellosis. Many patients are not seen at the beginning. Others are lost sight of after apparent recovery and one cannot be sure that there has been no relapse. The following is an analysis of 500 consecutive cases treated at the Central Civil Hospital.

Average duration of febrile stage	*Per cent of cases*
One month	20
Two months	25
Three months	40
More than three months	15

Mortality

The severity of brucellosis varies from year to year. There are years in which the disease appears to be more virulent and in which

the mortality is higher. The mortality curve as a rule runs parallel to the morbidity line and both seem to be influenced by the cyclical waxing and waning of the virulence of the microorganism. The official death rate, 6.1 per cent, is undoubtedly too high, if all the cases of brucellosis are taken into account. Many of the milder cases pass unrecognized and many more are not reported and do

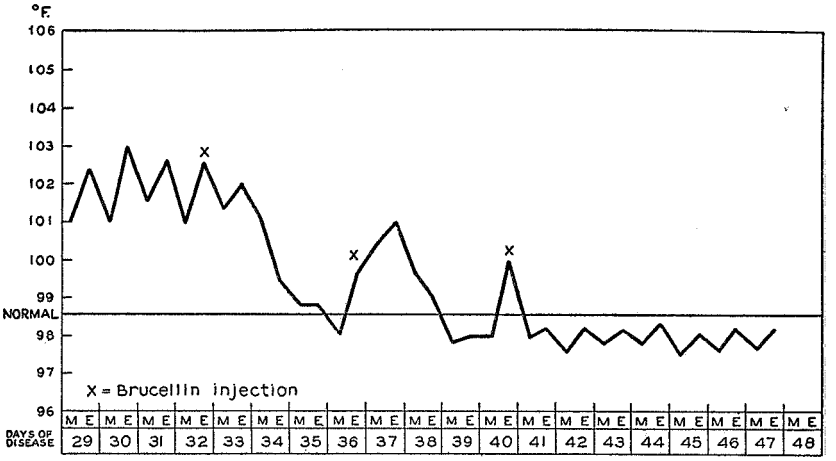


FIGURE 21. TEMPERATURE REACTION IN A PATIENT IN MALTA DURING TREATMENT WITH BRUCELLIN

not appear in the official statistics. It is mainly the severe cases, which have to be remitted to hospitals, and those in whom a fatal termination is feared, that are reported, and it is exactly among these that the highest mortality occurs. Taking all cases together the mortality averages between 4 and 5 per cent.

The disease is relatively mild in children and in old persons. In children, the chief complaint is pain in the sacroiliac joint; in old persons, the heart and the kidneys may not be able to stand the strain. The prognosis in septicemic cases is very bad and with the best of treatment the mortality is as high as 40 per cent. The outlook is particularly gloomy in pregnant women with abortion.

Hyperpyrexia persisting for more than a few days, persistent vomiting, suppression of urine, tympanites, a pulse over 120, a dry brown tongue, low muttering delirium, and tremors are ominous signs and usually herald the end.

The coexistence of tuberculosis and diabetes does not seem to aggravate the prognosis, but nephritis and a weak heart are redoubtable complications.

PART FOUR. TREATMENT

SPECIFIC AND CHEMICAL METHODS

The treatment of human brucellosis or undulant fever has been a perplexing problem to practicing physicians since the isolation and identification of its causative agent by Bruce. Although many agents have been reported on and proposed for the specific treatment of the disease, it has been difficult to evaluate their efficacy because of the nature of the disease and the fact that their use has been limited to a small number of cases. The fact that such a large number of remedies have been used in the past and that new ones are added to the list each year proclaims their ineffectiveness.

Wright was the first to study the efficacy of an immune serum prepared from a horse on infected human beings. His results were far from encouraging. Eyre (109) had a similar experience with an antiserum in the treatment of infected guinea pigs, monkeys, and human beings.

Gwatkin (158) has attempted to protect guinea pigs against *Brucella* infection by repeated injections of *Brucella* antiserums obtained from the guinea pig, rabbit, horse, and cow. The pigs were treated with the antiserums just before and after exposure to live organisms by way of the eye and mouth. While Gwatkin concluded that repeated injections of some of the antiserums protected the guinea pigs against infection, a critical analysis of the data presented does not justify such a conclusion.

Wherry, O'Neil, and Foshay (469) have presented data on 26 human cases of brucellosis treated with a *Brucella* antiserum derived from the goat. The serum was administered to certain cases intravenously, to others subcutaneously or intramuscularly in one or more doses of 20 cc. Of the total number treated, 6 showed no improvement and 4 others a relapse lasting from three days to two weeks. The duration of illness before treatment varied from three

to twelve weeks. The average duration of illness in the 20 cases after treatment was fifteen days. Since Hardy and associates (175) noted that 67 per cent of the 212 cases which they studied in Iowa had a duration of illness of only three weeks, it is not easy to evaluate the therapeutic results of the antiserum.

Flippin (132) prepared a *Brucella* antiserum by injecting cows intravenously with ascending doses of heat-killed suspensions of either *Br. abortus* or *Br. melitensis*. The pooled serum from the animals had an agglutination titer of 1:800. He reports 5 cases, the duration of symptoms varying from one to thirteen months, that were treated intravenously or intravenously and subcutaneously with 5 to 6 doses varying from 10 to 50 cc. All recovered in two to three weeks after beginning the treatment. Since most of the patients were showing an agglutination titer of 1:6,400 at the time, it is difficult to see how a serum of such a low antibody content had any influence on the course of the disease. Perhaps the same results might have been obtained with normal serum.

Creswell and Wallace (71) report remarkable results from the injection of brucellosis patients with whole blood from individuals who have recovered from the disease. The phagocytic test was used as an index in the selection of immune donors. They found that the phagocytic index paralleled the clinical condition of the patient. In other words, it was low during clinical manifestations of the disease and high following recovery.

Poston and Menefee (364) encountered an unusual case of *melitensis* brucellosis showing oral lesions along with the onset of elevated temperature, which they treated with 250 cc. of blood from an immune donor. Within twenty-four hours the temperature began to fall by lysis. The patient was symptom-free by the seventh day. No relapse occurred.

Vaccine (killed) therapy was first employed by Bassett-Smith, who considered it a valuable form of therapy. His opinion of its

value has not been shared by the majority of physicians in Malta in recent years. Rainsford (375) has given considerable attention to vaccine therapy in cases of the disease in Malta. It is his observation that if recovery is not obtained after two or three injections of vaccine its continued use is likely to result in more harm than benefit to the patient. The condition is aggravated rather than improved. He states: "The danger signs to be watched for when a patient is receiving vaccine treatment are: (a) a tendency for the temperature to become intermittent or continuous; (b) loss of appetite, listlessness of the patient; (c) increase of urobilinogen in the urine; (d) fall in the temperature or signs of improvement in the general condition."

O'Neil (342) has prepared and used a detoxified *Brucella* vaccine for treatment which does not elicit a reaction on injection. He and Calder (46) claim that it is very effective in shortening the course of the symptoms, especially in the chronic form.

Goldfain (151) has treated with a *Brucella* bacterin 38 patients with symptoms of chronic arthritis and laboratory findings indicating brucellosis. The bacterin was administered with the idea of desensitizing the patient. Of 23 cases studied completely, he reports that 7 became symptom-free after three months, 14 showed moderate to marked improvement, and 2 were not improved.

Simpson (409) is a strong advocate of vaccine (bacterin) therapy in the treatment of brucellosis. The procedure of treatment with this agent is similar to that described for Brucellin. He states that a proper vaccine brings about recovery in 60 per cent of those treated.

The fact that all kinds of bacterial vaccines have been tried on hospitalized cases by a great number of physicians on the Island of Malta, without any apparent success whatsoever, would indicate that they fall far short of being a satisfactory therapeutic agent.

Cambessédès and Garnier (50) claim to have had excellent re-

sults in the treatment of the disease with a *Brucella* endoprotein which is essentially the nucleoprotein fraction of the bacterial cell.

Non-specific protein therapy and chemotherapy, with the exception of typhoid vaccine, trypaflavine, metaphen, and sulfanilamide, have been of little if any value in shortening the course of the disease. Erwin, Hunt, and Niles (97) have used mixed typhoid and paratyphoid vaccine intravenously in brucellosis with reported success. The initial dose of 50 million killed organisms was repeated every five days until 4 to 6 injections had been given, the dosage being increased 25 to 50 million organisms each time.

Castaneda and Cardenas (58) report the treatment of 35 cases of brucellosis due to *Br. melitensis* with an antigen derived from the bacterial cells. A heavy suspension of the three species is ground in a glass tube containing steel balls. The cell-free supernatant is standardized according to its nitrogen content. The average duration of the disease in the 35 cases before treatment was ten months. After treatment had been begun the average duration was 3.2 months.

Trypaflavine was first used in the treatment of brucellosis by Izar (232) and later by Darré and Lafaille (74). Debono (see Chapter IV, page 139) reports favorable results from the use of trypaflavine when administered early in the disease. The product is used intravenously in a 2 per cent solution. The dosage varies from 5 to 15 cc.

Abbott, Abbott, and Abbott (1) have reported favorable results from the intravenous injection of metaphen. They recommended the injection of 10 cc. of a 1:1,000 dilution and repetition of the same dose if the temperature does not become normal.

The use of the sulfa compounds in the treatment of human brucellosis has been advocated and condemned by many physicians. The unfavorable reports just about neutralize the favorable ones. Blumgart (28) treated one acute case for nineteen days with sulfanilamide in doses varying from 25 to 75 grains daily. The symp-

toms disappeared on the nineteenth day. Stern and Blake (423) report the recovery of 3 acute cases in from forty-eight hours to four days after beginning treatment. The dosage varied from 60 to 80 grains daily. Traut and Logan (449) report recovery in two cases forty-eight hours after beginning treatment. Welch, Wentworth, and Mickle (467) say that 4 of 5 cases treated with sulfanilamide responded favorably. They have presented evidence that the drug stimulates specific opsonins in infection. Gaffney (139) reports 5 cases treated with sulfanilamide in doses varying from 22½ to 45 grains daily. He considered the results favorable although there were relapses in most of the cases. He believes that the drug shortens the duration of the disease. Bartels (14) has reported what would appear to be a dramatic recovery in a patient with *abortus* brucellosis following the oral administration of sulfanilamide; 90 grains were given the first day and 60 grains daily for nine subsequent days. The patient remained temperature free after the tenth day. The duration of symptoms before treatment was eight weeks, which is about the time the average acute case begins to show signs of improvement provided good care and bed confinement are employed in the treatment.

Toone and Jenkins (444) treated a *suis*-infected patient with a total of 450 grains of sulfanilamide over a period of eleven days. The temperature began to fall on the sixth day and continued downward until it became normal on the fifteenth day. No relapse occurred. Since the duration of symptoms was six weeks before treatment was instituted, it is problematical whether the treatment influenced the course of the disease. Neumann (333) has treated 4 cases of *melitensis* brucellosis with prontosil administered intramuscularly and 16 cases orally. No response was noted in the cases treated intramuscularly. It is claimed that 15 of the 16 treated orally recovered in seven days.

Debono (77) studied the action of the sulfa compounds on 26

cases, 16 of which were of the febrile undulating type, 4 of the septic type, and 6 of the mild intermittent type. They were treated orally for seven days with 4.5 grams of prontosil. No shortening of the course of the disease or beneficial effects were observed.

These cases were hospitalized and kept under close observation. The results have particular significance since Debono has few equals in his experience with the disease. He is convinced that there is an element of danger in sulfanilamide therapy in this disease and that its use in this instance should not be advocated.

Bynum (45) has also failed to note any favorable effects of sulfanilamide on the course of brucellosis. He treated 6 patients showing symptoms of either the acute or the chronic form with 60 to 80 grains daily for six days and continued with smaller amounts for seven days more.

The reported results from the use of chemo-therapeutic agents in the treatment of human brucellosis are conflicting and confusing. It may be said that whenever such agents are employed on a large number of cases under close observation positive therapeutic action is lacking. A satisfactory chemo-therapeutic agent for human brucellosis belongs to the future.

TREATMENT WITH BRUCELLIN

The study by the author and his associates of therapeutic agents for brucellosis in human beings has centered around a culture filtrate similar to the one used by Burnet (43) but considerably modified. The product has been given the name "Brucellin" (214). The value and limitations of this product and modifications in its use have been arrived at through the cooperation of a large number of physicians.

Preparation of Brucellin

For culture medium one may employ beef liver infusion broth

at a pH of 6.6. Each flask of broth should be inoculated with a smooth strain of each of the species of *Brucella.* The inoculated flasks are incubated at 37° C. for 100 days. They should be shaken at weekly intervals. Flasks showing no growth at the end of two weeks should be reinoculated.

At the end of the incubation period a small amount of the sediment is removed from each flask and cultured for purity by spreading over the surface of a liver agar or Tryptose agar plate. A fermentation tube containing beef broth should also be inoculated with 0.1 cc. of the suspended sediment to determine gas production. If after eight days of incubation the inoculated plates show no contaminating growth and the fermentation tubes no gas production, about 10 cc. of the supernatant liquid are withdrawn from each flask, filtered through a small Seitz filter, and tested for potency.

Potency Test

It has been found that the therapeutic value of Brucellin is directly proportional to its power to produce an allergic reaction in the skin of *Brucella* sensitized rabbits. The rabbits are sensitized by one intravenous injection of one cc. of living organisms, prepared by suspending the growth of one agar slant in 25 cc. of sterile saline solution. A period of thirty days is required for skin sensitiveness to develop. In view of the fact that a small percentage of rabbits fail to become skin sensitive, several should be injected separately with each of the three species at the same time.

The allergic activity of each flask of culture filtrate is determined by preparing dilutions of 1:5, 1:10, and 1:20 of the sample in sterile saline solution and injecting 0.1 cc. of these together with undiluted filtrate into the shaved skin of rabbits sensitized to *Br. abortus, Br. suis,* and *Br. melitensis.* The skin of the rabbits is examined after an interval of forty-eight hours. A positive reaction is

characterized by an area of induration and erythema at the points of injection. The size of the reactions varies from 3 mm. to 20 mm. and is exactly proportional to the dilution and allergic inciting power of the filtrate. A potent filtrate produces a reaction in all dilutions. A weak filtrate elicits a reaction up to the 1:10 dilution. A poor filtrate elicits a reaction only when undiluted or in a 1:5 dilution. Flasks of culture filtrate of low potency should be discarded.

The flasks of broth cultures containing potent filtrates may be stored in a cold room until they are filtered. Steps in the preparation of the filtrate are: addition of phenol to 0.5 per cent, centrifuging to remove the suspended bacteria, and filtering into a suitable container through a sterile Seitz filter. The bulk filtrate is tested for sterility by incubating the entire amount at 37° C. for seven days and by employing the method recommended for biological products by the National Institute of Health (328). If the filtered material is found sterile it may now be distributed into suitable sterile 2 cc. serum vials. The vialed filtrate is examined for sterility according to the method prescribed by the National Institute of Health and by incubating all vials at 37° C. for seven days. All vials showing cloudiness or suspended material are discarded. Five cc. of the vialed filtrate are injected intraperitoneally into a normal guinea pig to determine the presence or absence of toxic products. A potent filtrate may cause a drop in temperature of 2° to 3° C. in six hours. If the filtrate causes a drop in temperature of more than 4° C., it should be discarded.

The vialed filtrate is stored in a cold room. A potency test is made on sensitized rabbits at sixty-day intervals after preparation. If a lot of filtrate shows a decrease in potency it is discarded. It may be said here that no decrease in potency has been observed in any lot during two years of cold storage. One lot four years of age is now just as potent as on the day of its preparation.

Directions for Using Brucellin

Brucellin should be kept in the refrigerator when not in use. The liquid should be removed from the vial under strict aseptic precautions to maintain the sterility of the remaining portion. If the liquid becomes cloudy, the vial should be discarded. The syringe and needle should be sterilized by boiling. Brucellin contains phenol as a preservative.

Brucellin affects the course of the disease by producing a systemic allergic reaction which in turn is accompanied by a neutrophilic leucocytosis and increase in immune opsonins.

The efficacy of the agent depends on the existence and continuation of a state of sensitization in the patient while under treatment. If a state of sensitization is absent at the beginning of treatment or disappears during the treatment of a patient, the injection of Brucellin in a dose of any size will have little effect on the course of the disease.

In view of the differences in the reactions which Brucellin elicits in different patients, due principally to the degree of sensitization, no exact standard dosage can be set which will cover all cases.

From twelve years of experience with Brucellin, the following general procedure is recommended for its use in the treatment of the acute form of brucellosis.

When a certain diagnosis of brucellosis has been arrived at, an intradermal injection of about 0.1 cc. of Brucellin should first be given to determine the sensitiveness of the patient to this material. If no marked systemic reaction is elicited within twenty-four hours after the intradermal injection, one may give 0.5 cc. intradermally. If the 0.5 cc. dose elicits a systemic reaction, characterized by a rise in temperature during the following morning, muscular pains, chill, and sweating, the same sized dose in the same manner should be repeated after an interval of three days. If the second injection

produces a reaction similar to the first one, the 0.5 cc. dose should again be repeated after the same interval. This procedure should be continued until there is a tendency for morning and afternoon temperatures to remain subnormal during the intervals between injections. As a rule, at least 3 or 4 systemic reactions from Brucellin are required to bring the temperature below normal.

If the first 0.5 cc. injection fails to elicit a local and systemic reaction, the second injection may be increased to 1 cc. Inject part of the 1 cc. intradermally and part intramuscularly. If this amount produces the desired reaction, the third and fourth doses, given at intervals of three days, should be of the same size.

If there is no local or systemic reaction after the second or third injection, the patient has become temporarily desensitized. The state of sensitization should reappear after an interval of ten to fifteen days. Treatment may then be continued, spacing the injections (0.2 to 0.5 cc.) at intervals of fifteen days.

The temperature should be taken in the morning and afternoon of each day and for at least fifteen days after treatment is discontinued. In the absence of a daily temperature record during treatment, it is impossible for one to ascertain whether the course of the disease is being affected.

A daily temperature record for fifteen days after apparent recovery is of value in determining the occurrence of a relapse.

The procedure of treatment and dosage recommended for adults may be followed in the treatment of brucellosis in children.

The treatment of the chronic form of brucellosis presents a perplexing problem, due to the fact that the infection is of a focal form. About 25 per cent of the cases will respond to Brucellin when treated in the manner described under the acute form. The majority of the cases, however, show a relapse in fifteen to thirty days after the last third or fourth injection. It is advisable to treat patients with this form with 3 reacting doses at three-day intervals, then

space the injections (0.2 to 0.5 cc.) at intervals of fifteen days. This procedure of treatment should be continued for at least six months. Relapses can be prevented even though the patient remains infected.

Contraindications

Contraindications to the use of Brucellin are: cardiac diseases; brain tumor; kidney tumor; pernicious anemia; aplastic anemia; epilepsy; and diabetes.

Analysis of Cases Treated with Brucellin

Up to the present time more than 2,000 cases of brucellosis have been treated with Brucellin. The cases were located in North America, Mexico, and Malta.

It has not been possible to determine with accuracy the percentage of the total number of cases of the disease treated with Brucellin in which the duration has been noticeably shortened. From data collected on 100 treated cases prior to 1936 and a large number since 1936, it is apparent that Brucellin fails to affect the course in approximately 15 per cent of the cases treated.

Condensed data pertaining to 100 cases of brucellosis treated with Brucellin are presented in Table IX. Of the total, 65 were males and 35 were females; 23 were under eleven years of age, 9 were between eleven and twenty-two, and 68 were twenty-two years old or over.

It is difficult to evaluate the results of treatment in the 100 cases in terms of the number of injections given or their duration after beginning treatment. The data on the whole represent an attempt to arrive at the proper dosage and the number that should be given to stimulate the defensive mechanism of the body to bring about recovery.

From experience gained during the past two years there is no

TABLE IX

Summarized data on 100 cases of brucellosis treated with Brucellin

CASE NUMBER	SEX	AGE	DURATION BEFORE TREATMENT	LABORATORY EXAMINATION BEFORE TREATMENT			INTRADERMAL TEST*	NUMBER INJECTIONS BRUCELLIN	DURATION AFTER BEGINNING TREATMENT
				Blood culture	*Agglutinin*†	*Phagocytosis*‡			
1	F	33	2½ months	Negative	+1/500	None made	None made	4	18 days
2	F	42	10 months	Negative	+1/320	None made	None made	8	32 days
3	M	42	15 months	Negative	+1/640	12–6–5–3‡	4+	7	21 days
4	M	34	30 days	Negative	+1/1000	0–3–22–0	4+	4	14 days
5	F	27	30 days	+ suis	+1/500	None made	None made	2	10 days
6	M	26	4½ months	Negative	+1/500	0–0–0–25	4+	2	7 days
7	M	31	9 months	+ suis	+1/500	None made	None made	3	9 days
8	M	57	37 days	+ abortus	+1/320	None made	None made	4	30 days
9	M	49	3 months	Negative	+1/320	None made	None made	3	9 days
10	F	21	15 days	+ melitensis	+1/1000	0–2–19–4	4+	3	12 days
11	M	18	12 days	Negative	+1/200	0–4–8–13	4+	3	12 days
12	M	17	30 days	Negative	+1/640	1–2–4–18	4+	2	10 days
13	M	27	30 days	Negative	+1/640	None made	None made	3	39 days
14	M	53	1½ years	Negative	+1/160	None made	None made	10	30 days
15	F	21	22 days	Negative	+1/500	0–3–5–17	4+	4	12 days
16	M	60	60 days	+ abortus	+1/1000	0–2–3–20	4+	4	12 days
17	M	22	8 days	+ melitensis	+1/1000	3–4–14–3	4+	3	11 days
18	M	21	60 days	Negative	+1/2000	None made	4+	6	20 days
19	F	30	5 months	None made	+1/160	None made	4+	4	16 days
20	M	38	30 days	Negative	+1/1000	0–0–15–10	4+	2	6 days

* Intradermal test: 4+ = Edema one inch or more in diameter. Made with Brucellergen.

† Agglutination: + = complete; P = incomplete.

‡ Phagocytosis: total number of cells examined, 25; from left to right, first column, cells marked; second, cells moderate; third, cells slight; fourth, cells negative.

TABLE IX (*cont.*)

CASE NUMBER	SEX	AGE	DURATION BEFORE TREATMENT	LABORATORY EXAMINATION BEFORE TREATMENT			INTRADERMAL TEST*	NUMBER INJECTIONS BRUCELLIN	DURATION AFTER BEGINNING TREATMENT
				Blood culture	*Agglutinin*†	*Phagocytosis*‡			
21	M	37	3 months	Negative	+1/1000	None made	4+	1st course 5 2nd course 4	60 days
22	M	47	30 days	+ abortus	+1/640	None made	4+	3	15 days
23	F	20	2 months	Negative	+1/640	None made	None made	8	26 days
24	M	45	7 days	Negative	Negative	0–0–21–4	4+	2	10 days
25	F	17 mo.	9 days	None made	Negative	0–0–0–25	4+	4	12 days
26	F	3	75 days	Negative	Negative	0–0–0–25	4+	4	12 days
27	F	5	5 months	Negative	Negative	0–0–0–25	4+	4	12 days
28	M	6	4 months	Negative	Negative	0–0–2–23	4+	4	12 days
29	F	11	54 days	Negative	Negative	0–0–4–21	4+	4	26 days
30	M	44	9 months	Negative	+1/1000	0–4–12–9	None made	4	14 days
31	M	35	7 days	Negative	+1/100	10–7–8–0	4+	3	9 days
32	M	26	9 days	Negative	+1/500	0–0–4–21	4+	4	12 days
33	F	6	2 years	Negative	Negative	0–0–0–25	4+	4	10 days
34	M	7	17 months	Negative	Negative	0–0–0–25	4+	4	Recovery questionable
35	M	2½	1 month	None made	Negative	0–0–2–23	4+	4	14 days
36	M	3½	28 days	Negative	Negative	0–0–0–25	4+	4	12 days
37	M	22	3 days	Negative	+1/100	1–5–11–18	4+	4	12 days
38	M	26	1 day	Negative	+1/500	0–0–8–17	4+	4	12 days
39	M	4	11 days	+ suis	+1/100	0–0–8–17	4+	8	26 days
40	F	35	2 months	+ abortus	+1/500	None made	4+	3	9 days

* Intradermal test: 4+ = Edema one inch or more in diameter. Made with Brucellergen.

† Agglutination: + = complete; P = incomplete.

‡ Phagocytosis: total number of cells examined, 25; from left to right, first column, cells marked; second, cells moderate; third, cells slight; fourth, cells negative.

TABLE IX (*cont.*)

CASE NUMBER	SEX	AGE	DURATION BEFORE TREATMENT	LABORATORY EXAMINATION BEFORE TREATMENT			INTRADERMAL TEST*	NUMBER INJECTIONS BRUCELLIN	DURATION AFTER BEGINNING TREATMENT
				Blood culture	*Agglutinin*†	*Phagocytosis*‡			
41	F	61	2 years	Negative	+1/50	0–2–3–20	4+	14	60 days
42	F	29	6 months	Negative	+1/50	0–4–21–0	4+	6	19 days
43	M	5	1 year	Negative	P1/25	6–11–8–0	4+	9	30 days
44	M	44	17 days	None made	+1/500	None made	4+	13	55 days
45	F	28	8 months	None made	+1/50	None made	None made	7	30 days
46	M	8	22 days	Negative	Negative	13–9–3–0	4+	3	10 days
47	F	33	19 months	None made	No record	None made	4+	7	20 days
48	M	47	5 months	Negative	+1/640	None made	4+	4	10 days
49	M	45	18 days	None made	+1/640	None made	4+	8	30 days
50	F	23	8 days	+ melitensis	+1/50	10–12–3–0	4+	4	10 days
51	F	49	30 days	Negative	+1/500	None made	None made	3	11 days
52	M	59	6 months	+ abortus	+1/500	13–5–7–0	None made	1st course 3 2nd course 4	42 days
53	M	26	11 days	Negative	+1/640	None made	4+	4	11 days
54	M	4	6 months	Negative	Negative	0–0–2–23	4+	8	30 days
55	F	9 mo.	30 days	None made	Negative	0–0–3–22	4+	4	10 days
56	F	8	8 months	Negative	Negative	0–0–4–21	4+	4	15 days
57	M	2½	15 days	None made	Negative	0–0–0–25	4+	7	24 days
58	M	52	4 months	Negative	+1/640	1–10–14–0	4+	3	12 days
59	M	60	2 months	+ abortus	+1/640	11–10–4–0	4+	1st course 3 2nd course 2	36 days
60	F	11	8 months	Negative	Negative	0–0–18–7	4+	4	16 days

* Intradermal test: 4+ = Edema one inch or more in diameter. Made with Brucellergen.

† Agglutination: + = complete; P = incomplete.

‡ Phagocytosis: total number of cells examined, 25; from left to right, first column, cells marked; second, cells moderate; third, cells slight; fourth, cells negative.

TABLE IX (*cont.*)

CASE NUMBER	SEX	AGE	DURATION BEFORE TREATMENT	LABORATORY EXAMINATION BEFORE TREATMENT			INTRADERMAL TEST*	NUMBER INJECTIONS BRUCELLIN	DURATION AFTER BEGINNING TREATMENT
				Blood culture	*Agglutinin*†	*Phagocytosis*‡			
61	F	10	1 month	+ suis	+1/2500	0–12–12–1	4+	4	Illness continued 6 months
62	M	44	15 days	+ melitensis	+1/160	None made	4+	1st course 4 6 mo. later 2nd course 2	Recovery 8 days after 2nd course
63	M	14	3 years	Negative	Negative	0–0–0–25	4+	8	32 days
64	M	10	3 years	Negative	+1/20	0–0–0–25	4+	7	21 days
65	M	46	3 months	Negative	+1/1280	None made	None made	5	38 days
66	F	67	30 days	Negative	+1/500	9–3–7–6	4+	1st course 8 2 mo. later 2nd course 3	Relapse end of 2 months. Recovered 12 days after 2nd
67	F	57	40 days	Negative	+1/640	None made	None made	4	16 days
68	M	34	55 days	Negative	+1/640	None made	None made	8	24 days
69	M	42	5 months	None made	+1/500	None made	None made	1	Died 2nd day
70	F	3	1 month	Negative	Negative	None made	4+	4	12 days
71	M	38	4 months	Negative	+1/500	None made	None made	7	21 days
72	M	37	1 month	Negative	+1/320	None made	None made	4	12 days
73	F	9	3 months	None made	Negative	None made	4+	4	12 days
74	M	5	2 months	None made	Negative	None made	4+	5	15 days
75	F	54	2 months	None made	+1/500	None made	None made	4	12 days
76	M	55	25 days	Negative	+1/640	None made	None made	5	16 days
77	M	55	7 months	Negative	+1/1600	None made	None made	5	Died 18 days after 5th injection
78	F	8	3 months	Negative	Negative	0–0–0–25	4+	7	30 days
79	M	30	2 years	Negative	+1/500	0–1–17–7	4+	5	15 days
80	M	22	15 days	Negative	P1/25	11–7–7–0	4+	5	15 days

* Intradermal test: 4+ = Edema one inch or more in diameter. Made with Brucellergen.

† Agglutination: + = complete; P = incomplete.

‡ Phagocytosis: total number of cells examined, 25; from left to right, first column, cells marked; second, cells moderate; third, cells slight; fourth, cells negative.

TABLE IX (*cont.*)

CASE NUMBER	SEX	AGE	DURATION BEFORE TREATMENT	LABORATORY EXAMINATION BEFORE TREATMENT			INTRADERMAL TEST*	NUMBER INJECTIONS BRUCELLIN	DURATION AFTER BEGINNING TREATMENT
				Blood culture	*Agglutinin*†	*Phagocytosis*‡			
81	M	25	3 days	Negative	Negative	0–1–8–16	4+	3	9 days
82	M	27	4 days	Negative	Negative	3–10–11–1	4+	1st course 4 2nd course 4	27 days
83	M	24	5 days	Negative	Negative	0–0–0–25	4+	5	15 days
84	M	26	48 days	Negative	+1/2000	None made	None made	5	15 days
85	M	46	3 months	Negative	+1/500	0–6–6–13	4+	11	48 days
86	M	7	17 months	Negative	Negative	0–0–0–25	4+	4	15 days
87	M	45	30 days	Negative	+1/500	None made	None made	4	15 days
88	F	27	2 years	Negative	P1/100	None made	4+	6	20 days
89	M	57	1½ years	Negative	P1/500	0–4–21–0	4+	5	16 days
90	F	45	3 months	None made	Negative	0–5–20–0	4+	3	14 days
91	M	58	3 years	None made	Negative	0–0–3–22	4+	4	15 days
92	M	26	3 months	Negative	Negative	None made	4+	6	18 days
93	M	55	1 month	Negative	+1/640	None made	None made	4	20 days
94	M	45	10 days	+ abortus	+1/25	0–0–13–12	4+	7	21 days
95	F	25	15 days	Negative	+1/50	0–5–13–7	4+	5	15 days
96	F	47	25 days	Negative	+1/640	None made	None made	4	15 days
97	M	55	3 months	Negative	Negative	0–2–19–4	4+	3	12 days
98	F	2	7 months	Negative	+1/640	0–0–13–12	4+	6	20 days
99	F	40	2 months	Negative	+1/1000	None made	4+	8	26 days
100	M	53	5 months	+ suis	+1/1000	None made	4+	20	60 days

After Huddleson and associates (215)

* Intradermal test: 4+ = Edema one inch or more in diameter. Made with Brucellergen.

† Agglutination: + = complete; P = incomplete.

‡ Phagocytosis: total number of cells examined, 25; from left to right, first column, cells marked; second, cells moderate; third, cells slight; fourth, cells negative.

doubt that the course of the disease in many of the 100 cases would have been shortened if the size of doses used in the beginning had been larger, or if a fifteen-day interval had been allowed when desensitization occurred during treatment.

Duration of Symptoms in Treated Cases

In a disease like brucellosis it is difficult to evaluate the efficacy of specific treatment. It is a well-known fact that the symptoms in many cases are of only short duration. Again, if the disease is recognized early and the patient put to bed promptly, uneventful recovery takes place in from one week to three months. No one has presented any definite data by which the value of a specific agent used in the treatment of brucellosis might be measured, that is, the maximum time that should be required for it to bring about complete recovery. It is hardly reasonable for one to expect any agent to effect recovery in the same length of time in the acute form as in the chronic form, for the simple reason that the nature of the disease is not the same in the two. It is, therefore, obvious that the efficacy of any specific agent for brucellosis must be based on a large number of cases lasting from a few days to more than one hundred.

The 100 treated cases just mentioned have been divided into groups in Table X according to the approximate duration of illness before treatment with Brucellin was begun. This same grouping is maintained in recording the duration of symptoms after the beginning of the injections. Of 70 cases in which the duration of symptoms before treatment was less than 121 days, the persistence of symptoms in 51 (72.9 per cent) after the first injection of Brucellin was less than twenty-two days. The percentage recovering within twenty-two days was approximately the same regardless of whether the duration of symptoms before treatment was twenty-two days or one hundred twenty days. Approximately 25.7 per

cent of these 70 cases failed to recover within the twenty-two-day period, and one (1.4 per cent) showed no improvement under treatment.

The fact that it took longer than twenty-two days for 25.7 per cent of the group of 70 cases to respond to treatment was largely due to a lack of knowledge of the proper dosage, of the number

TABLE X

Duration of symptoms in 100 cases of brucellosis before and after treatment with Brucellin

DURATION OF SYMPTOMS BEFORE TREATMENT		DURATION OF SYMPTOMS AFTER BEGINNING TREATMENT					
		LESS THAN 22 DAYS		MORE THAN 22 DAYS		CONTINUED	
Duration	*Number of cases*	*Number of cases*	*Per cent of cases*	*Number of cases*	*Per cent of cases*	*Number of cases*	*Per cent of cases*
Less than 22 days	22	16	72.7	6	27.3	–	–
22 to 60 days	33	24	72.7	8	24.2	1	3.1
61 to 120 days	15	11	73.3	4	26.7	–	–
Less than 121 days	70	51	72.9	18	25.7	1	1.4
121 days to 6 months	8	4	50.0	3	37.5	1*	12.5
6 to 12 months	8	5	62.5	2	25.0	1*	12.5
12 months	14	8	57.1	5	35.7	1	7.2
More than 120 days	30	17	56.7	10	33.3	3	10.0
Total	100	68	68.0	28	28.0	4	4.0

* Terminated fatally.

of doses that should be given, of the criteria that should be followed in gauging the treatment, and of the failure in many instances to use the opsonocytophagic test to determine recovery or the progress of the patient toward recovery. Turning to the 30 cases in which symptoms had lasted more than one hundred twenty days before treatment was begun, it may also be said that these same factors explain why it took longer than twenty-two days for 33.3 per cent of the cases to recover.

If one excludes the six cases that failed to respond to treatment and the two that succumbed, it will be found that the average duration of illness per case before treatment was 159.3 days. The average duration of illness per case after treatment was begun was 18.3 days.

During the year 1937–38, a study was made to determine the therapeutic value of Brucellin under controlled conditions in brucellosis patients on the Island of Malta. The study was conducted by the author in cooperation with the Department of Health, Professors P. Xuereb, E. H. Ferro, J. E. Debono, Dr. C. H. Podesta, and several other physicians.

A total of 86 patients infected with *Br. melitensis* were studied. Of these, 28 were left untreated as controls. All patients were confined in bed in the Central Civil Hospital in Malta. A diagnosis of brucellosis was based on the results of physical examination, the agglutination and intradermal tests, and blood culture.

Since it was observed that a small percentage of cases (7 out of 86, or 8.1 per cent, in this study) recover from infection within three days after hospitalization, it was deemed advisable not to include a case for study in either group until the fourth day after admission. The patients were male and female; the ages varied from six to fifty-five years. The duration of the disease before treatment, in the 20 cases, varied from two weeks to one year.

The injection of Brucellin had little if any effect on the course of the disease in 7 cases. The average duration of symptoms in the remaining 51 cases after beginning treatment was twelve days, the shortest being three days.

Of the 28 untreated controls, 12 recovered within twenty days after admission to the hospital. The remaining 16 were still showing symptoms of the disease two months after admission.

The failure of a patient to respond to treatment was found to be due to the absence of the state of sensitization or to its complete

disappearance during treatment. During the course of this study it was discovered that the sensitization would often return if treatment was discontinued for a period of seven to ten days. Such patients when again treated with Brucellin responded rapidly to one or two injections (see temperature chart of Case 24, Figure 40, Appendix, page 335).

It has been the experience of physicians in Malta that a patient must be kept under close observation for twelve to fifteen days after the temperature returns to normal in order to determine recovery. Such a long period of observation is necessary because of the occurrence of apyrexial periods during the course of the disease which very often last from six to twelve days.

The cases used in this study were kept under observation for twelve to fifteen days after the temperature became normal or subnormal. Ten of the treated cases were questioned six months after treatment as to their state of health. None reported a return of symptoms.

The observation made during this study, that 7 out of a total number of 86 patients admitted to the hospital and diagnosed as having brucellosis recovered within three days, lends support to the contention that it is not possible to estimate the efficacy of any therapeutic agent on a small number of cases, especially when no controls are employed. The group of 7 patients that made a rapid recovery soon after admission to the hospital represents 8.1 per cent of the total number studied by the author.

It has been the author's observation that there is very often a direct relation between progress toward recovery from brucellosis and the phagocytic activity of the blood. When 60 per cent or more of the polymorphonuclear leucocytes in whole citrated blood (0.8 per cent sodium citrate) of the patient are able to phagocytize 40 or more *Brucella* cells *in vitro,* either recovery or a marked improvement in the condition of the patient occurs shortly afterward.

An exception to this rule is seen in *Br. melitensis* brucellosis. The blood of many patients during the course of the disease shows a high phagocytic activity for *Brucella.* Wherever it is possible and convenient, the phagocytic activity test should be employed in determining the patient's progress toward recovery from brucellosis. The practicing physician should become better acquainted with the possibilities of the test and the knowledge it furnishes.

Brucellosis has often been defined as a self-limiting disease with an average duration of one hundred days. The data assembled by Hardy and associates (175) on a large number of cases in Iowa tend to support this contention. A study of the available records on recent cases occurring in Malta shows that the duration of 85 per cent of the cases of brucellosis is less than one hundred days.

The duration of brucellosis in an individual without specific treatment depends largely upon its early recognition and the rigid discipline regarding bed rest to which the patient must submit until it is certain that the body temperature is normal. The average case of the disease is not recognized early, and unless the patient has been severely ill it is usually found that he has not remained in bed. As a consequence, these cases may develop into the chronic form and persist for months and even years.

Since many of the cases of brucellosis occurring in the United States do not conform to the usual "text book" description and do not react to the agglutination test, one might justly raise the question whether such cases are diagnosed correctly. The answer to this question will come when workers succeed in obtaining a higher percentage of blood cultures and will depend upon how accurate and specific the intradermal allergic test conducted with *Brucella* protein nucleate solution (Brucellergen) and the opsonocytophagic system recommended are in detecting *Brucella* infection in human beings.

Considerable data have already been collected by the author on

the specificity of the allergic and opsonic tests in cases of tuberculosis, tularemia, typhoid, chronic sinusitis, and intestinal infections, which show beyond question that in these diseases there is no cross skin sensitization detectable with *Brucella* protein nucleate solution. There is one disease, tularemia, in which the phagocytic activity of the blood is considerably increased for *Brucella.*

One might advance the opinion that recovery from brucellosis following Brucellin therapy was a sequence of what often occurs in using non-specific protein shock therapy in illnesses of a chronic nature. It has been the experience of the author that unless individuals are sensitized to *Brucella* they will show no systemic and little if any local reaction following the administration of Brucellin. Only a small percentage of individuals has been found sensitive to the beef liver broth as used in preparing Brucellin. Eventually, a preparation of Brucellin will be available that is free from the extraneous constituents that are to be found in the present product.

Artificial Fever Therapy

There were at one time many who advocated artificial fever therapy in the treatment of brucellosis, but like many other treatments it no longer has much support. Prickman and associates (368) treated 21 cases by this method and claim to have obtained recoveries in 80.9 per cent. Simpson (409) suggests fever therapy only for those cases that do not respond to vaccine therapy.

GENERAL THERAPEUTIC INDICATIONS OTHER THAN SPECIFIC TREATMENT

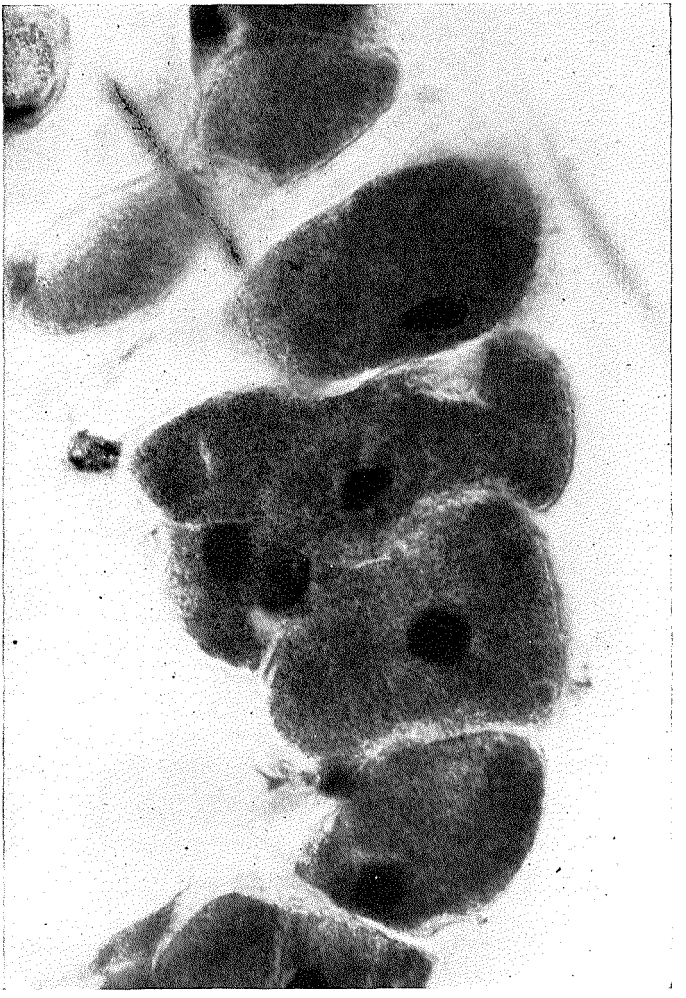
The general therapeutic indications for the treatment of brucellosis have been so ably presented by Gentry (141) and Rainsford (375) that the author has taken the liberty of making extensive quotations from their presentation of the subject (see also Chapter IV, Part Three, pages 138–139).

Recommendations by Gentry (141)

"GENERAL CARE—The patient should be isolated and all excreta disinfected. The sick room should be sunny, well ventilated, with an even temperature and a comfortable bed. In such a prolonged debilitating illness a trained nurse is essential. Light woolen bed clothing will be found advantageous, as it absorbs perspiration, avoids chilling and may lessen the frequency of the rheumatic pains. The mattress should be protected by a rubber sheet, and the bed clothing and linen should be changed whenever they become damp. The patient should remain in bed constantly during the acute stage, until the temperature has been normal for at least 10 days and a clean tongue and subnormal temperature indicate permanent improvement. Many relapses can be traced to allowing patients to get up too soon. The teeth should be kept clean and a soothing mouth wash prescribed.

"DIET—The diet is important and the patient's strength and nutrition should be maintained by giving him as much food as can be assimilated. Hughes (223) and others have laid stress on the appearance of the tongue as a guide to the state of the gastro-intestinal mucous membrane and as an index of the patient's digestive powers. With a fairly clean tongue and moderate fever, a more liberal diet can be given. In the acute stage the diet should be liquid, as milk, albumen water, fruit juices and egg nogs. Later, fresh fruits, cooked cereals, vegetable purees, eggs, custards, chicken and fish may be added. The patient should be urged to take water freely at all times. In chronic cases, with anemia and impaired digestive function, the maintenance of nutrition is often difficult.

"HYDROTHERAPY—The fever is best controlled by hydrotherapy. A warm cleansing bath should be given each morning. For a temperature of 103° F. a tepid or tap water sponge should be given



After Th. Smith (410)

FIGURE 22. THE EPITHELIAL CELLS OF CHORION BOVINE FETAL PLACENTA DENSELY PACKED WITH BRUCELLA

and, for a temperature of 104° F. or over, a cold sponge or wet pack may be required. Slight friction when drying the patient is beneficial. These measures keep the skin in good condition, improve the circulation, quiet the nervous irritability and promote general metabolism. Bassett-Smith cautions against checking the profuse sweats by hydrotherapy.

"TREATMENT OF SPECIAL CONDITIONS—Constipation is a marked feature and should be treated with mild laxatives, as liquid petroleum, cascara, or licorice powder supplemented by enemata if necessary. A bed pan should be used in the acute cases; later, a closed stool. Headache is relieved by the use of an ice cap. Nervous irritability may be benefited by bromides or small doses of luminal. Persistent insomnia may require occasional hypnotics. Arthritis is best treated by hot applications or radiant heat and light. The chronic cases with neuralgia or neuritis are benefited by conservative physiotherapy, including radiant light or cabinet baths and galvanism. Wasted muscles should receive light daily massage and electrical treatment, using the sinusoidal current. Morphine should be avoided, as a habit is easily induced in a prolonged illness of this character. The anemia should be treated by suitable diet and the administration of iron in the form of Blaud's pills or occasionally by intramuscular injections of iron or arsenic.

"CONVALESCENCE—During convalescence the patient should be encouraged to take gentle exercise out of doors if weather conditions are suitable. He should be warmly dressed and cautioned against over-exertion or exposure, which are apt to bring on a relapse. Change of climate often accelerates recovery."

Recommendations by Rainsford (375)

"Since undulant fever is primarily a bacteriaemia, it is rational

to suppose that the brunt of the attack has to be taken by the reticulo-endothelial system and that the liver, and to a lesser extent the spleen, has to play the major part in the defense of the body. Whatever form of specific treatment is to be adopted, whether it be pharmacological or biological, it is obvious that it will fail unless care is taken to preserve the efficiency of the natural defense mechanism of the body and to see that, as far as possible, these tissues are maintained in such a condition that they can react to the treatment given. With these principles in view, and with the specific object of preserving the liver from the effects of the toxins of these infections, the following general method of treatment was evolved:

"To preserve, if possible, the body defenses, patients were kept at rest in bed on a liberal but light nourishing diet, with a high carbohydrate and low fat and protein content, sufficient to allow the patient about 1,800 calories *per diem*. Care was taken to see that the diet contained all the essentials for maintaining health, that is, vitamins, micro-chemical constituents, essential amino-acids, etc. For these reasons butter, fruit, and green vegetables were allowed freely with half pint of milk a day ('Cow and Gate' dried) and small quantity, 4 to 6 oz., of red meat, 2 eggs and Marmite. In Malta, especially in the summer, when most of these cases arise it is very difficult to get green vegetables; fresh milk and fresh butter can never be obtained. 'Cow and Gate' dried milk, naturally rich in vitamin D, was used and tinned New Zealand butter. It was thought advisable to supplement this diet with Ostelin and sometimes with Bemax, but even then it is doubtful whether the vitamin A content was sufficient, as the butter was found to have a low 'blue value.' The vitamin C content was maintained by giving lemonade to which 4 oz. of glucose were added per pint. Patients were encouraged to drink 2 or 3 pints of this lemonade per day. In this way the calorific value of the diet was enhanced and a lib-

eral supply of glucose for the liver was ensured. Alkalis in the form of large doses of potassium citrate and potassium bicarbonate were also given by the mouth four-hourly. Sometimes, when patients complained of the sweetness of the lemonade, potassium bicarbonate was added to it with success. This treatment with glucose and alkalis was continued until the urine was free from an excess of urobilinogen for seven consecutive days, after which the use of alkalis was discontinued. Sometimes, in very resistant cases, this alkali treatment had to be persevered with for a month, but more usually, fourteen to twenty-one days' treatment with it sufficed to free the urine of urobilinogen. Care has to be taken during the period the patient is under this intensive alkali treatment that the blood calcium level does not fall, as it is very inclined to do. This tendency was counteracted with injections of calcium gluconate, 10 cc., given intramuscularly or intravenously once or twice weekly while the patient was taking alkalis. It was found to be unwise to continue this intensive alkaline treatment in chronic or resistant cases for more than four weeks. In this type of case, liver extract and intravenous glucose with insulin were found to give better results. The glucose was administered as a 10 per cent solution in 250 to 500 cc. doses, and the insulin was given by subcutaneous injection of 20 units half an hour before the administration of the glucose. Sometimes the insulin was given in 10 unit doses and the glucose, 50 grams by mouth, once daily. It was noticed that the cases which were most resistant to this alkali and glucose treatment were those that had been kept on a low diet for some considerable time before admission. One case which had been treated as enteric fever for forty-eight days before he was admitted to hospital still had an excess of urobilinogen in his urine five weeks after being treated with glucose and alkalis; he was invalided home after six weeks' treatment only very slightly improved. Aspirin was also found to have a marked effect in increas-

ing the amount of urobilinogen in the urine, and for this reason salicylates and aspirin were never employed for the relief of rheumatic pains, etc.

"SYMPTOMATIC TREATMENT—High fever was treated by hydrotherapeutic methods. The use of diaphoretics, for reasons already stated, was considered inadvisable. In the highly nervous and irritable patient potassium bromide was combined with the alkaline mixture. When the insomnia was persistent 'Allonal' was found to be most beneficial and appeared to have no ill-effects. Morphia had to be exhibited in a few cases for the relief of splenic pain. Constipation was prevented by giving honey, brown bread, green vegetables and fruit, and by the use of mild saline aperients in the mornings. Stimulants in the form of alcohol were given as seldom as possible since alcohol inhibits the storage of glycogen in the liver. When stimulants were required more reliance was placed on such therapeutic agents as camphor, digitalis, strychnine or caffeine, also tea and coffee. Brandy, however, had to be given to some of the chronic cases and to alcoholic subjects."

Large daily doses of vitamins A, B, C, and D are indicated in all forms of brucellosis. Blood transfusions (250 cc.) are recommended when the temperature tends to rise to about 104° F. in the afternoon for three or four successive days, or when there is a severe grade of anemia accompanying the disease. Low basal metabolism should be corrected by the administration of thyroid extract.

V

BRUCELLOSIS IN ANIMALS

PART ONE. BRUCELLOSIS BOVIS

HISTORY

Synonyms. Slinking of the calf, inzootic abortion, contagious abortion, infectious abortion, Bang's disease.

Infective Organisms. Br. abortus, Br. suis, Br. melitensis.

EPIZOOTICS of abortion in domestic animals have long been known to occur. Toward the middle of the eighteenth and nineteenth centuries opinion began to crystallize on the fact that the disease was of a contagious nature. As early as 1864 epidemics of abortion in cattle were known to have occurred in the region of the Mississippi River and Louisiana. One may find a clear picture of the disease as it occurred in 1864 in the discussion by Robert Jennings on *Cattle and Their Diseases.* At that time he was of the opinion that it was due to some sympathetic influence. In other words, when a pregnant cow would see another animal abort, she would abort in a few days or few weeks. In his discussion for the control of the disease he laid down a plan which is in no way different from that in use or recommended today. In controlling the disease he recommended the isolation of the aborting animal. In this instance he was of the opinion that when an aborting animal was isolated, the other animals had no opportunity to see the act or the occurrence of the abortion and as a consequence did not abort in sympathy.

Franck, as early as 1876, appears to have established in an empirical way the contagious nature of the disease by placing in the vaginas of pregnant animals portions of fetal membranes discharged in an abortion. In this way abortion was produced. Work-

ers in various countries sought in vain to find the cause of the disease. It was not until 1895 that the etiological agent of the disease was established for the first time. In that year Bang and Stribolt (10) found present between the uterus and the fetal envelope of the fetus in the thick, yellowish exudate, a very small Gram-negative bacterium which they designated as the abortion bacillus. They were able to cultivate the organism on artificial media. Following its isolation they produced abortion in pregnant cows by instilling cultures of the organism into the vagina and again recovering the organism from the exudate between the uterus and fetal envelope. The organism present was again isolated and reinjected into pregnant cows, again producing abortion. They also were successful in producing abortion in pregnant sheep and isolating the organism which was injected. Confirmation of Bang's discovery followed by Preisz (367) in Hungary, by McFadyean and Stockman in England (281), and by McNeal and Kerr (285) in the United States.

Another very important gap was bridged in the study of the nature of the disease in 1911 by a discovery made simultaneously by Schroeder and Cotton (400), and Smith and Fabyan (415). A large series of guinea pigs were inoculated with milk from presumably healthy cows in the search for the tubercle bacilli. To the great surprise of these very observing students of animal diseases, it was found that the milk produced lesions in the spleen and liver of the guinea pig which resembled tuberculosis. From the lesions in the various organs, the organism which Bang and his associates isolated from aborting cattle in Denmark was isolated. At this time these workers called attention to the possibility of *Brucella* being infectious for man. In 1916 Cooledge (61) demonstrated the presence of specific agglutinins in milk and showed that they indicated the presence of infection in the udder. His findings were later confirmed by many other workers.

PATHOLOGY

In the examination of the gravid uterus, fetal membrane, and fetus for evidence of *Brucella* infection, one should note the presence in the utero-chorion cavity of a sticky brownish, odorless exudate resembling soft caramel candy. This material consists of a collection of cellular debris containing *Brucella* in pure culture. One should observe the villi of the chorion and maternal placenta for evidence of necrosis. Another characteristic change may be seen in the smooth free intercotyledonary part of the chorion. When invaded by *Brucella* it becomes opaque, thickened, and leather-like in appearance. Hagan (165) is of the opinion that this is the most important gross change seen in the chorion when invaded by *Br. abortus*. Smith (410) has pointed out that an examination of the epithelial layer of the chorion reveals one of the most important distinguishing characteristics of *Br. abortus* invasion of the fetus. The epithelial cells are found choked with the organism. The organism appears to be multiplying within the cells.

Hallman (166) interprets the changes which take place in the bovine uterus and fetal membranes as a result of *Br. abortus* invasion in the following words.

"*Bact. abortus* may produce either acute necrotic or subacute or chronic placentitis, obviously depending upon the number and virulence of the organism and the resistance of the tissues. Because of the arrangement and relation of the fetal and maternal placentae, it appears that the acute, subacute, or chronic type may cause retention of the membranes. In acute necrotic placentitis, of course, not all the placental tissue is necessarily necrotic. The adjacent diseased, though living, villi and walls of the crypts are swollen, partly because of congestion and exudate and partly because of leucocytic infiltration and cellular proliferation. Retention in such cases is obviously explainable on a purely physical basis. There may

be other factors, as suggested by Williams, but surely this is an important one. In subacute or chronic placentitis there is also increase in size of the placental structures due to cellular infiltration and proliferation producing the same mechanical effect as in the acute condition."

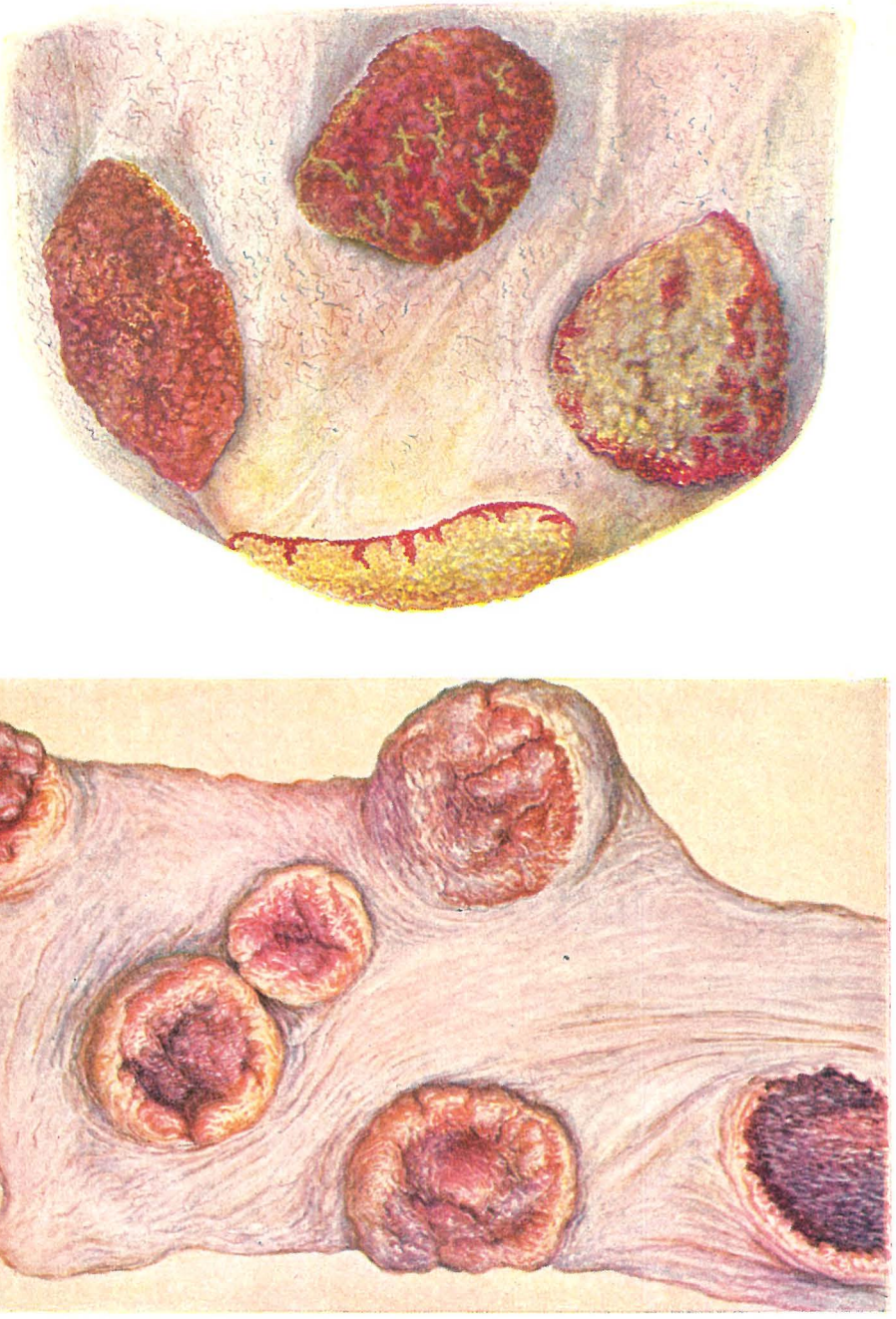
Pneumonia in the fetus due to *Brucella* has been studied at some length by Smith (411) and by Hallman and associates (167). The histopathological changes observed by the latter group of investigators are described as follows.

"Although bronchopneumonia is the prevailing type of fetal pneumonia, in some cases, it is not possible to determine the type because of the extension of the lesions. In some cases, congestion and fibrinous exudation are the prominent alterations. In others, cellular infiltration of the bronchioles, peribronchial tissues, alveoli, and alveolar walls predominates. The predominating cells are lymphocytes and endotheliocytes, although, in certain areas, many polymorphonuclears may be seen."

The invasion of the bovine udder by *Brucella* causes an acute to subacute and chronic inflammation in varying degrees (393). The lesions are located in the alveoli, in the interalveolar connective tissue, and along the lactiferous ducts. The histological changes appear to be of an acute or subacute type when the alveoli are involved, and of a chronic type when the interstitial tissue is involved. The changes appear to be progressive, that is, they involve the parenchyma first and the interstitial tissue later. The supramammary lymph glands, when involved, present a picture of chronic lymphadenitis.

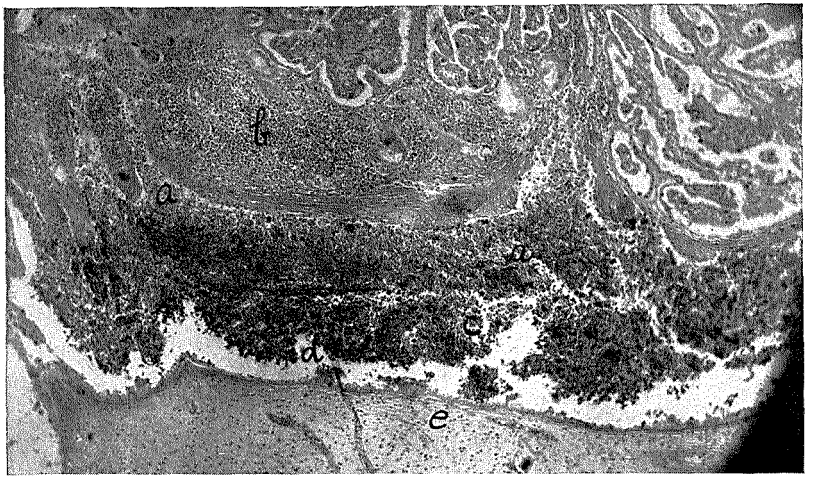
Ridala (383) has perhaps made a much more thorough and comprehensive study of the changes that take place in the udder of the cow as a result of *Brucella* infection than any other investigator. His findings are as follows.

"Changes due to *Br. abortus* may occur in all the quarters of the



After Poppe (363)

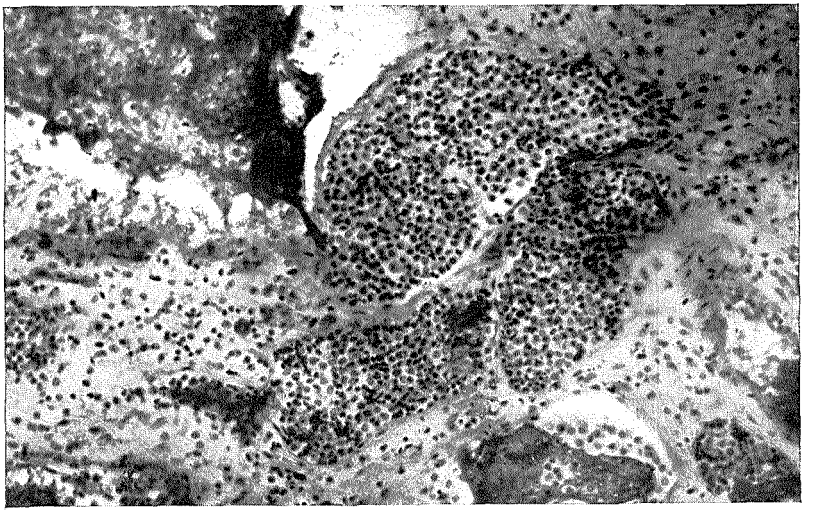
FIGURES 23 AND 24. CHARACTERISTIC LESIONS OF BRUCELLA INFECTION IN THE BOVINE CHORIONIC PLACENTAE



After Hallman (166)

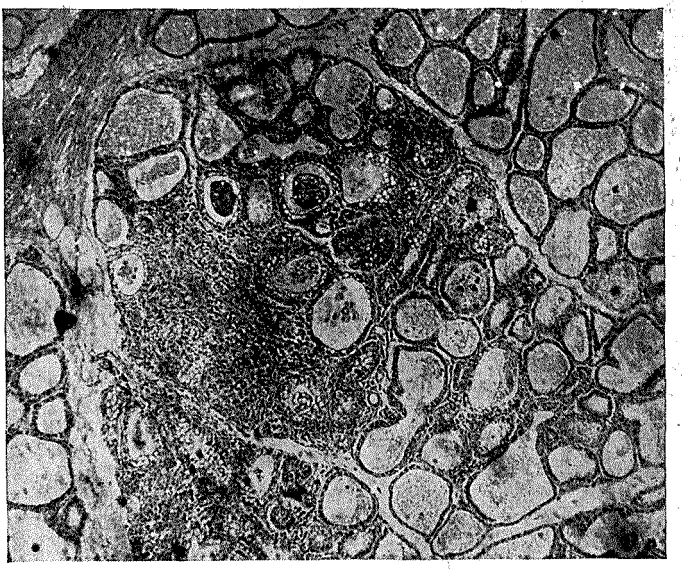
FIGURE 25. SECTION FROM PERIPHERAL PORTION OF BOVINE FETAL PLACENTA SHOWING CHANGES DUE TO BRUCELLA INFECTION. X 70

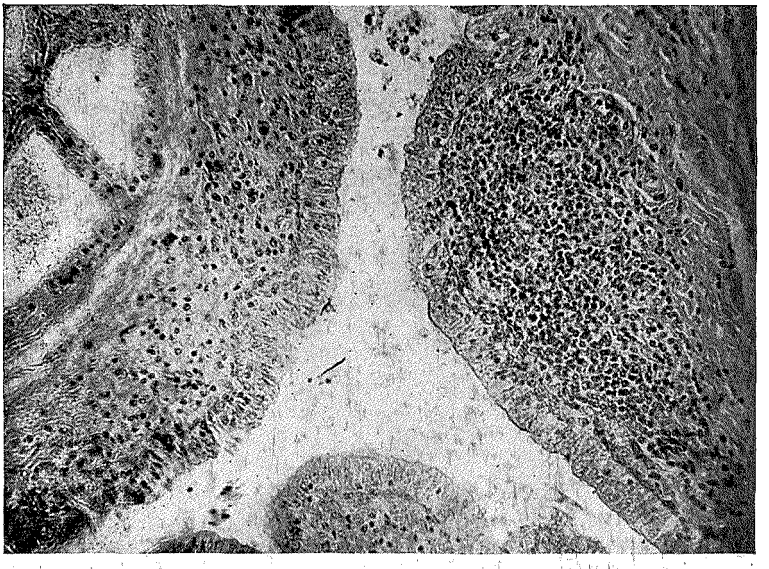
a. superficial necrosis of placenta; b. leucocytic infiltration and proliferation of the connective cells of the subnecrotic zone; c. exudate; d. degeneration and necrosis of chorionic epithelium; e. chorion



After Hallman and associates (167)

FIGURE 26. BOVINE FETAL PNEUMONIA DUE TO BRUCELLA. FIBRINOUS COAGULA AND ENDOTHELIOCYTES IN DISTENDED LYMPHATICS OF THE INTERLOBULAR SEPTA. X 200





After Runnells and Huddleson (393)

FIGURES 27 AND 28. SECTIONS OF THE UDDER SHOWING PATHOLOGICAL CHANGES DUE TO BRUCELLA

Figure 27. Areas of normal tissue at the periphery and a subacute inflammatory area at the center. x 60

Figure 28. A subepithelial focus of lymphocytic infiltration along a secondary lactiferous duct. x 250

udder in the lower and central parts, as well as in the milk cisterns regions.

"Changes caused by *Br. abortus* take place in the parenchyma of the udder, the lactiferous ducts, and the intersitial tissue.

"Changes are most frequent in the alveoli and the interalveolar connective tissue, the lactiferous ducts and the interlobular connective tissue showing fewer traces of change.

"The pathological changes due to *Br. abortus* are acute, subacute, and chronic, the latter predominating.

"In the parenchyma infected with *Br. abortus* there occur regressive changes (varying from fatty degeneration and necrosis to disintegration) and progressive ones (proliferation and dense cellular infiltration of the interalveolar connective tissue and of the epithelium of the alveoli).

"The acute changes in the parenchyma are accompanied by fatty degeneration or even complete disintegration of the epithelium of the alveoli, by desquamation, infiltration of polynuclear leucocytes, appearance of purulent exudate, particularly in the lumina of the alveoli, and occasional destruction and necrosis of groups of alveoli. Usually these changes are also accompanied by symptoms of chronic inflammation (infiltration of lymphocytes and plasma cells).

"The changes in the subacute inflammatory foci of the parenchyma of the udder resemble those in the acute foci, but the infiltration of polynuclear leucocytes is weaker and there are symptoms of chronic inflammation (proliferation of the epithelium of the alveoli, denser infiltration of lymphocytes and plasma cells, fibroblasts in small numbers, fibres of collagenic connective tissue, and an increase in the number of capillaries).

"The changes in the chronic inflammatory foci in the parenchyma of the udder resemble those in the subacute foci, but polynuclear leucocytes occur only singly, the proliferation of the fibrous

tissue is intense, in places very intense indeed, and there is little perivascular infiltration of plasma cells and lymphocytes.

"In the chronic, and partly also in the subacute inflammatory, foci of the udder the author found epithelioid cells due to *Br. abortus*—a fact never before described—including giant-cells of the Langhans type, and sometimes with necrosis, chiefly in the centre of the foci, but these foci of epithelioid cells differed from tuberculous foci in that there was no caseation.

"The changes in the lactiferous ducts are of varying intensity. They begin with a slight proliferation of the epithelium, intumescence and moderate interepithelial and subepithelial infiltration (of lymphocytes, plasma cells, and smaller numbers of polynuclear leucocytes), and end in very intense proliferation, flattening necrosis, and partial cornification of the epithelium of the lactiferous ducts—processes observed for the first time in connection with brucellosis infection and often causing obstruction of the lumen.

"In the very considerably changed epithelium of the lactiferous ducts, particularly in the subepithelial tissue, a certain amount of connective tissue is observed, the extent of which depends on the duration of the changes, and there is a dense, somewhat accumulated, cellular infiltration of lymphocytes, plasma cells, smaller quantities of fibroblasts, and polynuclear leucocytes.

"In the very markedly changed walls of the lactiferous ducts there are in places also foci of epithelioid cells with giant-cells of the Langhans type.

"Four cows had macroscopically observable cystiform formations proving to be dilated, and in many cases considerably altered lactiferous ducts, and dilated and confluent alveoli (retention cysts).

"The interlobular connective tissue proliferates in varying degrees, according to the extent to which changes have taken place in the parenchyma of the udder.

"In the interlobular connective tissue, in which proliferation has

taken place, focal and diffuse infiltration (of lymphocytes, plasma cells, and single mast cells, a moderate amount of fibroblasts, and comparatively few leucocytes), indicative of chronic inflammation, is observed in varying intensity.

"The original focus of the changes of the udder is apparently found in the parenchyma of the udder, the changes in the interlobular connective tissue and in the lactiferous ducts being of secondary nature.

"Varying amounts of amyloid corpuscles appear both in the changed and in the normal parts of the udder in the lumina of the alveoli.

"In the altered parts of the udder (the foci in the parenchyma and the altered lactiferous ducts, as well as in the lymph nodes), the author discovered *Br. abortus* in groups and sparse sprinklings between cells and in cells, by means of staining histological sections by the Giemsa method, as modified by the author, and by Loeffler's methylene blue and the carbol fuchsin staining methods."

Jepsen and Jorgensen (234) have noted the following pathologic-anatomical changes in orchitis in the bull due to *Br. abortus.*

"In one case the testis shows one large or several smaller areas of dry necrosis with only slight tendency to softening, but with marked tendency to encapsulation with production of large amounts of fibrous tissue. In this form the swelling of the enlarged testicle keeps unchanged for months; but gradually the retraction of the fibrous tissue around the necrosis makes the swelling subside—even to such a degree that a testicle with encapsulated necrosis may fall off in size so much that it comes below the normal size of the testis. The other form is characterized by a marked tendency to purulent liquefaction, so that the completely necrotic testicle lies like a sequestrum in a pus-filled cavity formed by the fibrous thickened tunica vaginalis, being suspended above merely

by the remnants of the spermatic cord. In these cases the swelling in the scrotum has a more or less distinct fluctuating character and it may sometimes go on to perforation with evacuation of the purulent content and fistula formation; in other cases the changes persist in the same state for months."

PERIOD OF INCUBATION

It is unfortunate that so little attention should have been given in the past to the importance of determining the size of the infecting dose of organisms in experiments on animals in which the objects in view were to compare virulence of strains and to determine infectivity by different routes of exposure. The Mediterranean Fever Commission as early as 1907 showed that the infective power of *Br. melitensis* is high. But in the case of *Br. abortus* there has always been a difference of opinion as to its invasive ability. This doubt existed in the face of the experimental data obtained by Hagan (163, 164), who showed that fewer than 100 *Br. abortus* organisms were required to infect the guinea pig and only nine the rat experimentally. The question of its invasiveness for cattle was partially answered by McEwen and associates (279) in an experiment designed to determine the minimum infective dose of *Br. abortus* for cattle. After breeding, five groups of ten heifers each were inoculated by way of the conjunctiva with different sized doses of bacteria. Group I received 1,460,000,000 bacteria; Group II, 14,600,000; Group III, 1,460,000; Group IV, 146,000; and Group V, 1,460. In Groups I and II, nine animals became infected; in Groups III, IV, and V, seven, five, and two animals, respectively, became infected. The results show that the minimum infecting dose of a given strain for the average susceptible cow is in the neighborhood of 1,000,000 bacteria. The results obtained by McEwen and associates (279) are summarized in Table XI. It is of interest to note that the time of the first appearance of a significant agglutina-

TABLE XI

Infective dose of Br. abortus *for pregnant heifers*

GROUP	ORGANISMS INOCULATED	ANIMALS SHOWING SEROLOGIC EVIDENCE OF INFECTION AFTER EXPOSURE*					ANIMALS SHOWING BACTERIOLOGIC INFECTION
I(a) 10 animals	$1{,}460 \times 10^6$	14th day: 7	28th day: 3				9
II(a) 10 animals	$1{,}460 \times 10^4$	28th day: 3	46th day: 4	65th day: 2			9
III(a) 10 animals	$1{,}460 \times 10^3$	80th day: 1	106th day: 3	123rd day: 1	227th day: 1		7
IV(a) 10 animals	$1{,}460 \times 10^2$	65th day: 1	80th day: 1	106th day: 1	123rd day: 1	156th day: 1	5
V(a) 10 animals	1,460	205th day: 1					2
I(b) 8 animals	15^6	13th day: 5	27th day: 1	53rd day: 1	82nd day: 1		8
II(b) 6 animals	5^6	35th day: 5	One failed to react				5

* Agglutination titer of 1:40 or higher. (a) McEwen and Priestley (279). (b) Hutchings (227).

tion titer after exposure to infection is directly proportional to the size of the dose of bacteria. Exposure to a large dose of bacteria elicits a significant titer in fourteen to twenty-eight days. When the exposing dose is less than 1,000,000 bacteria, significant titers do not appear until eighty to one hundred six days.

There are also set forth in Table XI the results obtained by Hutchings (227) from exposing each of two groups of pregnant heifers to 5^6 and 15^6 organisms respectively. It may be noted that a much smaller number of organisms induced evidence of infection in a shorter period of time than in McEwen's experiments.

The period of gestation at which abortion takes place varies from two months to nine months.

ROUTES OF INFECTION AND CHANNELS OF ELIMINATION

Bang and Stribolt (10) in their first studies of the nature of the abortion disease of cattle demonstrated that infection could be induced by instilling pieces of infected placentae or pure cultures into the vagina of pregnant animals. This fact was confirmed by many others, and for many years was considered the chief route through which breeding cattle became infected. This idea was no longer held tenable after Birch and Gilman (24) demonstrated that pregnant heifers could be infected readily by the ingestion of feed sprinkled with a suspension of living *Br. abortus*. Then Thomsen (435) and later Schroeder (399) showed that cattle could be infected experimentally by way of the conjunctiva. Another important route through which the organism could gain entrance to the body was disclosed when Bang and Bendixen (12) succeeded in showing that living organisms would pass through the unbroken skin of cattle.

Br. abortus, as a rule, gains entrance to a herd of cattle free from the disease when an infected animal is added to the herd. It is generally believed that the only time in which an infected animal is a

source of danger to susceptible ones is during and following the act of aborting. However, a considerable amount of experimental data exists today which show that infected animals are a source of infection at all times. The introduction of infection into a herd free from the disease at a time when a large number of animals are pregnant results in a serious epidemic of abortions. The majority of animals abort only once, and during subsequent years may conceive and produce normal calves even though they carry the organism in the various tissues of the body. They may remain infected throughout their lives.

TABLE XII

Susceptibility of pregnant and non-pregnant heifers to Br. abortus *infection*

GROUP	TOTAL ANIMALS	STATE OF PREGNANCY	ROUTE OF EXPOSURE	ABORTING	INFECTED
I	12	+	Vagina	11	11
II	4	—	Vagina	None	None
III*	3	—	Eye, vagina	None	None
IV	4	—	Eye, vagina	None pregnant	None
V*	4	—	Vagina or mouth	None	None
VI*†	6	—	Vagina or eye	None	None

After Edgington and Donham (96)

* Reexposed during pregnancy. † Cows.

Calves up to the time of breeding age are relatively insusceptible to the disease; however, there are instances in which the organism has been recovered from the tissues and discharges or excretions of experimentally infected calves (52).

Non-pregnant heifers and cows rarely become infected after exposure to *Br. abortus,* and when they do become infected and are bred they seldom, if ever, abort. Conclusive proof that non-pregnant animals possess a high degree of resistance to infection was obtained by Edgington and Donham (96). In a series of experiments, summarized in Table XII, 12 pregnant heifers (Group I) and 15 non-pregnant heifers (Groups II–V) were exposed to large

doses of the organism. Seven of the heifers exposed before breeding (Groups III and V) were reexposed during different stages of gestation. Of the 12 exposed during pregnancy, eleven became infected and aborted. The heifers exposed before breeding not only failed to abort during pregnancy, but apparently failed to become infected. The investigators obtained similar results on six cows (Group VI) which were exposed before and after breeding.

When a pregnant animal becomes infected, that is, after the organism passes the epithelial barrier of the skin or mucous membrane, the organism passes to the pregnant uterus, the udder, and lymph glands by way of the blood stream.

Although many have apparently succeeded in infecting bulls by way of the prepuce, no one has observed that the infection of cows has been caused by infected bulls at service. King (249) used 3 bulls, 2 of which were excreting *Br. abortus* in the semen, in an attempt to infect 12 cattle by mating. Eleven were impregnated, but none became infected. The collected data are conclusive in showing that cattle may become infected by way of the mouth, the eye, and the skin.

The two important channels through which *Brucella* is eliminated from the infected cow are the uterus at the time and shortly after abortion and the infected udder. The organism may be eliminated in the milk from the infected udder during the life of the animal.

Brucella has been found present in the urine and feces of infected animals, but it is not yet known whether these two excretions are important channels of elimination.

Kruger (258), examining the tissues from all parts of the body of a large number of infected animals, succeeded in isolating *Brucella* from muscular parts of the diaphragm (kidney peg). Tissues taken from other parts of the animal, such as lymph glands and muscles, were negative.

Doyle (85) has made an extensive study of the location of *Br. abortus* in the tissues of thirty-two naturally infected cows. In addition to the tissues referred to above, *Br. abortus* was isolated from the iliac gland in 52 per cent and from the pharyngeal lymphatic gland in 28 per cent of the cows examined. His results are summarized in Table XIII.

TABLE XIII

Tissues of infected cattle from which Br. abortus *has been isolated*

TISSUES EXAMINED	TOTAL EXAMINED	POSITIVE	
		Number	*Per cent*
Spleen	32	3	10.6
Liver	32	1	3.0
Supramammary glands	25	16	64.0
Pharyngeal glands	26	7	28.0
Submaxillary glands	26	3	12.0
Prescapular glands	7	1	14.0
Popliteal and precrural glands	19	2	10.0
Mediastinal glands	24	2	8.0
Hepatic glands	19	1	5.0
Iliac glands	23	13	52.0
Knee hygroma fluid	3	2	66.0
Bile	32	1	3.0
Left forequarter	23	7	28.0
Right forequarter	23	12	48.0
Left hindquarter	23	11	44.0
Right hindquarter	23	15	60.0
Pooled milk	8	4	50.0

After Doyle (85)

Bang, Bendixen, and Orskov (13) studied the distribution of *Br. abortus* in the bodies of a group of heifers that were fed a suspension of virulent organisms. One animal was killed on the sixth, one on the eleventh, the sixteenth, the thirtieth, the forty-eighth, the fifty-second, and the ninetieth day after exposure and the tissues cultured to determine the location of the organism. They found that early in infection the organism is located in the lymph glands

around the head and intestines; on the thirtieth day it is well distributed throughout the body; on the ninetieth day it is found only in the udder and mammary lymph glands.

Birch and Gilman (26) examined at autopsy the tissues of several cows that had been infected artificially. They recovered *Br. abortus* from the supramammary glands in 7 of 17 animals, from the genital mucosa of 4, from the oviduct of one, from the lymph nodes about the head in 2, and from the mediastinal and bronchial nodes in one.

CLINICAL MANIFESTATIONS AND NATURE OF THE DISEASE

The primary symptom of the disease in cattle is the premature expulsion of the fetus. Opinions differ considerably as to the number of cattle that abort following infection. There are considerable data which show that about 70 per cent of pregnant cattle abort following initial infection.

When *Br. abortus* invades the gravid uterus, fetal membrane, and fetus of a cow, inflammatory changes of an acute, subacute, or chronic nature take place in the tissues which may play an important role in causing the premature expulsion of the fetus. Although the anatomical changes interfere with the proper nourishment of the fetus, they are not sufficient to cause death *in utero* in fetuses expelled at the sixth month of gestation or thereafter.

The author and his associates, in a study of the toxic effects of the endoantigen of *Brucella* cells, have shown that when it is injected into pregnant cows or guinea pigs premature expulsion of the fetus will follow in from twelve hours to three days. The endoantigen when injected into animals in a sufficient amount will cause a contraction of the smooth muscle fibers. There is a possibility that the toxic endoantigen may be produced in the infected gravid uterus and cause the contraction of smooth muscle fibers, and in turn cause the premature expulsion of the fetus.

One common sequel to abortion is retention of the fetal mem-

brane. The retained membrane later becomes invaded with other bacteria, often causing serious acute and chronic inflammation of the uterus and adjacent reproductive organs.

Many animals which are infected, although they do not abort, become sterile. That is, they may fail to conceive at oestrum or may show no sign of oestrum.

After the disease has once gained entrance to a herd there is a tendency in subsequent years for the number of abortions to decline.

Boyd, Delez, and Fitch (34), Van der Hoeden (454), Magnusson (288), and others have observed the occurrence of bursitis in *Brucella* infected cattle. The knee joint is the one most frequently affected. *Br. abortus* has been isolated from fluid removed from the area involved.

Humphreys and Moore (225) examined the serous fluid from serous swellings (hygromata) of 42 cattle. *Br. abortus* was recovered in culture from 27 of the swellings. Thirty-six of the animals were positive and 6 were negative to the *Brucella* agglutination test.

Van der Schaff and Roza (456) have encountered an interesting disease in Zebu cattle in Java due to *Br. abortus*. The infected cattle show the symptom of abortion in the female and orchitis in the male. In addition, a peculiar enlargement of the joints, particularly the knee, is a common occurrence. It is termed "thick knee disease" in Java. They were able to recover *Br. abortus* from the affected joints and also live specimens of *Onchocerca bovis*. The cultures of *Br. abortus* isolated agreed with the commonly recognized ones in CO_2 requirements and H_2S production. They acted like *Br. melitensis* on dye differential media.

Calves born of infected dams are negative to the agglutination test before the ingestion of colostrum, but directly after or within four hours after the ingestion of colostrum agglutinins may be demonstrated in the blood serum. Little and Orcutt (271) were

the first to show that agglutinins which appear in the blood of newborn calves come from the ingestion of colostrum containing agglutinins and not as a result of infection. The agglutinins do not persist in the blood of the calf longer than twelve weeks on the average.

Fitch and associates (124) made a seven-year study of 56 female calves from infected dams to determine the possibility of infection manifesting itself at some period of pregnancy. After maturing they were observed through from one to six pregnancies. All animals remained free from infection during the period of observation.

The organism may be demonstrated by cultural methods in the milk from the udder directly after aborting. Milk from infected udders will also show the presence of specific agglutinins. Fitch and associates (123) have made a systematic study of the occurrence of *Br. abortus* in the blood stream of 7 heifers, nine to ten months of age. They were able to recover the organism more often shortly after exposure and at the time of the first appearance of agglutinins in the blood. *Br. abortus* was isolated from the blood of 3 up to the time of parturition and from 3 others before as well as after parturition. This study leaves no doubt about the presence of *Br. abortus* in the blood stream of infected cattle for long periods of time.

DIAGNOSIS

The expulsion of a premature calf or abortion is suggestive of *Brucella* infection. However, one must keep in mind that a large number of abortions are not due to *Brucella,* but to other infectious microorganisms and to protozoa. The physical appearance of the exudate in the fourth stomach of the fetus often suggests *Brucella* infection. The exudate in infection nearly always has a cloudy appearance and is of a lemon-yellow color. Microscopic examination will show the presence of many clumps of a small rod-like organism. They may also be found in the chorionic epithelial cells

(165, 410). If the fetal membranes are available for examination one usually finds a considerable leather-like thickening between the placental areas (Figures 23 and 24). The villi of the placental areas usually have a necrotic or eroded appearance; however, these changes are not always characteristic of *Brucella,* but may occur following the invasion of the fetal membranes by many other microorganisms. The organism may be demonstrated in the exudate from the stomach of the fetus or the fetal organs by cultural methods (see Chapter II). The application of the agglutination test is a satisfactory means of demonstrating that the abortion is due to *Brucella* (see Chapter VI, Part One).

Much effort and time have been devoted to the diagnosis of the disease by means of allergic tests used in the skin, subcutaneously and intravenously. McFadyean and Stockman (281) were the first to attempt to diagnose the disease by means of an allergic test similar to the tuberculin test. The chief difficulty in the use of any allergic test in the diagnosis of the disease in cattle is that those animals which have recovered from infection will give a reaction similar in nature to that in animals actively infected. The results of such a test as observed by various workers are so contradictory that today no satisfactory agent or test of this nature has been developed (see Chapter VI, page 239).

TREATMENT

The increased interest in Bang's disease and the better understanding of the problem on the part of the farmers and herd owners have been responsible in a large degree for the decreased use of abortion remedies. Some states have passed laws prohibiting the use of abortion cures. Livestock sanitary officials in some of the states have waged a relentless war against these fake remedies.

More recently the sulfa compounds have been studied to determine their possible therapeutic value in both small experimental

animals and cattle. The results obtained by various investigators do not by any means agree.

Chinn (59) and Menefee and Poston (298) claim that they were able to obtain sterilization of the tissues of guinea pigs infected with *Br. melitensis* by the oral administration of sulfanilamide. Morales-Otero and associate (321) obtained no significant results in mice infected with *Br. melitensis* by using either sulfanilamide or sulfamethylthiazol. Hamann and Huddleson (169) failed to note any appreciable influence on *Br. abortus* infection in the guinea pig after the oral administration of sulfapyridine. Kolmer and associate (253) studied the effect of sulfanilamide, neoprontosil, sulfapyridine, and aldanil in mice inoculated with a heavy suspension of the three species of *Brucella.* Only in those inoculated with *Br. abortus* was death prevented. No attempt was made to measure the sterilizing effect of the drugs. Wilson and Maier (474) found that sulfanilamide would not sterilize the tissues of guinea pigs until a total of 400 mg. was administered over a period of three weeks.

The therapeutic effect of sulfanilamide has been studied in *Br. abortus* infected cattle by Hamann and Huddleson (168) and by Miller and associates (310). The results failed to indicate that the drug had any influence on the disease.

The relation of nutrition and vitamin deficiencies in animals to resistance against and recovery from brucellosis has received considerable study. Hart, Hadley, and Humphrey (179) conducted a well-controlled experiment to determine the influence of nutrition on the susceptibility of cattle to brucellosis. It was found that a high plane of nutrition, involving the feeding of alfalfa hay, minerals, cod liver oil, and iodized salt had no effect whatsoever in increasing the resistance of cattle to infection with *Br. abortus* above that of a control group of animals fed a ration of low protein and mineral content.

Hoagland and Buck (195) fed groups of albino rats rations defi-

cient in vitamins A and B and in calcium and phosphorus, and compared their resistance to experimental *Br. abortus* infection with that of a control group fed non-deficient rations. No significant difference in resistance between the two groups was noted. Gwatkin and MacLeod (160) injected infected cattle and guinea pigs with wheat germ oil, but no change in the course of the disease was noted. Hart and Guilbert (180) have found the livers of aborted fetuses to be deficient or lacking in vitamin A, but no data were obtained which would indicate that the deficiency in the fetal liver also occurred in the infected cow.

Thus, it may be said that there are yet no data available to substantiate the prevailing belief that rations deficient in proteins, minerals, or vitamins play an important part in the prevention or treatment of bovine brucellosis.

PART TWO. BRUCELLOSIS SUIS

HISTORY

Infective Organism. Br. suis.

Hutyra as early as 1909 isolated a species of *Brucella* (later identified as *Br. suis* by the author) from fetuses of aborting sows in Hungary. Hutyra (228) failed to report this finding until many years after Traum (447) had published his findings of a similar discovery in 1914. Traum isolated and identified what he believed to be *Br. abortus* from aborted swine fetuses collected by Dr. E. C. Schuble in northern Indiana. The organism which was isolated grew well on culture media under aerobic conditions.

In 1916 Good and Smith (152) made an extensive study of the cause of abortions in twenty sows in Kentucky. A species of *Brucella* (later identified as *Br. suis*) was isolated from the aborted fetuses under aerobic conditions which according to the methods then available was identified as *Br. abortus*. They satisfied Koch's postulates with the cultures that were isolated, thus proving beyond any doubt that *Brucella* is infectious for swine. The findings of Good and Smith have been confirmed by Hayes and Traum (185), Doyle and Spray (83), Connaway, Durant, and Newman (60), Graham, Boughton, and Tunnicliff (154), and many others.

For many years the species infecting swine was classified as *Br. abortus,* although Schroeder (399), Cotton (63), Smith (412), and many others had noted repeatedly that cultures from swine produced, upon inoculation into guinea pigs, gross changes in the organs different in character and size from those produced by cultures isolated from bovine tissues.

It was not until 1928 (203) that a satisfactory laboratory method was evolved that would differentiate cultures isolated from swine (*Br. suis*) from those isolated from cattle (*Br. abortus*).

Brucella infection in swine has been reported by Marcis (293)

in Hungary, by Reboulleau, Placidi, and Verge (377) in France, by Nagel (327) in Switzerland, and by Thomsen (439) in Denmark. No one has reported the isolation of *Br. abortus* from naturally infected swine.

PATHOLOGY

The first observations pertaining to vertebral lesions in swine due to *Br. suis* were reported by Giltner (148). Feldman and Olson (115) in a more extensive study of *Brucella* vertebral lesions in swine found a specific spondylitis associated with the disease. The lesion was an encapsulated abscess-like structure occupying an irregular cavitation in the body of the vertebrae. The chief location of the lesions is in the intervertebral discs in the lumbar and sacral region in the form of irregularly shaped cavitations and they extend to the spinal dura mater. The cavitations are filled with a thick, creamy, cellular material without odor. *Br. suis* may easily be isolated from the thick exudate. The size of the lesions varies from 0.5 to three centimeters in diameter.

Thomsen (439) has observed that the most conspicuous changes in *Brucella* infected sows occur in both the pregnant and the nonpregnant uterus. The stratium propium of the uterine mucosa shows numerous whitish-yellow nodules, ranging in size from tiny points to hempseed. On section the nodules are firm in consistency and contain a central purulent or caseous focus. Thomsen has designated the condition as "miliary brucellosis of the uterus of the sow." In addition to the uterine change, Thomsen has noted that the Danish strain of *Br. suis* produces large abscesses in various organs of the body of the hog such as the spleen, subcutis, thorax, and tendon sheaths. In some instances abscesses up to the size of a child's head were seen. In the infected boar he has observed necrotic inflammatory processes involving the epididymitis, testis, and seminal vesicle.

Creech (70) describes one case of swine brucellosis in which

the lesions involved the kidney, liver, and hepatic lymph gland. The gross changes consisted of encapsulated nodules, cross-sections of which were of a slightly yellowish or cream color. The histological examination of the affected tissues showed "the lesions to consist of amorphous central masses, varying in size, with a rather distinct line of demarcation between the necrotic centers and the adjacent tissue structure. Immediately surrounding the degenerated and necrotic areas, just beneath the capsule, were zones of cellular infiltration, consisting for the most part of small, round, or connective tissue cells and leucocytes, many of the latter being eosinophile cells. The hepatic gland also showed masses of eosinophiles and small areas of necrosis. A number of small non-encapsulated centers also were noted in the kidney. Some of these areas contained masses of blood or hemorrhages. In sections from the hepatic lymph gland, occasional cell fusions, suggestive of giant-cell formation, were noted. In the larger nodules the necrotic centers showed early stages of calcification."

ROUTES OF INFECTION AND CHANNELS OF ELIMINATION

The route through which hogs become infected naturally is a matter of speculation. It is generally assumed that the oral route is the most probable one because of their habits of living close to the soil.

The demonstration of *Br. suis* in the semen of infected boars by Thomsen (439) strongly suggests that the vagina may be an important route of infection.

Br. suis is eliminated from the infected hog with the aborted fetuses, vaginal discharge, urine, semen (439), and milk (60).

CLINICAL MANIFESTATIONS

Although there is definite proof that *Br. suis* is responsible for serious outbreaks of abortion in hogs in certain sections of the

United States, it has also been observed that the disease may exist in a herd of hogs for a considerable length of time without any clinical manifestation of the presence of the disease. In a study of the natural course of the infection in several large groups of hogs in Michigan tested during the past several years, the symptom of abortion was not observed.

Data have been obtained which indicate that the disease is self-limiting in certain herds of hogs. In one study conducted at Michigan State College on a naturally infected herd of swine, over a period of six months, the majority of animals recovered from the disease in sixty to ninety days as determined by the agglutination test.

DIAGNOSIS

The presence of *Br. suis* infection may be determined by the isolation of the organism from infected tissues (see Chapter II, page 27) or by means of the serum agglutination test (see Chapter VI, Part One, page 214). One must take into consideration reactions in a low titer as well as those occurring in a high titer in order to control the disease by means of the agglutination test.

PART THREE. BRUCELLOSIS CAPRINUS AND OVINUS

HISTORY

Infective Organisms. Br. melitensis, Br. abortus.

Since Zammit (481) demonstrated natural *Br. melitensis* infection in the milch goat, it has remained the important infecting species for this animal, and for milch sheep as well, in all parts of the world. The failure to demonstrate natural infection in goats or sheep with *Br. abortus* or *suis* to any extent does not imply that they are resistant to infection with these species of *Brucella.* It has been demonstrated that experimental infection can be readily induced in the milch goat with anaerobic strains of *Br. abortus.*

Eyre has voiced the opinion that *Br. melitensis* infection in the milch goat had its origin in the Persian hills. Although Nicolle has expressed the belief that caprine brucellosis has followed the emigrations of the English race and the exportation of Maltese goats, there is evidence which indicates that brucellosis in man and in goats was introduced into Spain and adjoining countries in the early fifteenth century or before the English had entered the Mediterranean littoral to any great extent. The disease was then brought from Spain to the Americas by the early Spanish invaders. The first evidence of its presence in the United States was established by Gentry and Ferenbaugh (142) in 1911. Although they found it present in Texas in that year, there is circumstantial evidence which indicates that it had been present in that state and adjoining states for many years previously.

NATURE OF THE DISEASE AND DIAGNOSIS

The Commission on Mediterranean Fever failed to observe any objective sign or symptom of the disease in infected goats during the period of their study. Dubois (88) was the first to report the occurrence of abortions as a frequent symptom of the disease.

Later Holt and Reynolds (196) in a study of the disease in Arizona and New Mexico, and Taylor and Hazemann (428) in a similar study in southern France, noted that abortion was a frequent symptom. Dubois (88) has also observed lameness and a mild mastitis in *Br. melitensis* infected goats.

The Commission on Mediterranean Fever found that goats could be infected experimentally by almost any route. The results of their study of the channels of elimination led them to conclude that the organism was eliminated with the urine. In recent years in a more careful study Taylor and Hazemann (428) and Polding (359) have not been able to confirm their conclusion. The data obtained by these investigators show that the vaginal discharge at the time of aborting, and for a short time thereafter, plays an important role in the dissemination of the organism.

During the past three years Polding (358, 359) has made an intensive study of *Br. melitensis* infection in goats in Malta under experimental conditions. He has been kind enough to summarize his findings.

Evidence of Invasion by Br. melitensis *in Mature Goats*

"The mature pregnant goat, artificially infected by subcutaneous infection of a smooth strain of *Br. melitensis,* offers the first measurable response to invasion, by the change of the blood serum agglutination reaction. The change is preceded by a lag period, during which there is no response and which on the average lasts about seventy-two hours. Thus a low serum titer may be expected on the third day after invasion. From this point serum changes are very rapid, the titer rising within the next forty-eight hours to a dilution in the neighborhood of 1:1,280. Thereafter the rate of change slows, and peak titers of upwards of 1:2,560 are reached by the eighth to the twelfth day. In our hands, 16 goats infected in this manner showed little individual variation.

"Turning to pregnant goats, exposed to infection by contact, evidence of invasion in about 95 per cent of animals is again offered by the serum response. A small error occurs, as the blood of a few animals exposed to infection by contact fails to agglutinate, but may prove to be slightly infected by subsequent isolation of the organism in small numbers on rare occasions. Thus, in our experience, out of 32 contact-exposed goats, two animals failed to offer a serum reaction when tested weekly, yet *Br. melitensis* was on one occasion isolated from their milk. The first serum change among contact-exposed goats may be seen within the first fortnight of exposure. On the other hand this change may be delayed for as long as one hundred days. The majority of first responses occur toward the end of the first month of exposure. The serum response in these animals, unlike that of the artificially infected goat, takes the form of a slow, irregularly rising serum titer with preliminary subsidiary peaks. In most cases final peaks are at about 1:2,560 dilution. The final major peak usually coincides with the termination of pregnancy.

"When the exposed animals are not pregnant, response takes the form of a low (1:20 to 1:80) rise in serum titer. These responses are usually very transient, a serum being 1:20 one week and negative thereafter. Occasionally, however, cases may occur where responses are moderately high (1:160) and the subsequent decline lasts for some months. It is thought, however, that animals showing such response may have been in the very early stages of pregnancy and have aborted unseen.

"The long periods that elapse between exposure and first response, naturally, do not represent the true period between invasion and serum response. Evidently, invasion may occur at any period after exposure, as donor animals remain infective for some months. On the contrary, consideration of the response of artifi-

cially infected animals, and of the quick response of some of the contact-infected animals, which in some cases may take place in as short a time as nine days, leads to the conclusion that first evidence of invasion may be expected within the fortnight following first exposure.

Period of Incubation in Mature Goats

"Reverting to artificially infected pregnant adults, it has been said that the serum response peaks at the eighth to twelfth day. At a point just before this peak, a bacteremia commences. It will persist for upwards of a month. We have observed that when animals of this description have been placed in contact with disease-free goats, transmission of the disease has occurred within nine days. Allowing three days for serum response to develop in the receptors, one must assume that artificially infected donors become infected within six days and, it must be noted, prior to first signs of abortion. On the other hand, in pregnant or recently kidded animals, whose serum responses are slight or irregular, it may be necessary to culture all possible tissues, and in the case of milk and vaginal secretions prepare daily cultures before isolation can be effected. Even under these circumstances failure to isolate in a proportion of cases must be expected.

Clinical Symptoms in Mature Goats

"In *Brucella* infections the most compelling clinical symptom is abortion. Its economic importance is not refuted, but it is our opinion that it has received far too much attention from the epidemiological point of view. The incidence of abortion, in naturally infected animals at any rate, seems largely fortuitous, and it is felt that no true insight into the nature of the disease can be gained, where abortions alone are counted, and bacterial invasion is neg-

lected. Some authorities have denied that abortion follows *Br. melitensis* infection in the Maltese goat. In experiments at this station, of 15 pregnant artificially infected goats, all aborted; of 22 pregnant females exposed to invasion by contact, nine aborted. Four of these animals were but slightly infected, having negligible serum reaction and eliminating organisms in small numbers, for a shorter period, than many animals that kidded at full term. On the other hand, 6 acutely infected goats, with high serum reaction and frequent heavy elimination of *Br. melitensis,* kidded normally. It is probable, indeed, that some factor exists that controls the occurrence of abortion, but until it is completely understood it seems folly to rely on abortion as an index of the extent of infection.

"Clinical symptoms other than abortion exist in all acutely infected goats. Pyrexia occurs within forty-eight hours of generalized infection. Animals rapidly lose condition, they stand with a dejected expression and drooping head, and their coat becomes rough. Pregnant goats become uneasy, and from their constant biting at a region high up in the left flank appear to be suffering pain at this point. Slight diarrhea may be seen. In lactating goats, the milk loses its normal characteristics, becoming a clear serum with clots suspended in it. While the physical appearance of the milk remains unchanged, cultures of the serum yield a confluent growth of *Br. melitensis* in pure culture. A retention of the placenta has not been observed but a copious discharge from the vagina is common for two to three weeks after kidding.

"The above serious symptoms are most noticeable when infection and kidding occur early in the year during the cold season of January, February, and March. Animals kidding and infected as late as June are less gravely ill and recover more rapidly. For these reasons, the peak individual rate of elimination of *Br. melitensis* may be placed early in February, and since this is the time of the

year when the largest majority of goats kid, the greatest elimination will occur at this time. At first sight, therefore, it seems odd that the peak of human infection falls during the months of July and August. Explanation of this discrepancy may be sought among the following points: Goats kidding in February would generally not be used for commercial purposes for about two weeks, owing to the presence of colostrum and the suckling of the kid. The time that man will be subjected to the greatest exposure will therefore be some time around the beginning of March. A period of three weeks' incubation at the minimum is to be expected in man. Among the Maltese where there is a fair degree of racial immunity this may be much longer. Earliest symptoms could, therefore, be expected at the end of March or the beginning of April and in actual fact these are probably first noticed at the commencement of the hot weather at the end of May or the beginning of June. Finally public health records, from which the peak period in man is assessed, refer to the notification of the disease to the authorities which occurs when patients enter hospital or visit a doctor. The Maltese, however, will often neglect to take these steps until he becomes seriously ill and, since the onset of the disease is probably insidious in the racially immune, attention is not called to the condition until July or August.

Persistence of Infection in Mature Goats

"Although the blood serum response may be taken as an indication of invasion by *Brucella* organisms, it is a matter of great difficulty to assess the exact point when infection may be said to have ended. Infection, it is true, ceases to be of interest to the epidemiologist when the organism finally ceases to be eliminated from the body, but it is impossible to say where such cessation has a sufficiently definite relationship to the fall or disappearance of serum agglutinins, for one to be able to dogmatize about the end of in-

fection using this reaction as an index. Indeed, once goats have suffered from an acute generalized infection our records have shown that they seldom regain an absolutely negative blood serum reaction during the remainder of their useful life. The peak serum reaction of an acutely infected animal persists, with minor fluctuations and a slow overall decline, for at least the period of lactation following infection. For example, goats infected in February of 1938, and responding with a serum peak titer in the order of 1:2,560, showed an average serum reaction of 1:640 in August of the same year. Many of them, however, retained a titer of 1:2,560 in this month. Goats infected in the early months of 1937, having completed a further pregnancy in 1938, still retained titers in the order of 1:160 in the summer of 1938. Animals infected during 1929, 1930, and 1931 offered titers of 1:80 or thereabouts as late as 1935–36. Ten such animals were slaughtered and postmortem tissues cultured. *Br. melitensis* was isolated from the supramammary gland of one animal. Animals less severely infected, on the whole, show the same proportionally slow decline over similar periods, but since their original peak titers would be in the region of 1:160 at the end of eighteen months, their serum would react in the rather neglected orders of 1:20 to 1:80.

"A small number of moderate reactors, however, and almost all of the non-pregnant reactors, return to a negative serum reaction after the lapse of two or three months, and one is encouraged to seek proof that these are examples of true recovery. It seems quite reasonable to suspect, therefore, that a persistent serum reaction following generalized infection represents the presence of a latent focus of infection, although this may be difficult to demonstrate.

"In an attempt to throw further light on this problem, efforts have been made to assess the period during which *Br. melitensis* is eliminated in the secretions of goats that have become systemi-

cally infected while pregnant. It must be remembered, that even in the most acutely affected goat, when the height of the infection is passed (usually within a month), elimination becomes sporadic. For this reason single negative cultures are worthless, and even daily cultures of the milk and vaginal secretions over long periods must only be regarded as moderately informative. A group of 12 goats had their milk plated every third day during the first, third, and fifth months of the lactation following the pregnancy during which they were infected. All but one returned positive plates on the majority of days of the first month. There was some falling off in the rate of elimination toward the end of the month, but the number of colonies derived from approximately 0.2 cc. of milk was generally more than 100. The milk had degenerated in character in all but three goats. During the third month, but five goats returned colonies, two on one occasion, one on two occasions, one on three occasions, and one on five occasions. Colonies as a rule numbered less than ten. During the fifth month, three of the five goats eliminating during the third month returned less than ten colonies on two or three occasions. From these returns it will be seen that elimination could only be regarded as constant during the first month or six weeks of lactation. Thereafter most goats appear to cease to produce the organism, whilst the few that continue to do so offer few colonies on rare occasions.

"A second group of ten goats were similarly examined during the lactation following the second pregnancy after infection. Of these, one goat remained a fairly constant eliminator of *Br. melitensis* up to the fifth month of lactation; two others returned less than ten colonies on one occasion each during the first week of this lactation, but on no occasion thereafter. The organism was not recovered from the remaining seven goats. During the first lactation of infection, all these ten goats had been constant eliminators.

"The vaginal secretions of these two groups were cultured at the same time as the milk. While the results were similar, elimination in these secretions ceases earlier, no positive cultures being recorded during the fifth month after termination of pregnancy. The genital secretions and placenta of goats terminating their second pregnancy of infection do not appear generally to return the organism in culture, although cases of such have been seen.

"In conclusion, therefore, bearing in mind the bacteremia already mentioned, it may be said that acute generalized *melitensis* infection of the mature Maltese goat commences to localize during the second month after the termination of the pregnancy during which it was infected. In the majority of goats, localization would appear to exclude the udder and uterus by the fifth month after termination of pregnancy, but a minority of goats will retain localized udder infection to the end of this lactation. The termination of a second pregnancy after infection will not as a rule cause an exacerbation of the disease, but a small proportion of the animals will carry over elimination of *Br. melitensis* in the genital secretions into the second lactation. Considering all available data, therefore, it is the writer's opinion that infection may remain localized in the glands draining the genital areas for several years following acute infection.

Evidence of Invasion and Infection in Immature Goats

"Evidence of invasion of *Br. melitensis* infection into kids can usually be obtained from the blood serum agglutination reaction. Confirmation of infection by cultural methods can be effected by direct culture of tissues, in the case of fetuses or stillborn kids; but in the absence of products of puerperal activity, confirmation in the live kid may only be effected with difficulty, by frequent hemoculture or specialized methods of isolation from urine and feces.

"In the case of fetuses aborted from acutely, or moderately, affected dams, all tissues appear to be infected. On the other hand, abortions in slightly infected dams may also occur, and fetuses of such dams may not prove to be infected. When kids of acutely affected dams are carried to full term, and are stillborn, or die shortly after birth, postmortem culture of the tissues will reveal infection.

"Kids of infected dams that survive parturition have been examined for serum response only. Few have been tested at birth prior to suckling, owing to difficulties of bleeding at such time. The majority have been tested first between three and four weeks of age, and were subsequently tested at monthly intervals. Returns have shown, with two exceptions, that all kids born of acutely infected dams, at the experiment station in Malta, had no blood serum agglutinins for *Br. melitensis* at three weeks of age. In all cases these kids were not only ingesting enormous numbers of organisms and milk serum agglutinins from the mother's milk, but were additionally exposed to the same contact infection from vaginal secretions and placentas that proved capable of infecting 85 per cent of adult goats around them. The two exceptions offered a serum response in the neighborhood of 1:320, that declined to negative within three months of life.

"At a second test, three of the non-reacting kids mentioned above developed moderate agglutination titers, and again these titers persisted for a comparatively short period. One is forced to conclude, therefore, that the newly born kid which escapes prenatal infection is highly resistant to infection by ingestion or ordinary contact for the first few weeks of life. Kids have not been artificially infected by *melitensis* strains during the present work. Two groups, however, of kids aged four and one-half months and five and one-half months respectively, have been infected by subcutaneous inoculation of a virulent strain of *Br. abortus*. The serum response in

these animals developed in a manner exactly like that in artificially infected adults, while modified clinical symptoms developed lasting some weeks.

Persistence of Infection in Immature Goats

"We have already stated that such kids as become serum reactors through natural causes during the first month or two of life rapidly and completely lose their serum reaction. We conclude this represents true recovery. The serum response of the two artificially *abortus* infected older groups of kids mentioned in the last paragraph of the above section presents an interesting study. Both groups responded with a typically high adult rise but, unlike adults, which hold their agglutinins after peaking for long periods, the kids' serum showed a rapid uniform decline. Further, in sharp contrast, the younger age group continued its decline, so that all members of the group failed to agglutinate within six or eight months after injection. The older group retained low serum agglutinins beyond this time, until they were fully mature and had commenced their first pregnancy. This pregnancy, however, did not provoke an exacerbation of serum agglutinins. Thus, the extent of invasion being equal, and it may be very great, we must conclude that in immature goats not only resistance to invasion but also the ability completely to recover from infection are directly proportional to the youth of the goat."

That goats are susceptible to *Br. abortus* infection has been proved by Doyle (86). He exposed 48 pregnant goats to *Br. abortus* by way of the conjunctiva. Of the total, 36 aborted. *Br. abortus* was recovered from the fetuses. In contrast to what one finds in *Br. melitensis* infection in the goat, only 3 showed *Br. abortus* in the blood stream. *Br. abortus* was recovered from the milk of 4 goats, 114, 216, 259, and 386 days respectively after infection.

Br. suis has not yet been isolated from naturally infected goats.

PART FOUR. BRUCELLOSIS IN OTHER ANIMALS

BRUCELLOSIS EQUINUS

Infective Organisms. Br. suis, Br. abortus.

The first published report of the isolation of *Brucella* from the horse was in 1924 by McNutt and Murray (286), who isolated a species of *Brucella* from the aborted fetus of a mare. Several years later the culture was identified as *Br. suis.*

For many years veterinarians have recognized and treated a type of disease in horses known as fistula of the withers and poll evil. Fistula of the withers is a chronic inflammatory disease involving the supraspinous bursa which overlies the second to fifth thoracic spines. Poll evil is a disease similar in nature and involves the atlantal bursa which lies between the supraspinous ligament and the dorsal arch of the atlas. These two conditions have been ascribed to many causes. It was not until 1928 when Rinjard and Hilger (384) reported on fifteen cases of this condition in horses that *Brucella* was found to be the most constant etiological agent. Of the fifteen horses tested, ten showed a high agglutination titer. A species of *Brucella* was cultured from two. Later the organisms were identified as *Br. abortus.* Fitch and associates (122, 126) have made several important contributions toward identifying the cause of the disease in horses in the United States. In one study, four of forty-eight horses were found to have specific *Brucella* agglutinins in their blood. In a later study, sixty-one horses were examined of which forty-eight were positive to the agglutination test. From seven of these, either *Br. abortus* or *Br. suis* was isolated from tissue taken from the area involved.

Van der Hoeden (452, 454) in Holland has made a very thorough study of natural and experimental *Brucella* infection in horses. Of 424 animals examined for natural infection, 254 reacted

to the agglutination test. He succeeded in culturing *Brucella* not only from the fistulae of the neck and the poll, but from abscesses at the fetlock and sternum. The species of the organism which he isolated was *Br. abortus*. No one has as yet isolated *Br. melitensis* from horses. However, Eyre and associates (110), in examining horses and mules in Malta, found a considerable number that reacted to the agglutination test, and since *Br. melitensis* is the only species found on this island, it is quite possible that it was the infecting species.

Karlson and Boyd (241) made very thorough bacteriological examinations of 5 horses that reacted to the *Brucella* agglutination test. *Br. abortus* was isolated from the feces of two, from an abscess of the withers in one, and from a sternal abscess of another. *Brucella* could not be demonstrated in the urine or blood stream.

Stone (425) examined 18 horses showing either fistula of the withers or poll evil for the presence of *Brucella. Br. abortus* was isolated from 11.

The organism is eliminated from the infected horse in the purulent exudate from the suppurating fistula of the shoulder and suppurating abscesses. The route through which horses become infected is not known.

The serum agglutination test appears to be a reliable method for detecting infection when suitable material is not available for bacteriological examination. A serum agglutination titer of 1:100 is of diagnostic significance.

During recent years there has been an attempt to attribute the cause of periodic ophthalmia in horses to *Brucella.* Jones (239) examined 95 horses showing eye lesions characteristic of periodic ophthalmia and found no evidence of *Brucella* infection. Those who have studied horses infected with *Brucella* have not noted eye lesions occurring at the same time.

BRUCELLOSIS GALLINUS

Infective Organisms. Br. melitensis, Br. abortus, Br. suis.

As early as 1907, Fiorentini (118) reported natural *Br. melitensis* infection in a flock of fowl in Italy. The symptoms shown by the diseased fowl were: fever, emaciation, loss of appetite, and loss of feathers. Evidence of *Brucella* infection was based on positive agglutination tests and the isolations of *Br. melitensis* from the spleen of those that were ill.

In 1910 Dubois (87) studied a highly fatal disease among chickens on a farm in France on which there were also milch sheep infected with *Br. melitensis*. The symptoms shown by the diseased chickens were: difficulty of motion, loss of appetite, debility, gradual immobility, drooping of wings, attacks of diarrhea, and emaciation. The blood serum of many of the chickens was found to contain agglutinins for *melitensis* in titers ranging from 1:50 to 1:600.

It was not until 1928 that the fowl was again associated with *Brucella* infection. In that year Huddleson and Emmel (209) conducted a series of experiments designed to determine the pathogenicity of *Br. abortus* for fowl. They found that the fowl could be infected quite easily, but that the disease was very different in nature from any heretofore described. Soon after experimental infection, the birds developed diarrhea and paleness about the head, comb, and wattle. They became very weak and some showed marked paralysis. There was a marked decrease in the egg production. The infected birds usually died within eighteen to ninety-six days. Specific agglutinins developed within seven to ten days after exposure to a titer of 1:100. Within thirty days the agglutination test was negative and was always so at the time of the death of the infected birds. McNutt and Purwin (287) attempted to confirm the results obtained by Huddleson and Emmel but were unable to do so. They succeeded in producing specific agglutinins

following exposure but were unable to produce any sign of a disease. Gilman and Brunett (146) succeeded in establishing experimental *Brucella* infection in chickens sufficiently so that the organism could be recovered up to one hundred and forty-one days after exposure. These investigators succeeded in isolating *Br. suis* from several naturally infected fowl coming from flocks in the state of New York.

BRUCELLOSIS CANINUS

Infective Organisms. Br. melitensis, Br. suis, Br. abortus.

Eyre and associates (110) were the first to observe that the dog was susceptible to experimental *Br. melitensis* infection during the study of the disease on the Island of Malta. The first case of *Br. suis* infection in a dog in the United States was reported by Planz and Huddleson (353). The case was a male fox terrier three and one-half years old. The dog was brought to the hospital for an examination due to the fact that he had appeared listless and languid for more than six weeks. The symptoms changed from day to day. Physical examination showed the right testis swollen to twice its normal size. The rear legs were stiff and painful. The temperature was 105.8° F., pulse 100. The enlarged testis was removed surgically and found to contain a large cavity 5 by 15 cm. in diameter, filled with a sanguinolent pus slightly viscid. From the pus, *Br. suis* was isolated. Following the removal of the diseased testis the condition of the dog improved rapidly.

Van der Hoeden (455) examined blood serum from 425 dogs in Holland and found agglutinins for *Brucella* in 16.3 per cent. *Br. abortus* was cultivated from one naturally infected dog. He succeeded in infecting dogs experimentally by way of the mouth, the skin, and the conjunctiva. On postmortem examination the organism was recovered from the spleen, liver, mesentery, lymph gland, and bone marrow. The disease was also transmitted to nor-

mal dogs through contact with infected ones. He considers the dog as a possible source of the disease in animals and man.

Thomsen (438) examined fifty-eight dogs from farms on which the cattle were infected with Bang's disease. Of the total number examined, nine were found to react positively to the agglutination test. He succeeded in infecting three dogs experimentally. The only clinical sign of the disease was an undulating temperature. At necropsy the dogs showed multiple yellowish nodules beneath the kidney capsule.

Davis (75) reported a case of brucellosis in a dog in Colorado. The clinical and physical findings in this case were not unlike those reported by Planz and Huddleson (353). The affected left testis was removed and found to contain a large abscess 3.75 by 25 cm. in diameter which contained a semipurulent inflammatory exudate.

The organism is eliminated from infected dogs in the urine, as reported by Thomsen (438) and Van der Hoeden (455).

The agglutination test is a satisfactory method for detecting *Brucella* infection in the dog. An agglutinin titer of 1:100 or higher indicates active infection.

PART FIVE. EXPERIMENTAL BRUCELLOSIS IN THE GUINEA PIG

The simultaneous discovery by Smith and Fabyan (415) and Schroeder and Cotton (400) that guinea pigs were susceptible to *Brucella* infection has led to the universal use of this animal for experimental studies and for determining the presence of the organism in tissues of animals and human beings.

ROUTES OF INFECTION

For many years the intraperitoneal route was used for infecting guinea pigs. Then Schroeder (399) found that infection could be produced by way of the conjunctiva. Hardy and associates (175), and Bang and Bendixen (12) demonstrated that *Brucella* would pass through the unbroken skin of guinea pigs, giving rise to generalized infection. In recent years the conjunctival and skin routes have been the ones chiefly employed in infecting the guinea pig for experimental purposes.

PATHOLOGY

Gross Changes

The gross changes have been so fully described by Henry, Traum, and Haring (191) that their description is given here.

"Macroscopically the spleen may be enlarged slightly or may be enlarged up to six times its normal size. The surface is usually nodular, but in cases of an acute nature the spleen may be smooth. The nodules in the early stages of their development are hemorrhagic, later becoming encapsulated, gray, discrete, and may have a necrotic center.

"Just beneath the capsule on the surface of the liver, small gray glistening nodules may usually be found. These nodules range in size from 0.5 to 2.0 mm. in diameter, are discrete, and may or may

not have an opaque center. In exceptional cases the entire liver may be studded with the nodules, but usually from 10 to 50 occur.

"Macroscopic lesions in the female genital organs are so rare that they are of no importance for routine diagnosis. The reverse is true, however, in the case of the male organs. In most cases of intraperitoneal inoculation of male guinea pigs, lesions may be found in the testicles proper, in the epididymis, or in the walls of the sac surrounding the organ. Adhesions of the testicles or epididymis to the sac are frequent. Abscesses containing creamy yellow or white pus are occasionally found in the testicle proper, but more frequently in the epididymis. The tubules of the epididymis become enlarged and may be disintegrated sufficiently so that they do not appear sharp in outline as do the tubes in a normal organ. Abscess formation may occasionally be found in the cremaster muscles with no macroscopic change in the epididymis. Atrophy of one or both testicles also occurs, with or without abscess formation.

"Noticeable enlargement of the sublumbar lymph nodes almost invariably accompanies testicular involvement. This increase in size usually varies from two to six times normal. The nodes are often hyperemic and occasionally definitely hemorrhagic. Abscess formation is rather rare in the lymph nodes of animals that are inoculated with small numbers of bovine *abortus* and kept only six weeks before slaughter. The precrural and inguinal lymph nodes often show alteration similar to that found in the sublumbars. Macroscopic changes in other lymph nodes of the body are relatively rare, although occasionally enlargement of axillary, mesenteric, and bronchial nodes is noted.

"In a rather small percentage of cases, clear glassy irregularly shaped areas are found just beneath the pleura. These vary from 1 to 5 mm. in diameter. They usually show an opaque center. When the lesions in the lungs are large or numerous, some enlargement of the bronchial lymph nodes occurs.

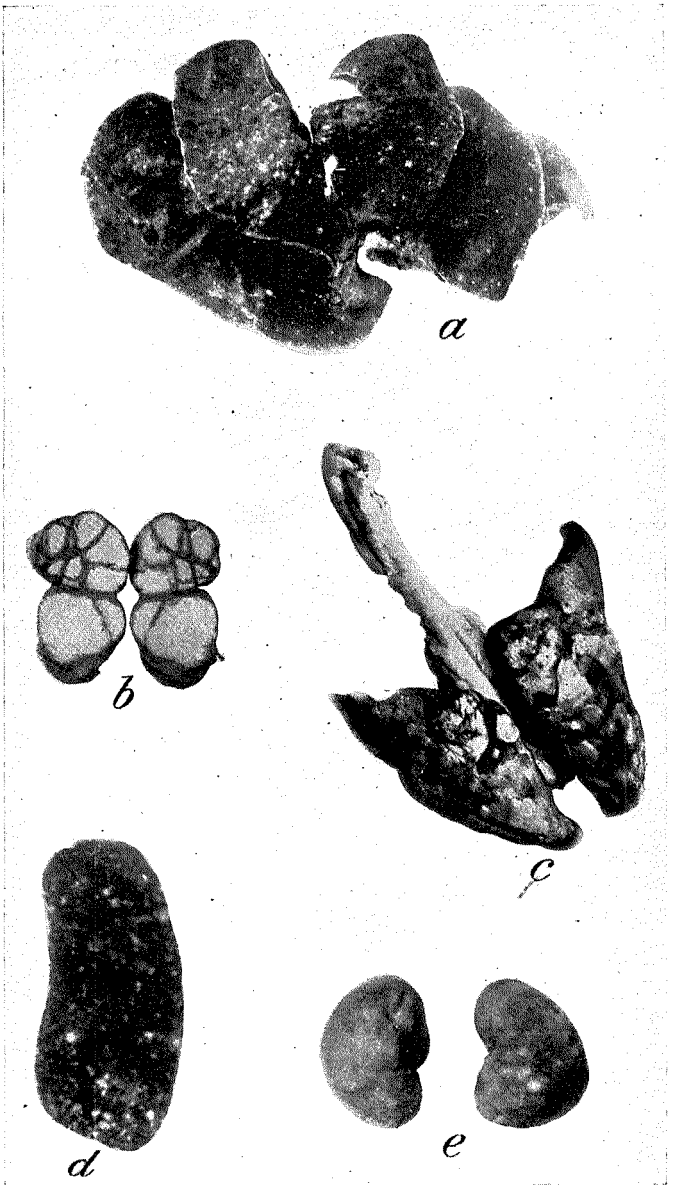
"Lesions of the joints are extremely rare in animals inoculated with bovine tissues and material, and slaughtered at the end of six weeks. With massive infecting doses such as cultures or infected utero-chorionic exudate, swollen carpal and tarsal joints occur rather frequently.

"In addition to the previously mentioned manifestations, other less common lesions may from time to time be encountered. In this group would be included an abscess 1 cm. in diameter filled with creamy pus, on the wall of the urinary bladder, which upon culture yielded only *abortus;* subcutaneous or intramuscular abscesses in the abdominal wall at the point of inoculation which may be caused by *Br. abortus;* and a large subcutaneous abscess behind the ear, open to the outside, which also yielded *Br. abortus.*

"In the examination of large numbers of guinea pigs for lesions of *brucelliasis,* it will be found that those outlined above may appear singly or in almost any combination; however, the spleen, liver, and testicle lesions are ordinarily rather uniform as to extent and occurrence. The liver lesions are the most characteristic of all the lesions described. It is rare indeed to find typical small gray translucent nodules on the surface of the liver without finding *abortus* agglutinins in the blood or obtaining *Br. abortus* in cultures made from the spleen. On the other hand, the absence of liver lesions is not necessarily indicative of freedom from *Brucella* infection."

The foregoing description of the lesions due to *Br. abortus* applies to the *Brucella* group in general.

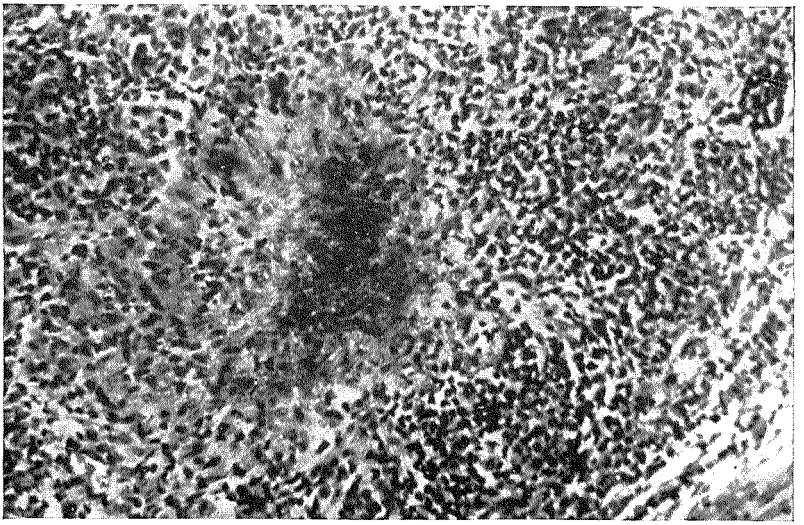
The chief difference between the lesions produced by *Br. suis* and *Br. abortus* infection in the guinea pig lies in their size and degree of abscessation. It is not uncommon to find *Br. suis* producing abscesses one to two centimeters in diameter in the liver, spleen, epididymis, lymph nodes, and joints of the guinea pig. *Br. abortus* rarely produces abscesses of this size.



After Schroeder and Cotton (400)

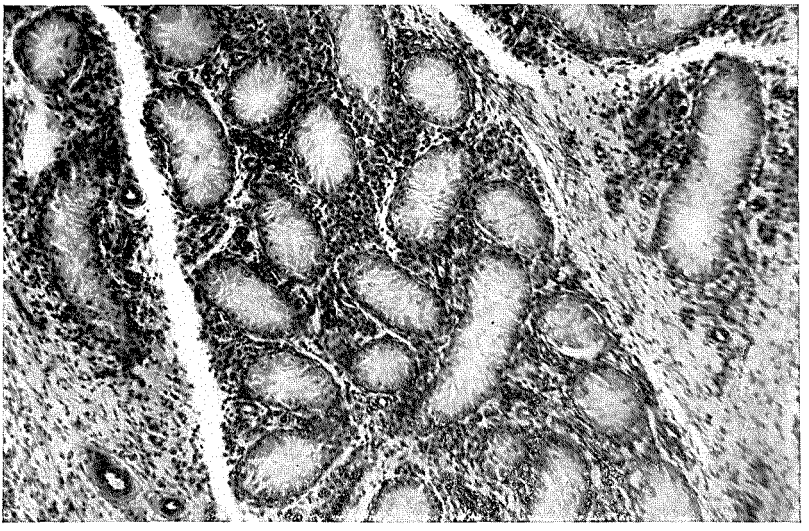
FIGURE 29. ORGANS OF GUINEA PIG SHOWING LESIONS DUE TO BRUCELLA

a. liver; b. testes; c. lungs; d. spleen; e. kidneys. White spots in the kidneys are fibrous nodules in the degenerated cortex. Testes are necrotic throughout, except for bands of connective tissue



After Hallman and associates (167)

FIGURE 30. FOCAL INTERSTITIAL EPIDIDYMITIS WITH NECROSIS DUE TO BRUCELLA INFECTION IN THE GUINEA PIG. X 328



After Hallman and associates (167)

FIGURE 31. DEGENERATION OF EPITHELIUM OF CONVOLUTED SEMINIFEROUS TUBULES AND HYPERPLASIA OF THE INTERSTITIAL CELLS DUE TO BRUCELLA INFECTION IN THE GUINEA PIG. X 125

Histopathological Changes

The histological changes which occur in the tissues of the guinea pig infected with *Brucella* were first minutely described by Fabyan (111). A summary of his description of the changes follows.

"It may be said that the essential changes associated with this disease are of a chronic, inflammatory character, and resemble those of tuberculosis, often to a startling degree, both in character and in focal dissemination. The lesions are small, usually microscopic, and occur chiefly in the perivascular areas in most of the viscera of the body.

"The focal lesion consists of a group of epithelioid elements, i.e., cells with large vesicular, usually oval nuclei, poor in chromatin. The cytoplasm stains very poorly, and is ill defined. Among these cells may be a few nuclei with large peripheral blocks of chromatin and probably lymphoid cells.

"The numerical relation between these two kinds of cells varies from case to case and from organ to organ. Some foci are composed mainly of large cell elements, while the periportal and perivenous cell masses are largely of lymphoid elements. In some foci, plasma and giant cells may be noted. Occasionally polynuclear leucocytes are seen. Mitoses are numerous. In the more chronic cases the tissue changes have progressed beyond the stages described and signs of organization into connective tissue are present."

For methods of inoculation and bacteriological examination of guinea-pig tissues, see Chapter II, page 27.

VI

LABORATORY DIAGNOSIS OF BRUCELLOSIS

PART ONE. SEROLOGICAL METHODS

THE first application of the agglutination test in the confirmatory diagnosis of *Brucella* infections was made in cases of brucellosis in man by Wright and Semple (479) in 1897. Later, Zammit (481, 482) found that this test could be used to a high degree of accuracy in detecting infection in the goat by using either blood serum or milk. Grinsted (155), a Danish veterinarian, was probably the first to use the agglutination test in detecting infection in cattle. This was followed by a report of its use in Great Britain by McFadyean and Stockman (282), and in the United States by Larson (261). McFadyean and Stockman and Larson also reported on the value of the complement-fixation test for the same purpose. These two serological tests in their applications to *Brucella* infections have been exhaustively studied by many investigators in Europe and North America. Their value and limitations are now well established. The complement-fixation test as a routine procedure in detecting *Brucella* infection has fallen into disuse because of the time and attention that are required for its operation and because it does not furnish more information than the agglutination test, which is simpler to perform and gives results just as dependable.

Two methods of performing the agglutination test to detect *Brucella* infection in human beings and animals are available: the test-tube method and the rapid method. Both have been studied critically by many workers and found satisfactory.

THE AGGLUTINATION TEST FOR HUMAN BEINGS

The technics of the two methods to be described for cattle are

equally applicable to human blood serum. In the performance of the agglutination test for the detection of *Brucella* agglutinins no advantage is obtained through the use of separate or combined antigens made of each species. An antigen made of an S strain of *Br. abortus* is satisfactory for detecting agglutinins produced by all three species of *Brucella.* There should be little if any difference in the maximum agglutination titer of a serum when tested against antigens of the different species if they are smooth.

In certain Mediterranean countries where the infection in animals and man is caused by *Br. melitensis,* the antigen used in the agglutination test is made of a strain of *Br. abortus.* Strains of *Br. melitensis* tend to dissociate into antigenic variants and in this state are unreliable for use as antigens as they agglutinate nonspecifically.

Brucella agglutinins as a rule do not appear in the blood until ten days after the onset of the disease. Occasionally they cannot be detected during the course of the disease. This is especially true of the chronic form. It has been the author's observation, from a comparative study of the disease on the Island of Malta and in the United States, that agglutinins are found more often in the *Br. melitensis* type of infection than in the *Br. abortus* type. Very few, if any, *Br. melitensis* cases fail to show agglutinins during the course of the disease.

The interpretation of a positive agglutination test in human blood in terms of active infection is often not an easy matter. Many individuals have *Brucella* agglutinins in their blood from a past infection or from recent exposure to infective materials. This is especially true of veterinarians, packing-house workers, and breeders of livestock. Dooley (81) has presented a very interesting report pertaining to the significance of agglutinin titers in the blood serums of 263 boys in a boys' school where milk infected with *Br. abortus* had been consumed. The study was begun after two of the boys

TABLE XIV

Serum agglutination of 15 persons consuming Br. abortus *infected milk whose serum gave a positive reaction in a dilution of 1:320 or higher*

	1930				1931									
CASE	*March*	*May*	*October*	*December*	*January*	*February*	*March*	*April*	*May*	*June*	*September*	*October*	*November*	*December*
1	–	–	Negative	1:2,560	1:12,000	1:5,120	1:1,280	–	–	1:160	Negative	–	–	Negative
2	–	–	–	–	1:2,560	1:5,120	–	1:320	–	1:80	–	–	–	–
3	–	–	–	–	1:1,280	1:2,560	–	–	1:640	–	1:80	–	–	1:20
4	Negative	–	–	–	–	–	1:640	–	–	1:640	–	1:80	–	1:80
5	–	–	–	–	1:160	1:640	–	–	–	1:80	–	–	Negative	–
6	–	–	–	–	–	1:320	–	–	1:120	–	–	–	–	Negative
7	–	–	–	–	–	1:5,120	1:5,120	1:2,560	1:1,280	1:640	–	–	–	–
8	–	Negative	–	–	1:2,560	1:5,120	1:2,560	1:640	1:320	1:320	1:120	1:120	–	1:120
9	–	–	–	–	–	–	1:2,560	–	–	1:1,280	–	1:120	–	–
10	–	–	–	–	–	–	–	–	1:320	1:320	–	–	1:120	–
11	–	–	–	–	–	–	–	1:1,280	–	–	–	1:120	–	–
12	–	–	–	–	–	–	–	1:1,280	–	1:320	–	–	–	Negative
13	–	–	–	1:40	–	–	–	1:640	–	1:120	–	–	–	–
14	–	–	–	–	1:1,280	–	1:320	–	–	1:20	Negative	–	–	Negative
15	–	–	–	–	–	–	–	1:640	–	1:320	–	1:20	–	1:20

After Dooley (81)

developed the *Br. abortus* type of brucellosis and covered a period of fourteen months. Of the total number examined 41 per cent developed specific serum agglutinins in titers ranging from 1:40 to 1:12,000; the serums of fifteen boys agglutinated in the dilution of 1:320 or higher (see Table XIV). None of the boys, with the exception of the two mentioned, showed any clinical manifestations of active *Brucella* infection.

TABLE XV

Results of agglutination test on 100 cases of brucellosis

SERUM AGGLUTINATION TITER	NUMBER		
	Less than 11 years of age	*More than 11 years of age*	*Total*
Negative	18	11	29
Less than 1:50	2	2	4
1:50 to 1:100	1	8	9
More than 1:100	2	55	57
No test	–	1	1

After Huddleson and associates (220)

Cases of brucellosis with and without positive blood culture have been encountered in which agglutinins were either not detected or were present in only a low titer during the course of the disease. The author has studied 100 cases 29 of which showed no agglutinins in the blood serum in a titer of 1:20 or higher (see Table XV). Taylor, Lisbonne, Vidal, and Hazemann (431) carried on in France a study of the relation between positive blood culture findings and agglutination titers on 1,412 blood specimens from human beings. Table XVI shows the results of the culture of these specimens classified in relation to agglutination. The authors state:

It will be noted that the percentage of positive haemocultures augments in keeping with the agglutination titre, until it reaches 1/160. After this dilution, there is no significant difference. Among the specimens which agglutinated in 1/80 or above, 55.5% gave a positive

TABLE XVI

Comparison of agglutination reaction and hemoculture of human blood specimens received for diagnosis

AGGLUTINATION REACTION	*Number*	HEMOCULTURE POSITIVE	
		Number	*Per cent*
Negative	569	11	1.9
Positive 1:10	29	4	13.8
Positive 1:20	41	9	22.0
Positive 1:40	47	15	31.9
Positive 1:80	90	41	45.6
Positive 1:160	117	67	57.3
Positive 1:320	145	80	55.2
Positive 1:640	145	82	56.6
Positive 1:1,280	229	133	58.1
Total	1,412	442	31.3

After Taylor and associates (431)

NOTE: Contaminated specimens not included. Within a period of one month, 8.3 per cent were contaminated. Specimens agglutinating 1:80 or higher gave 55.5 per cent positive blood cultures.

haemoculture. Of the strains isolated, 95% classified as the *melitensis* variety, the remainder as *abortus*.

They succeeded in isolating *Brucellae* from thirty-nine specimens which failed to agglutinate, or agglutinated in titers of less than 1:80, and of these thirty-nine specimens, twenty-seven were collected thirty days or more after onset.

Gilbert and Dacey (144) and Carpenter and Boak (55) have also found cases of brucellosis in which the agglutination test was negative.

Francis and Evans (137) have observed that cases of tularemia may show agglutinins in a low titer for *Brucella*. In personal communications the author has been informed of two other cases of tularemia in which the blood serums showed agglutinins for *Brucella* as well as *Pasteurella tularense*. Both cases gave a negative skin reaction.

The interagglutinability between strains of *Brucella, Pasteurella,* and *Pfeifferella* by specific serums is a debatable question among many investigators. While Mallmann (290) has observed a high degree of interagglutinability between strains of these groups, Wilson (473) and others have been unable to detect any antigenic relationship between them.

Wong and Chow (478) have noted that the serum of patients infected with *Vibrio cholerae* and those treated with cholera vaccine will agglutinate *Brucella* antigen. In countries where cholera is present, one should be cautious in the interpretation of a positive *Brucella* agglutination on human serum.

Shaughnessy and Grubb (406) find that about 2 per cent of patients showing agglutinins for *Eberthella typhosa* and *Bacillus proteus* X19 show agglutinins for *Brucella* antigen. *Brucella* agglutinins were found in 3 to 9 per cent of tuberculous patients.

A serum agglutination titer of 1:50 or higher, obtained on a patient showing clinical evidence of infection, should be considered as presumptive evidence of active *Brucella* infection. One should rely as much on the history of the case and other confirmatory tests as on the agglutination test in detecting active *Brucella* infection in human beings.

If the serum agglutination titer of an individual, following the disappearance of all clinical symptoms and signs of the disease, is found to be slowly decreasing as shown by a repetition of tests at thirty to sixty-day intervals, it is evidence of recovery from infection. Fluctuating agglutination titers in individuals working with infective materials indicate exposure to *Brucella.*

THE AGGLUTINATION TEST FOR CATTLE

The Test-Tube Method

The technic for performing the test-tube agglutination test varies to some extent in different laboratories. The results of the test,

TABLE XVII

Specific agglutinability of variant Brucella *cultures*

NUMBER	TYPE	SERUM NO. 1					SERUM NO. 2					SERUM NO. 3				
		1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500	1:25	1:50	1:100	1:200	1:500
A154	N	–	–	–	–	–	+	P	–	–	–	+	+	+	+	+
A36	V	–	–	–	–	–	+	P	P	–	–	P	–	–	–	–
A132	V	–	–	–	–	–	+	P	–	–	–	P	–	–	–	–
S400	N	–	–	–	–	–	+	P	–	–	–	+	+	+	+	+
S429	V	–	–	–	–	–	+	+	P	T	–	–	–	–	–	–
S422	V	–	–	–	–	–	+	P	T	–	–	+	+	+	+	+
M784	N	–	–	–	–	–	+	P	T	–	–	+	+	+	+	+
M623	V	–	–	–	–	–	+	P	P	T	–	P	P	P	P	P
M323	V	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–

A = *abortus;* S = *suis;* M = *melitensis.*
Agglutination: V = variant culture; N = normal culture. + = complete; P = incomplete; T = trace; – = none.

however, are in close agreement. A satisfactory method for performing the test-tube agglutination test has been proposed and recommended after exhaustive study by appointed referees of the 1931 conference of the Official Research Workers in Animal Diseases of North America. The referees' report was submitted by the Committee on Bang's Disease to the thirty-fifth annual meeting of the United States Live Stock Sanitary Association. The Association adopted the report as submitted (381).

The method of collecting blood samples from cattle and the technic for performing the test-tube agglutination test as recommended by the Committee follow.

"1. Blood should be collected from the jugular vein by means of a hypodermic needle. Containers should be clean, dry, and sterile. A convenient receptacle for collecting blood is a ½ inch x 4 inch test tube. The size of the hypodermic needle is a matter of individual preference. It should be of sufficient size to permit an ample flow of blood, not smaller than 16-gauge. Preferably a separate needle is employed for each animal or the needle may be thoroughly washed in an antiseptic solution before the bleeding of each animal. Some laboratories have special boxes for sending out the containers for collecting blood samples. The boxes can be sterilized. It is advisable not to fill the tube more than one-half to two-thirds full of blood, and allow it to remain quiet on its side at an angle of approximately 30 degrees until it has become well coagulated. The sample must not be allowed to freeze. If the blood is to be sent to a laboratory, when three to four days will elapse before it is tested, it is advisable to pour off the serum. Under usual circumstances, however, the serum can best be sent on the clot in the original container. It is highly important, however, that the blood serum shall be as free of hemoglobin as possible.

"2. The tubes must be properly labeled. It is satisfactory to number the tubes consecutively (1, 2, 3, etc.) and on a clean sheet of paper identify each tube with the individual animal by tag num-

ber, registry number, or further description. It is necessary to take the greatest care to see that each specimen of blood is properly identified. It is highly desirable to have special sheets prepared for the identification of the animals. These sheets may be made up in a way suitable for the individual state or other organization.

"3. The antigen should be prepared from cultures of typical strains of *Br. abortus* (Bang) that show no tendency to spontaneous agglutination and manifest normal agglutinability. Strains selected should be cultivated and studied under usual laboratory conditions to determine that they do not contain dissociated forms. Either a monovalent or polyvalent antigen may be employed. The culture medium selected shall be such that the strains will grow well on it. There is very little preference of the medium ordinarily employed, namely, nutrient agar containing glycerin, serum infusion agar, liver infusion agar, or potato agar. The cultures should be incubated only until maximum growth is obtained, which is usually from forty-eight to seventy-two hours, at 37.5° C. The bacteria may be grown either in the atmosphere or in a modified atmosphere containing 10 per cent CO_2. Glass test tubes, Blake or Erlenmeyer flasks, or flat bottles may be used as containers for the culture medium on which the bacteria are grown. It is necessary that the purity of the culture be definitely determined. Contaminated cultures have been the source of much difficulty in the agglutination test.

"4. Bacteria should be removed from the culture medium with a phenolized saline solution containing 0.85 per cent C.P. sodium chloride and 0.5 per cent C.P. phenol crystals. Bacteria may be loosened from the medium with a blunt instrument or soaked loose by permitting the phenolized saline to stand on the culture for two to three hours. Suspensions of bacteria should be carefully filtered through paper or cotton, spun glass, or gauze, to remove any particles of agar. Unheated antigens are entirely satisfactory and are recommended. Heating of the antigens at 65° to 100° C.

for two hours does not seem to alter sensitivity. If the antigens are heated, however, extreme care must be exercised to exclude all agar from the suspension, to guard against the so-called agar thermo-agglutination of the bacteria. The antigen may be diluted to the desired concentration immediately after the bacteria are washed from the medium, or stored in concentrated suspension and diluted with carbolized saline solution as needed. Antigen should not be unduly exposed to the sunlight. For use, the concentrated antigen is diluted with phenolized saline solution until its density is 0.04 per cent bacteria by centrifuge-tube method (128, 129). This density compares with tube number 1 of the McFarland nephelometer (284), 200 parts silica standard (422), or approximately seven centimeters on the Gates apparatus (140). Antigen so diluted which has satisfactory agglutinating properties retains them, at refrigerator or room temperatures, for at least one year. If a concentration different from this is employed it should be so stated when the results of the test are reported. Each lot of antigen should be tested out carefully with sera of known titers, high, medium, and negative. It should give clear-cut reactions with all three; otherwise it should be discarded.

"It is not usually necessary to alter the pH concentration of the antigen. Sensitivity of antigens having a comparatively broad zone in pH concentration is not appreciably altered (a pH from approximately 4.7 to 8.9).

"5. Either the multiple dilution method or the decimal system of dilutions may be employed. The dilutions shall be 1:50, 1:100, 1:200 or 1:250, and higher if desirable.

"6. The tests should be held at approximately 37.5° C. for at least forty-two hours before reading. Tests of sera, containing amounts of hemoglobin which are appreciable to the unaided eye, should be held at room temperature. Tests of such hemolyzed sera which do not show complete agglutination in all dilutions used in testing (at least up to and including 1:200) should be considered

not satisfactory, and no diagnosis made. Water-bath or hot-air incubation at temperatures up to and including 55° C. is acceptable, providing the temperature does not at any time exceed this level. Unstoppered test tubes are satisfactory for conducting the test.

"7. All tests incubated at 37.5° to 55° C. must be held until the second day (approximately forty to forty-seven hours) before final observation, at which time the tests may be read. Maximum titers of a few low-to-medium agglutinin-content sera are not obtained until the third day. Tests held at room temperature must remain sixty-eight to seventy-two hours before final observation.*

"8. Tests should be observed both in racks and held in the hands and shaken. The agglutination results in individual serum-antigen mixtures should be recorded as complete agglutination by a plus sign (+), incomplete agglutination by P, and no agglutination by a minus sign (—). Very slight traces of agglutination should be ignored.

"The reading of tests should be carried on only by individuals who have had wide experience in this work. Extreme care should be exercised in the observation of tests to minimize the discrepancies resulting from improper or careless observation."

The Committee recommended that the following interpretations be given to agglutination reactions obtained in cattle:

DILUTIONS				
1:25	*1:50*	*1:100*	*1:200 or 1:250*	DIAGNOSIS
–	–	–	–	Negative
+	P	–	–	Suspicious
+	+	–	–	Suspicious
+	+	P	–	Suspicious
+	+	+	–	Positive
+	+	+	P	Positive

Agglutination: + = complete; P = incomplete; – = none.

* The holding of tests at 55° C. for forty hours or over is not necessary. Incubation at 55° C. for four hours and holding at room or ice-box temperature for twenty-four hours is sufficient. Results of tests conducted at room temperatures are questionable.—Author's note.

At the 1932 conference of the Official Research Workers in Animal Diseases of North America, the referees on the test-tube agglutination test recommended that the previous report be amended as to the interpretation of the test. It was recommended that an animal showing a complete reaction in no higher than 1:25 dilution be considered suspicious. This recommendation was adopted by the conference.

They further stated: "All suspicious animals should be retested within a period of ten to thirty days. It is impossible to determine on a single test whether the titer is on the upgrade or whether it is more or less constant. Retests will answer this question in most cases."

The interpretation of agglutination reactions in cattle in the suspicious category depends upon whether the agglutination titer is (1) ascending, (2) descending, (3) remaining approximately stationary. More than one test on a given animal is required to establish the direction in which the titer is moving. Fitch and associates (125) have accumulated considerable data on the status of suspicious reactions in 136 animals. Only 9 showed bacteriological evidence of infection and these had a serum agglutination titer of 1:100. This titer in an animal usually implies active infection.

Those animals which continue to show a suspicious to negative serum agglutination reaction may be highly resistant to brucellosis. The studies which Beach (15) made on 19 previously infected animals, 18 of which he was unable to infect, lend considerable support to this view.

Those animals which show a suspicious reaction in successive tests conducted at thirty to sixty-day intervals may be considered free from infection. One negative test obtained on an animal in a herd containing infected animals does not necessarily imply that the animal is free from infection. It has been definitely shown that when an animal is exposed to infective material, agglutinins indica-

tive of infection may not appear in the blood until after a period varying from three weeks to four months. A very small percentage of infected animals do not show agglutinins, either in the blood or in the milk.

There are many factors which might influence the results of the test-tube agglutination test. These have been given considerable study by Fitch and his associates (128, 129), by Henry and Traum (190), and by the author.

Some of the factors which may influence the results of the agglutination reaction follow.

1. Presence of hemoglobin in the serum. *Effect:* causes non-specific clumping of the organisms.

2. Heating serum to 56° C. or above. *Effect:* lowers the agglutination titer of the serum.

3. Age of serum. *Effect:* lowers the agglutination titer of the serum.

4. Increase in turbidity of the antigen. *Effect:* tends to lower the agglutination titer of the serum.

5. The use of certain preservatives in the antigen such as formalin and tricresol. *Effect:* prevents or retards the clumping of the organisms.

6. Insufficient period of incubating serum and antigen mixture. *Effect:* maximum titer of serum not obtained.

7. Presence of dissolved agar in a dilution of 1:50,000 in the antigen suspending liquid. *Effect:* causes non-specific agglutination of the organisms.

8. Employment of antigenic variant strains for antigen. *Effect:* such strains are poorly agglutinated by specific serum; they may be agglutinated by negative serum (see Table XVII, page 220).

The Rapid Method

The rapid agglutination test (202) has been used as a routine procedure for more than eight years in the diagnosis of *Brucella* infection in all species of animals and man in North and South America and in European countries. Its practical applicability, accuracy, and simplicity as a means of detecting specific *Brucella*

agglutinins in blood serum, milk serum, and whole blood have been studied critically by many investigators and found satisfactory. The method has been approved and adopted by Livestock Regulatory Boards in several states of the United States as an official test for detecting Bang's disease in cattle.

Many slight variations have been introduced into the technic of preparing the antigen and the manner of performing the test by several investigators. The changes that have been introduced do not materially affect the end results of the test. The results should coincide with those of the tube test.

The procedure for the preparation of the antigen and the performance of the test which the author has found highly satisfactory follows.

APPARATUS AND MATERIALS. *Serum Pipettes.* The standard 0.2 cc. pipette, graduated in 0.01 cc., is preferred, as this size necessitates only one pipetting for each serum sample.

Standardized Dropper Pipette for Antigen. This may be prepared by drawing out thick-walled glass tubing of ⅛ inch bore and cutting the capillary end at 0.07 inch or gauge 15 by U.S. standard gauge No. 283. The delivery end should not be flamed, but left square. The outside diameter of the pipette governs the size of the drop. A dropper pipette of the diameter just mentioned delivers approximately 0.03 cc. of antigen in each drop.

Glass-Plate and Dark-Field Illumination Box (Figure 32). The glass plate is made from double-thickness window glass. Plate glass is not suitable for the test as the surface tension between it and serum is so low that the serum cannot be easily confined to the squares. The plate is cut to a width of 6 inches and to a length of 12 inches. It is then ruled off into inch squares by a diamond point.

The box is 14 inches long, 9½ inches wide, and 5 inches deep. One side of the top (next to operator) is covered to a width of

2½ inches. Under this covering a light socket is placed for a 10-inch show-case bulb which affords indirect illumination for the glass plate on top of the box. The light is controlled by a snap switch on the connecting cord near the box. The inside of the box just behind the light contains a chromium-plated metal mirror in order to enhance the illumination and distribute it evenly over the glass plate. The remainder of the inside of the box, the bottom and front, is painted black. This arrangement produces a very satisfactory black background and enables one to see better the material on the glass plate which is illuminated indirectly. The light is turned on only during the reading of the reactions. To the right of the plate, holes have been made for wax pencil, antigen bottle, and toothpicks.

SERUM AMOUNTS. In the standardization of the antigen and in the routine performance of the test proper, the serum has been used in amounts that could be handled with only one pipetting, so that when the serum was used with the proper amount of antigen in a test-tube test, the serum dilutions would approximate those now in use in most laboratories.

PREPARATION OF ANTIGEN. A single smooth strain of *Br. abortus,* of which the agglutinability and absence of dissociation have been determined previously, is grown on beef liver infusion agar, pH 6.6, for seventy-two hours at 37° C. Flat, small-mouth, one-liter bottles are preferred as containers for the culture medium as a large amount of growth will be needed. At the end of the cultivation period all water of condensation is syphoned off. This may contain considerable dissolved agar which oversensitizes the antigen. The growth is then removed by adding just sufficient (about 25 cc.) distilled water containing 12 per cent sodium chloride, 20 per cent glycerin (Baker's Analyzed, c.p., or Merck's

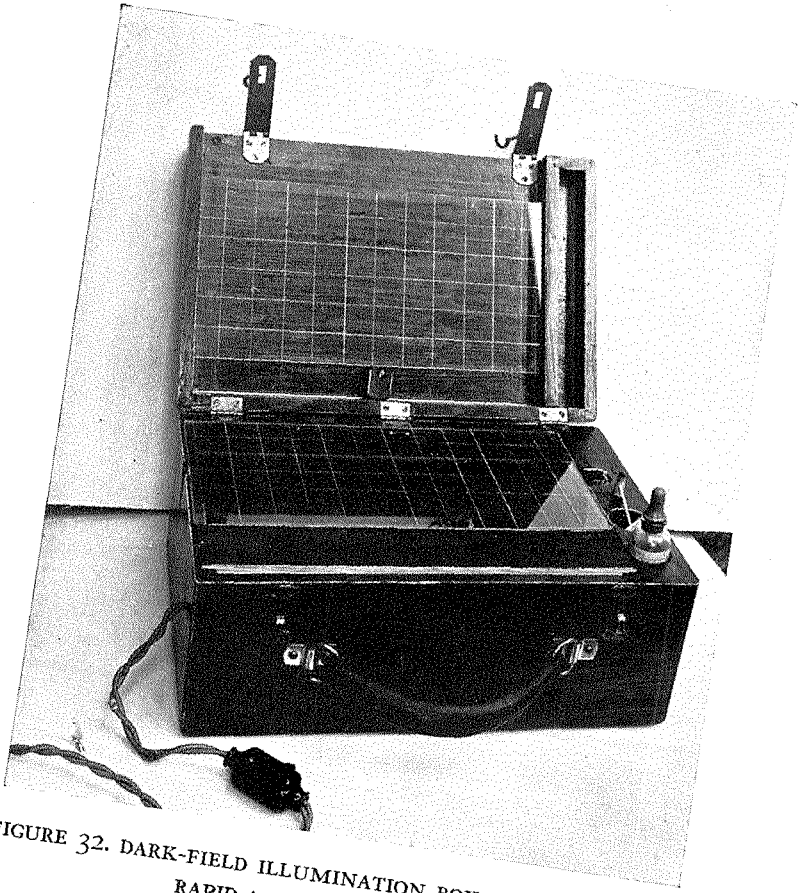


FIGURE 32. DARK-FIELD ILLUMINATION BOX FOR PERFORMING RAPID AGGLUTINATION TEST

Blue Label), and 0.5 per cent phenol to each bottle to loosen the growth from the medium. The high concentration of sodium chloride is employed to increase the sensitivity of the antigen. The added glycerin prevents rapid drying of the antigen-serum mixtures on the glass plate. A cotton swab attached to the end of a wire may be used to hasten removal of the growth from the medium. In the removal, one should avoid breaking up the agar.

The suspension is next filtered through several layers of closely woven cotton cloth by suction to remove pieces of the medium or other foreign material that may be present. At this stage, crystal violet and brilliant green are added from a one per cent aqueous stock solution so that the final dilution of the dyes in the bacterial suspension is 1:25,000. Crystal violet inhibits the growth of Gram-positive organisms and brilliant green inhibits the growth of Gram-negative organisms which may contaminate the antigen during its preparation or when its container is opened. The concentrated suspension of bacterial cells is placed in a flask and heated at a temperature of 60° C. for one hour in a water bath to kill the bacteria. It is then cooled rapidly and tested for sterility. The suspension should be concentrated to a bacterial volume of approximately 20 per cent for standardization by employing a slight modification of the method proposed by Fitch and associates (128). The method is as follows:

Withdraw 1 cc. of the suspended bacterial cells and dilute with 9 cc. of 12 per cent saline solution containing 20 per cent glycerin and thoroughly mix; withdraw 2 cc. amounts of the 1:10 dilution and place in each of 2 capillary centrifuge tubes (Machlett and Son). Add sufficient 12 per cent saline to the centrifuge tube to bring the total volume to 10 cc.; mix, stopper, and centrifuge at 2,700 r.p.m. for 2 hours. The capillary portion of the tube is graduated in 0.002 cc. to a total of 0.05 cc. The volume of sedimented bacteria in the capillary portion of the tube represents the quantity of bacteria in 0.2 cc. of the original suspension. By converting the amounts to a percentage basis, one arrives at the

volume per cent of cells in the original suspension. If the volume of sedimented cells reads 0.036 to 0.04 cc., the original suspension contains between 18 and 20 per cent of cells and is satisfactory for standardization. If the capillary tube contains less than 0.036 cc. of cells, the original suspension must be concentrated by removing a calculated amount of supernatant liquid by centrifugation. It is unnecessary to centrifuge the total volume of the suspension. The amount that should be centrifuged to bring the suspension to the required cell concentration may be calculated from a proportion formula.

TABLE XVIII

Procedure for titrating antigen with supernatant liquid

TUBE	ANTIGEN	SUPERNATANT	SERUM AMOUNTS IN CC.				
1	0.5 cc.	0.0 cc.	0.08	0.04	0.02	0.01	0.004
2	0.5 cc.	0.1 cc.	0.08	0.04	0.02	0.01	0.004
3	0.5 cc.	0.2 cc.	0.08	0.04	0.02	0.01	0.004
4	0.5 cc.	0.3 cc.	0.08	0.04	0.02	0.01	0.004
5	0.5 cc.	0.4 cc.	0.08	0.04	0.02	0.01	0.004
6	0.5 cc.	0.5 cc.	0.08	0.04	0.02	0.01	0.004

NOTE: One drop of each antigen mixture is added to each five amounts of serum.

If the volume of cells in the suspending fluid is found to be greater than 20 per cent, the original suspension need not be diluted to 20 per cent before determining its sensitivity according to the procedure which follows.

TITRATING ANTIGEN FOR SENSITIVITY. The titration is made by following Table XVIII. The respective dilutions of the cells are made in small clean test tubes with the supernatant liquid. Each dilution of cells should be examined for its agglutinability against four different serums, the titers of which should be 1:500 or higher, 1:100, 1:25, and negative. The titers are determined previously by the test-tube method. The serum containing no agglutinins for *Brucella* should be examined first. The proper amounts of serum are placed on the squares running the short way of the plate. In order

to take care of the six dilutions of the cells, it is necessary to place the proper amounts of the serum on the squares in six rows. One drop of antigen is added to the serum on each square. The serum and antigen are mixed slowly by means of a common wooden toothpick, starting with the smallest amount of serum and passing to the next largest amount until the five amounts have been thoroughly mixed. A separate toothpick is used for each row. When the mixing is completed, the light in the box is turned on. The plate is slightly raised from the top of the box and tilted backward and forward slowly for two to three minutes. If there is perceptible clumping with the five amounts of negative serum in any one of the different dilutions of antigen this indicates oversensitization due to absorbed agar. It is useless to carry the examination further as it is not possible to adjust the antigen to the point where non-specific clumping can be eliminated. The lot of antigen should be discarded.

If clumping does not occur with a negative serum, one may now proceed to titrate the antigen dilutions with the high titer serum. In the titration one may find that the tube containing the more concentrated suspension is too dense to permit one to interpret the reaction with the smallest amount (0.004 cc.) of serum. This, of course, is undesirable. It is necessary to have the antigen as concentrated as possible and yet obtain clear clumping with each of the amounts of serum used in a period of two to three minutes after mixing.

For the purpose of illustration, let us suppose the antigen dilution in tube 3 is completely clumped with all amounts of the high titer serum. This is the antigen dilution, then, that should be used in determining its agglutinability with the other types of serums previously mentioned, namely, serums of a 1:25 and 1:100 titer. In the titration of the low titer serums, one will as a rule find that the clumping of the antigen does not break off immediately in a

certain dilution of the serum as is often the case in the test-tube method. Some degree of clumping usually continues in the next square containing a smaller amount of serum. For example, if complete clumping has taken place at the end of the stated period in 0.08 cc. of serum, there will also be found incomplete clumping in the 0.04 cc. amount and possibly a trace in the 0.02 cc. amount.

The results obtained in titrating the low titer serums with the antigen may fall into one of three categories. The rapid test may give the same readings as the test-tube method, it may show complete flocculation beyond the test-tube titers, or it may not flocculate as high as the test-tube titers.

If the titers are correct, the antigen should now be examined further with several serums of different titers. If the results continue to be satisfactory, the original suspension of cells may be brought to the proper concentration by means of the supernatant liquid. This should also be titrated against several serums. If the new suspension is also found satisfactory, it may be distributed in 20 cc. containers and stored in the ice box when not in use.

If the diluted cell suspension in question appears too sensitive when titrated with the low titer serums, it will be necessary to set up tubes of antigen as in Table XVIII, and instead of adding increasing amounts of the supernatant liquid to the tubes, add distilled water containing 0.5 per cent phenol. The sensitivity of the antigen is lowered by reducing the concentration of the salt solution. The sensitivity tests must be repeated with a high titer serum as well as with low titer serums.

If the cell suspensions do not flocculate as well with the 1:25 and 1:50 titer serums as desired, two treatments should be tried to obtain an increase in sensitivity. The first consists in reheating a small portion of the cell suspension to a temperature of 60° C. for a period of ten minutes. Cool and examine.

If this treatment does not alter its sensitivity a second procedure

should be tried. This consists in adding increasing amounts of hot 10 per cent gelatin to constant amounts of the cell suspension in small test tubes as outlined in Table XIX. The cell suspension should be placed in the tubes first and the gelatin added afterward. Mix by shaking vigorously. First, titrate the 1:25 titer serum with each of the antigen-gelatin mixtures. The addition of gelatin should increase the sensitiveness of the antigen markedly. If more than one tube of the antigen-gelatin mixture appears satisfactory, it is desirable to use only the one that contains the smallest amount of gelatin for further titration of serums. The use of too large an

TABLE XIX

Procedure for titrating antigen with gelatin

TUBE	ANTIGEN	GELATIN	SERUM AMOUNTS IN CC.				
1	0.5 cc.	0.01 cc.	0.08	0.04	0.02	0.01	0.004
2	0.5 cc.	0.02 cc.	0.08	0.04	0.02	0.01	0.004
3	0.5 cc.	0.03 cc.	0.08	0.04	0.02	0.01	0.004
4	0.5 cc.	0.04 cc.	0.08	0.04	0.02	0.01	0.004
5	0.5 cc.	0.05 cc.	0.08	0.04	0.02	0.01	0.004

NOTE: One drop of each antigen mixture is added to each five amounts of serum.

amount of gelatin must be avoided as it may increase the sensitiveness to a point where flocculation of the antigen occurs to a slight degree with negative serums. Moreover, as the serum-antigen mixture dries out on the plate the gelatin appears to flocculate, which, to the inexperienced observer, might be taken for clumping of the antigen.

The addition of gelatin to the antigen should be avoided if possible as the antigen becomes too sensitive after standing for six months.

It may be stated here that it is seldom found necessary to apply all the measures just described in order to adjust an antigen to the desired agglutinating sensitiveness for all types of serum. They are presented for the purpose of solving the difficulties occasionally

met with in the preparation of rapid antigen. One may often find it necessary to add gelatin to the antigen to increase its sensitiveness. This correction is necessary for strongly positive serums as well as for weaker ones, since very often one encounters a 1:500 titer serum agglutinating much more slowly than others of this titer.

In adjusting the antigen one should employ a slowly agglutinating 1:500 titer serum as encountered by Donham and Fitch (79).

The keeping quality of the antigen is one of its important features. It has been demonstrated on several occasions that it is still reliable eighteen months after preparation when kept intermittently at room and ice-box temperatures.

TESTING BLOOD SERUM. The serum samples to be tested and the antigen are brought to room temperature (about 25° C.) before the test is begun. The antigen is then shaken thoroughly before beginning the test and at two-hour intervals during the testing of samples. In the routine performance of the test one should adhere strictly to the use of the same amounts of undiluted serum from which dilutions of 1:25, 1:50, 1:100, 1:200, 1:500 are obtained by the slow method with 2 cc. of antigen. The serum amounts used are 0.08, 0.04, 0.02, 0.01, and 0.004 cc. The agglutination titer of a given serum obtained by the rapid method should conform exactly or closely to that of the test-tube method when properly performed. If one desires to interpret the results in dilutions other than those just mentioned by using different amounts of serum, it will be necessary to standardize the antigen for the amounts in question.

The glass plate is arranged for handling twelve serums. The best results are obtained by testing only six at a time. Each of the five amounts of serum is placed on a square of one of the rows on the plate, beginning at the top or bottom of the row and working across to the opposite side. A drop of antigen is placed on each

amount of serum. Care is taken to hold the dropper pipette in a vertical position, as holding it at another angle will make a considerable difference in the amount of the delivered drop. When the addition of the antigen to all serums has been completed, slow mixing is begun by using a separate toothpick for each serum, starting at the smallest amount and working upward to the largest. On completion of this performance, the plate is lifted from its position on the box and slowly tilted back and forth for two to three minutes. It is then placed in its former position and the light turned on to read the results.

The reactions stand out very clearly as shown in Figure 33. The row of squares containing the designated amounts of serum sample 1 shows a negative reaction. No flocculation of the antigen has taken place. In the second row from the left, 0.08 cc. of serum of sample 2 has completely agglutinated the antigen, and in 0.04 cc. the antigen has been incompletely agglutinated. The antigen shows complete agglutination with 0.08 and 0.04 cc. and incomplete with 0.02 cc. of serum sample 3. The antigen shows complete agglutination with three amounts of serum sample 4, with four amounts of serum sample 5, and with five amounts of serum sample 6. The agglutinated antigen in 0.004 cc. of a strongly positive serum, such as sample 6, often becomes very viscous and does not separate into individual clumps as it does with larger amounts of the same serum.

The light should be turned off as soon as the results have been read to prevent the plate from becoming too warm and causing too rapid evaporation of the next group of serums when they are placed on the plate. On the completion of the test, the material is removed from the plate by scrubbing with a good triple phosphate cleansing powder. The plate is then thoroughly rinsed in tap water and afterward in distilled water. It is wiped dry with a moist chamois skin.

The results obtained by the rapid method are given the interpretation indicated under the test-tube method.

TESTING MILK SERUM. Milk samples are collected, preferably from individual quarters of the udder, in clean test tubes or vials. The strippings should never be used. The tubes should be filled to only one-half their volume. Let the samples stand from six to eight hours to permit the cream to separate. Remove as much of the cream as possible with a pipette. Add rennin, allowing two drops for each 5 cc. of milk, to each tube of milk and mix thoroughly. The tubes may be placed either in an incubator at 37° C. or in warm water at the same temperature. If the tubes are placed in a slanting position, the curd will settle to one side, and the clear milk serum with which the test is to be made will separate. The serum will form in about two hours and may then be examined in the same manner and amounts as blood serum. In order to obtain a serum free from particles of casein, the samples should be placed in an ice box or cold place for from six to eight hours before testing. Sour and decomposed milk should not be used, as the results are not reliable. Neither should milk be used in which the curd has become partly digested, as this interferes with the test. Colostrum is unsatisfactory because of the difficulty of separating the serum. The presence of infection in the udder is indicated by complete agglutination in .04 cc. or less of milk serum.

If milk samples are sent to a laboratory for examination and more than ten hours intervene before their arrival, they should be iced. Traum and Henry (448) found boric acid an excellent preservative for milk samples. If this agent is used, icing of milk samples is not necessary.

INTERPRETATION OF MILK SERUM TEST. Cooledge (61) in his early work was of the opinion that the presence or absence of agglu-

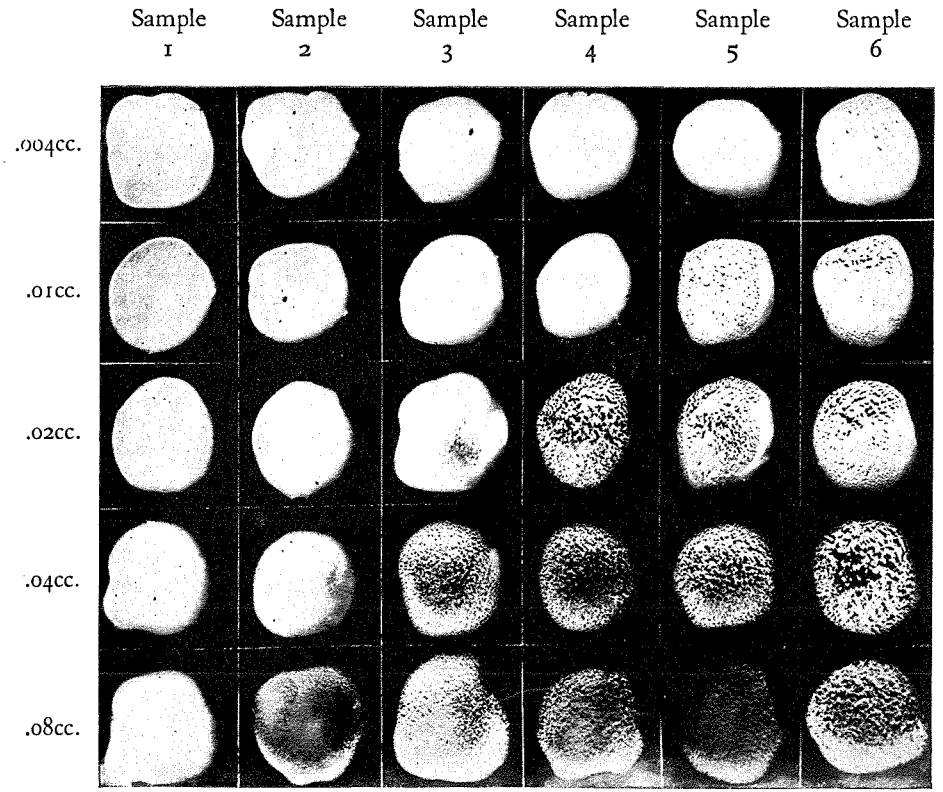


FIGURE 33. DIFFERENT DEGREES OF AGGLUTINATION OBTAINED WITH THE RAPID TEST

tinins in milk was indicative of the presence or absence of infection in the udder. Gilman (145) concluded from his study of a large number of infected cattle with infected udders that there was some relationship between the agglutination titer of the whey and the presence of *Brucella* in the milk. Milk from quarters showing an agglutination titer of 1:80 or higher were usually actively infected, but the organism was found present in rare instances only in titers below 1:80. Henry, Haring, and Traum (189) found a high percentage of animals with infected udders whose milk showed an agglutination titer below 1:50. In no instance was the organism found in udders in which the milk showed a negative reaction. Over a considerable period of years it has been found by the author and his associates that the agglutination titer of the milk is of little value in indicating the presence of the organism. In other words, the milk from an udder with an agglutination titer of 1:25 is quite likely to be as heavily infected as one with an agglutination titer of 1:500. One herd consisting of a large number of infected animals with infected udders has been studied for the past three years. In this herd about 50 per cent of the animals with infected udders have consistently shown a negative agglutination reaction when a test was conducted with whey obtained from the milk by means of rennin. All the animals showed a very high blood serum agglutination titer.

THE AGGLUTINATION TEST FOR ANIMALS OTHER THAN CATTLE

Although the procedure of performing either the test-tube or rapid agglutination test on cattle serum may be used for other animals, the results cannot always be given the same interpretation. In many goats that have been found infected with *Brucella* the agglutination test may be negative even after repeated tests. A negative test, therefore, does not always signify that infection is absent.

The agglutination test may be used to detect infection in the hog and horse. When the blood serums of these animals show agglutinins in a titer of 1:100 or higher, active infection is indicated. From experience with the test in hogs, the author and his associates have found them infected when a positive agglutination was obtained in a titer no higher than a 1:25 dilution as well as in higher titers of the serum.

PART TWO. ALLERGIC METHODS

ALLERGIC TESTS

Allergic tests as a means of detecting *Brucella* infection in cattle were first studied by McFadyean and Stockman (283). They used an allergic agent prepared according to a method similar to that used in preparing mallein and tuberculin. It was injected both subcutaneously and intravenously. Meyer and Hardenbergh (301) and others have studied the value of allergic agents injected subcutaneously and intravenously into infected cattle. Thus far such tests as indicators of infection in cattle have proved unreliable.

Fleischner and Meyer (131) and Stafseth (421) have employed salt solution suspensions of heat-killed *Br. abortus* intradermally to detect infection in experimentally infected guinea pigs. They found that a positive intradermal test was a reliable index of infection.

Giordano (150) has reported favorable results from the use of an allergic agent made from a heat-killed suspension of *Brucella* cells in detecting human brucellosis.

Burnet (43) has used an intradermal test to detect infection in man with apparently satisfactory results. The agent which he employed was made from filtrates of the organism grown in broth for twenty days.

Foshay (133) has employed a bacterial-specific antiserum intradermally for detecting residual antigen which may be present or remain in the skin following the invasion of the tissues by a microorganism. The specific-antiserum intradermal test is read fifteen to twenty minutes after injection. Data obtained by Foshay indicate that the test may be used in detecting past as well as present infection and is species specific.

Rainsford (374) has had considerable experience in using a culture filtrate or "melitin" in detecting *Brucella* allergy in human

beings in Malta. This agent seldom detects allergy in the early stages of the disease. In other words, a positive reaction is not obtained in a case until improvement is noted.

Levin (266) has used a suspension of *Brucella* cells previously extracted with fat solvents for skin-testing individuals for *Brucella* allergy. This agent, while detecting allergy satisfactorily, produced an area of central necrosis at the point of reaction in a great many instances. Obviously such a reaction is undesirable from the standpoint of the clinician.

Morales-Otero and Gonzalez (319, 320) have prepared a soluble protein from *Brucella* cells by a grinding and extraction method similar to that described by Siebert for the preparation of purified protein derivative from tuberculin. The *Brucella* protein contains approximately 13.14 per cent of nitrogen and 1.2 per cent of carbohydrate. The substance will provoke a positive skin reaction in sensitized animals and humans when 0.005 mg. is injected into the skin.

The chief objection to the use of suspended heat-killed *Brucella* cells intradermally in human beings is that the area of the skin involved in the reaction very often becomes necrosed and sloughs, leaving an unsightly open wound for several weeks.

LIMITATIONS OF THE ALLERGIC AND SEROLOGICAL TESTS

Since the widespread occurrence of brucellosis in man in the United States was established, considerable attention has been given to the diagnosis of the disease, chiefly by cultural, serological, and allergic methods. Data are being accumulated which indicate that these methods are not always satisfactory in detecting many cases of the disease in man. Again, there are times when the results of the serological or allergic tests are misleading so that a correct diagnosis is not possible.

Many physicians often observe the occurrence of a symptom-

complex in patients not unlike that of acute or chronic brucellosis, but are confused in making a diagnosis because the serum reaction to an agglutination test for *Brucella* infection is negative. If in addition to the agglutination test an intradermal test is performed with a suitable agent and a positive reaction is obtained, this evidence alone is not sufficient to warrant a diagnosis of brucellosis, because all individuals who have been infected with *Brucella* in the past as well as those who are actively infected may show a skin reaction to a satisfactory *Brucella* allergen (see Table XX).

The results of the agglutination test may also be misleading because individuals are often found to have *Brucella* agglutinins in their blood serum when they show no symptoms of *Brucella* infection (see Table XXI). These are chiefly laboratory workers, practicing veterinarians, farmers, and packing-house employees. By way of illustration let us consider the status of veterinarians with respect to the disease. Those who are engaged in cattle practice come in contact with *Br. abortus* to a greater extent than any other single group of people. It has already been shown by serological and intradermal tests made on veterinarians in Europe and this country that a considerable percentage have *Brucella* agglutinins in varying degrees in their blood and give a positive intradermal test. While very few of them give a history of having a clinical course of the disease, many report the occurrence of skin eruptions and malaise after removing retained placentae from aborting cows. The malaise is usually characterized by dulness, headache, sweating, and aching in the muscles and joints. There may occur an elevation of the temperature. These symptoms may occur from contact with the diseased tissues or from the ingestion or inhalation of the organism alive or dead, or from the injection of sterile broth filtrates on which the organism has grown. It is necessary, of course, that the specific allergen pass through the epithelium of the skin, the respiratory tract, or the digestive tract.

TABLE XX

Allergic tests on persons exposed to contact infection with Br. abortus, Br. suis, *and* Br. melitensis

OBSERVER	PLACE	NUMBER TESTED	NUMBER REACTING	PER CENT REACTING	OCCUPATIONAL GROUP
C. Dubois and N. Sollier (1931)	Nunes, France	14	4	28.57	Veterinarians
		11	5	45.45	Sheepherders
		112	10	8.90	Agriculturists in contact with infected sheep
		16	7	43.70	Slaughter-house employees
I. F. Huddleson and H. W. Johnson (1930)	Michigan	50	10	20.00	Veterinarians
Lerche and F. Roth (1933)	Breslau, Germany	44	41	93.18	Veterinarians, laboratory assistants
W. Levin (1930)	Oregon	21	2	9.52	Medical students
		269	7	2.60	Inmates of sanatoriums consuming infected milk
K. F. Meyer, B. Eddie, L. Veazie, and B. Stewart (1932–1933)	San Francisco, California	227	108	47.57	Packing-house employees
		388	204	52.60	Packing-house employees
		58	35	60.34	Veterinarians
		7	4	57.14	Meat inspectors
		115	19	16.50	Milk handlers
		55	6	10.90	Medical students
G. Straube (1932)	Rostock, Germany	54	6	11.11	Inmates of psychiatric clinics
Wichels and P. von Gara (1933)	Pomerania	59	11	18.64	General population
	Total	1,500	479	31.90	

After Meyer and associates (300)

TABLE XXI

Serological examination of serums from persons specially exposed to contact infection with Brucella *organism*

OBSERVER	PLACE	NUMBER EXAMINED	NUMBER REACTING	PER CENT REACTING	TITER OF REACTION	HIGHEST TITER OBSERVED	OCCUPATIONAL GROUP
J. H. Dible and M. Pownall (1932)	Liverpool	100	12	12.00	1:40 or over	1:640	Slaughterers
A. V. Hardy, M. G. Hudson, and C. F. Jordan (1929)	Iowa	217	40	18.40	1:40 or over	1:2,560	Packing-house employees
A. V. Hardy, C. F. Jordan, I. H. Borts, and G. C. Hardy (1930)	Iowa	120	5	4.20	1:40 or over	1:40	Veterinarians
Herrmann, *et al.* (1934)	Armenia	399	35	8.70	1:100 or over	1:5,000	Veterinarians and agricultural students
Hitchner (1929)	Maine	235	29	12.30	1:80 or over	–	University students (heavy milk drinkers)
I. F. Huddleson and H. W. Johnson (1930)	Michigan	49	28	57.10	1:50 or over	1:500	Practicing veterinarians
I. F. Huddleson, H. W. Johnson, and E. E. Hamann (1933)	Iowa	167	18	10.77	1:25 or over	1:500	Packing-house employees
C. F. Jordan (1931)	Iowa	120	54	45.00	1:5 or over	1:40	Veterinarians
C. F. Jordan (1931)	Iowa	220	68	30.90	1:10 or over	1:1,280	Packing-house employees
C. F. Jordan (1931)	Iowa	138	41	29.70	1:5 or over	1:640	Consumers of contaminated milk supply
C. H. Kitselman (1934)	Kansas	88	26	29.50	1:100 or over	1:400	Veterinary students
M. Knoth (1930)	Saxony	107	17	15.80	1:50 or over	1:100	Veterinarians exposed to contact
F. A. Lentze (1930)	Breslau	57	13	22.80	1:120 or over	1:1,920	Male stock-farm employees
Lerche and F. A. Lentze (1933)	Breslau	130	8	6.15	1:120 or over	–	Milkers
Lesem (1931)	San Diego	409	35	8.55	1:40 or over	–	Milk handlers
W. N. Makkawejsky, J. A. Karkadinowsky, N. J. Michejeff, A. J. Gawriloff, and W. G. Dawydowsky (1931)	Russia	354	27	7.60	1:100 or over	1:800	Farm hands on 15 farms infected with *Brucella abortus*
J. W. Martin and J. T. Myers (1931)	Nebraska	200	18	9.00 (15.00)	1:80 or over	1:1,280	Packing-house employees
K. F. Meyer and B. Eddie (1932–1934)	San Francisco	115	3	2.60	1:40 or over	1:80	Milk handlers
	Total	3,225	477	14.79	–	–	

After Meyer and associates (300)

Individuals in the general population as well as those in particular groups may show the symptoms just mentioned when exposed to *Brucella* by way of the skin or digestive tract. Killed organisms as well as live ones contained in food will produce an allergic reaction the symptoms of which are quite similar to those seen in chronic brucellosis. It is now a well-established fact that many cases of *Brucella* allergy have been and are constantly being mistaken for chronic brucellosis.

An attempt has been made to arrive at a satisfactory method of detecting *Brucella* infection in those who show neither a positive blood culture nor agglutinins in their blood and of clarifying the status of those individuals who show symptoms characteristic of the disease when exposed to *Brucella* allergens. It has been found that when the results of a suitable allergic skin test and the opsonocytophagic power of the blood in a phagocytic system are considered together, they may furnish the necessary information in the majority of cases (217).

THE BRUCELLERGEN TEST

For the past ten years the author has been studying and using the protein nucleate fraction of *Brucella* cells as an allergic agent for detecting *Brucella* skin allergy in human beings. Data collected on more than 20,000 individuals who were either normal or actively infected show that it is a highly satisfactory and specific agent for detecting *Brucella* allergy. The agent will hereafter be referred to as "Brucellergen."

Preparation of Brucellergen

A smooth strain of either *Br. abortus, Br. suis,* or *Br. melitensis* is grown for seventy-two hours at 37° C. on liver infusion agar in flat 32-ounce bottles. At the end of the incubation period the growth is washed from the surface of the medium with distilled water. The

cells are immediately recovered in a Sharples laboratory centrifuge and extracted with 500 cc. of anhydrous ether for two successive five-day periods to remove ether-soluble lipids. The solvent is decanted and the residue collected on a Buchner funnel. The ether-extracted cells are then dried *in vacuo* over H_2SO_4 at 37° C. The dried cells are transferred to a ball mill and ground to obtain complete disintegration. The powder from the ball mill is transferred to a large beaker containing 2 liters of distilled water buffered to a pH of 7 with N/1 NaOH for each 10 grams of powder and resuspended by stirring for four hours with a mechanical stirrer. The suspension is allowed to stand for twelve to fifteen hours in a cold room, after which it is passed through a Sharples centrifuge to separate the insoluble material. The final liquid should be clear and slightly yellow. The protein nucleate in the clear extract is then precipitated at a pH of 3.9 by the addition of a 1:1 dilution of acetic acid. Precipitation is allowed to continue for twenty-four hours in the cold. The precipitate is separated from the supernatant liquid by decantation and centrifugation. It is suspended in 1,000 cc. of cold water by drawing it through a fine cloth by suction. The suspended material is redissolved by the addition of N/1 NaOH to a pH of 6.8. The insoluble fractions are removed by centrifuging. The clear solution is again precipitated at a pH of 3.9 by the addition of 1:1 acetic acid, centrifuged, and the supernatant discarded. The precipitate is again redissolved in 1,000 cc. of distilled water by the addition of N/1 NaOH at a pH of 6.8 and all insoluble material removed by centrifuging. The resolution and precipitation procedure should be repeated once more. The precipitate from the last precipitation is dissolved in distilled water by the addition of N/1 NaOH at a pH of 6.8 and the concentration adjusted to approximately one per cent. Phenol is added to a final concentration of 0.5 per cent and the solution is sterilized by passing through a sterile Seitz filter. The one per cent solution should be stored in

sterile pyrex bottles in a cold room. A 100-gram portion of dried cells yields from 13 to 15 grams of protein nucleate.

Standardization of Brucellergen

The stock solution is made slightly insoluble for allergic standardization by acidifying with sterile N/1 HCl until it becomes visibly cloudy. A small portion of the suspended material is withdrawn from the bottle and progressive dilutions from 1:1,000 to 1:32,000 made in sterile physiological salt solution containing 0.5 per cent phenol.

The allergic potency of the material is determined by injecting 0.1 cc. amounts of each dilution intradermally into previously sensitized rabbits. Rabbits are sensitized by the intravenous injection of one cc. of a 1:100 dilution of a forty-eight-hour agar slant growth of a virulent culture of *Brucella.* About thirty days are required for skin sensitiveness to develop. Tests may be repeated on rabbits at intervals of fifteen days.

The skin tests for determining the potency of a given lot of Brucellergen are read forty-eight hours after injection. A positive reaction is characterized by a raised area of erythema and induration varying from 3 to 20 mm. in diameter. There is usually a progressive reduction in the size of the reaction. As a general rule, Brucellergen does not produce a noticeable skin reaction in rabbits beyond a dilution of 1:32,000. Normal rabbits should not show a skin reaction to a dilution of 1:1,000 or beyond.

The dilution of Brucellergen that will serve for diagnostic purposes in human beings is the first progressive one that produces a skin reaction of 5 mm. in diameter in sensitized rabbits. For example, if the 1:4,000 dilution produces a 10 mm. reaction, and the 1:8,000 and 1:16,000 produce a 5 mm. reaction, the 1:8,000 dilution is the one chosen for diagnostic purposes.

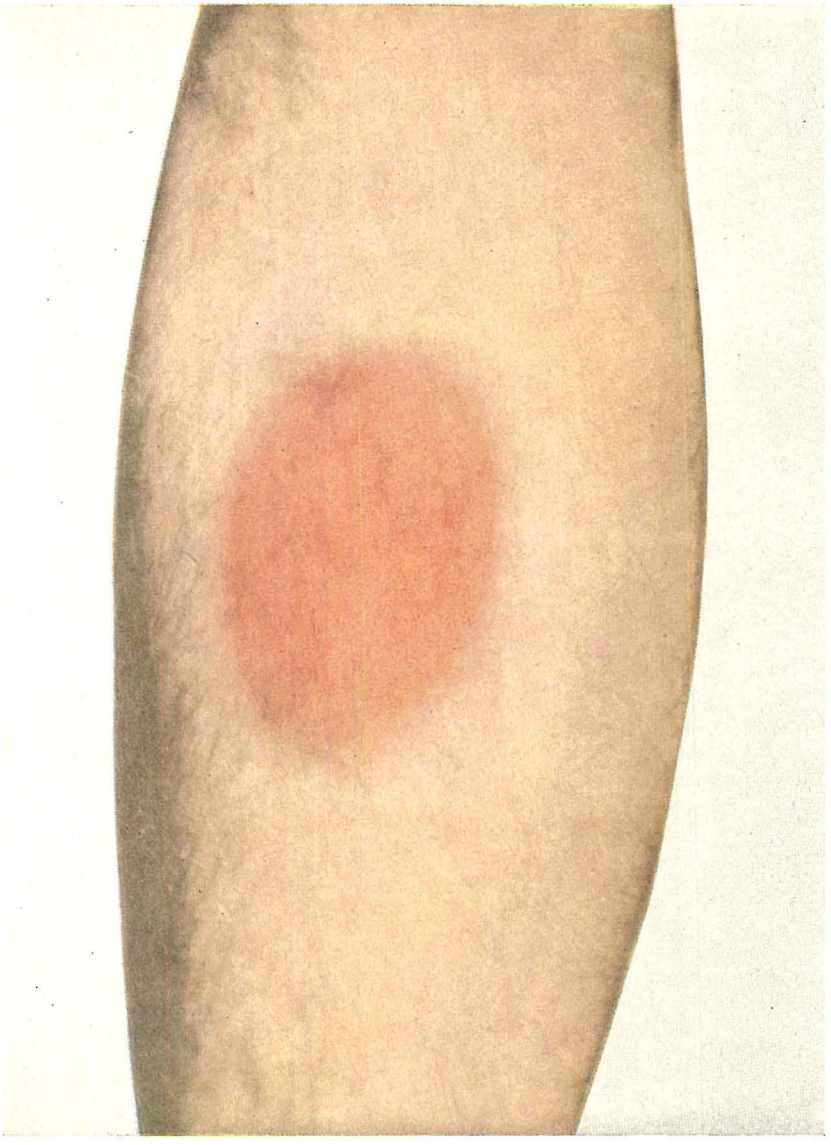


FIGURE 34. A POSITIVE BRUCELLERGEN SKIN REACTION

If a lot of Brucellergen fails to elicit a reaction 5 mm. in diameter in a dilution of 1:8,000, or if on successive tests conducted at intervals of sixty days after preparation it fails to elicit a 5 mm. reaction in the dilution just mentioned, the lot should be discarded.

After the diagnostic concentration of Brucellergen has been determined, a sufficient amount of the insoluble material may be made up to the desired dilution in sterile physiological salt solution (0.5 per cent phenol) vialed in small, sterile non-soluble glass vials, and stoppered with suitable sterile rubber stoppers. The vialed material should be tested for sterility according to the method described under *Regulations of Biological Products,* section 36, of the National Institute of Health (328). The vialed material should be stored in a cold room.

Certain lots of Brucellergen have been found to lose their allergic potency two years after preparation. Despite its long stability, the potency of each lot in use should be determined at sixty-day intervals.

Technic and Interpretation of the Test

Brucellergen should be kept in the refrigerator when not in use. *The vial should be thoroughly shaken before the liquid is withdrawn.* The liquid should be removed from the vial under strict aseptic precautions to maintain the sterility of the remaining portion. The syringe and needle should be sterilized by boiling.

The test is made by injecting about 0.1 cc. of the fluid intracutaneously into the lateral surface of the forearm, using a 26-gauge needle. The local reaction is characterized by a circumscribed erythema, edema, and induration and may vary in diameter from 2 to 10 cm. (See Figure 34.) The reaction should be read at the twenty-fourth and forty-eighth hour after injection. It may persist for seven days. There is rarely, if ever, any necrosis or sloughing

of the tissue at the point of the local reaction. In the infected, the local reaction may be accompanied by a more marked manifestation of the present symptoms. Those who are hypersensitive will show a systemic reaction along with the local reaction. Those who have not been sensitized to *Brucella* and who are probably susceptible to infection show no local or systemic reaction. One often sees in certain normal individuals during the first twenty-four hours an erythema about one to two centimeters in diameter with no edema around the point of injection. It has the appearance of a non-specific reaction.

Skin reactions to the Brucellergen test are recorded and interpreted as follows:

E = Erythema, no significance.
2+ = Edema and erythema 2 cm. in diameter, positive.
3+ = Edema and erythema 2 cm. or more in diameter, positive.
4+ = Edema and erythema 2 cm. or more in diameter, and mild systemic reaction, positive.
5+ = Edema and erythema 2 cm. or more in diameter, and marked systemic reaction, positive.

Brucellergen does not produce skin sensitiveness to subsequent skin tests. One skin injection of Brucellergen may give rise to *Brucella* agglutinins in a low titer in a small percentage of individuals. The agglutinins disappear in about sixty days.

In a recent study of the efficacy of Brucellergen in detecting allergy in 106 *melitensis* brucellosis patients on the Island of Malta, all except 6 were found to show a typically positive reaction. From the results of this study one may expect to find a negative Brucellergen skin reaction in 5.5 per cent of actively infected brucellosis patients. The efficacy of the agent in detecting *Brucella* skin sensitization depends on the existence and continuation of this state in an infected individual. If skin sensitization is absent, as it sometimes is, Brucellergen will fail to elicit a positive reaction.

Supporting Data

The allergic response to different dilutions of Brucellergen in *Brucella*-sensitive individuals is shown in Table XXII. In order to compare concentrations of Brucellergen, the injections were made simultaneously into the skin of one or both arms. Brucellergen in a dilution of 1:2,000 produced a positive reaction in all 125 individuals tested. Of these, 118 were tested with a 1:10,000 dilution and four failed to show a reaction. Five out of 125 persons tested failed to show a reaction to the 1:25,000 dilution and six out of

TABLE XXII

Allergic response to different dilutions of Brucellergen in Brucella-*sensitive individuals*

TOTAL INDIVIDUALS TESTED	DILUTIONS OF BRUCELLERGEN							
	1:2,000		1:10,000		1:25,000		1:50,000	
	Pos.	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*
67	67	–	–	–	64	3	–	–
60	60	–	58	2	–	–	54	6
58	58	–	56	2	56	2	–	–

After Huddleson, Gould, and associates (210)

sixty were negative to the 1:50,000 dilution. The tabulated results show that the efficacy of Brucellergen in detecting *Brucella* allergy decreases as the dilution increases.

Data are presented in Tables XXIII and XXIV to show the relation between the degree of serum agglutination and the degree of intradermal reaction in individuals classified as immune to *Brucella* and in others classified as infected with *Brucella.*

The data are arranged to show the relationship, first, between the size of the intradermal reaction and the titer of specific agglutinins in the blood; and second, between a positive intradermal test and the presence of specific agglutinins in the blood. It is apparent from the tabulated percentages that the size of the intrader-

TABLE XXIII

Relation between the degree of serum agglutination and the degree of intradermal reaction in individuals classified as immune to Brucella

DIAMETER OF REACTION	NUMBER OF INDIVIDUALS	AGGLUTINATION TITER 1:25 −		1:25 T AND P		1:25 +		1:50 +		1:100 +		1:500 +	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
5 to 25 mm.	45	8	17.8	17	37.8	7	15.6	7	15.6	4	8.8	2	4.4
25 to 75 mm.	110	20	18.1	35	31.8	15	13.6	18	16.3	15	13.5	7	6.7
More than 75 mm.	67	10	14.9	15	22.3	17	25.4	17	25.4	5	7.5	3	4.5
Total	222	38	17.1	67	30.2	39	17.6	42	18.9	24	10.8	12	5.4

After Huddleson, Gould, and associates (210)

Agglutination: + = complete; P = incomplete; T = trace; − = none.

TABLE XXIV

Relation between the degree of serum agglutination and the degree of intradermal reaction in individuals classified as infected with Brucella

DIAMETER OF REACTION	NUMBER OF INDIVIDUALS	AGGLUTINATION TITER 1:25 −		1:25 T AND P		1:25 +		1:50 +		1:100 +		1:500 +	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
5 to 25 mm.	196	152	77.6	28	14.3	6	3.0	3	1.5	4	2.0	3	1.5
25 to 75 mm.	290	194	63.3	65	22.4	17	8.6	6	2.0	5	1.7	3	1.0
More than 75 mm.	137	90	65.8	34	24.8	4	2.9	2	1.5	4	2.9	3	2.1
Total	623	436	70.0	127	20.5	27	4.2	11	1.7	13	2.1	9	1.5

After Huddleson, Gould, and associates (210)

Agglutination: + = complete; P = incomplete; T = trace; − = none.

mal reaction and the presence of specific agglutinins bear no relationship to each other. There are, however, noticeable differences between the various agglutination titers obtained and the total number showing a positive intradermal reaction, particularly below and above the 1:25 titer, in both Tables XXIII and XXIV. Of those classified as infected 90.5 per cent failed to show complete agglutination in the 1:25 dilution (Table XXIV). Only 5.3 per cent of those classified as infected and 35.1 per cent of those classified as immune showed agglutinins in significant titers, that is, in 1:50 dilution and above. If the agglutination test alone had been used to determine past and present infection it would have failed to detect 86.9 per cent of the 845 individuals studied.

In tabulating the frequency of the sizes of the intradermal reactions, it was found that approximately twice as many fell between 25 and 75 mm. in size as under or over this size. This difference applies to both the infected and immune group.

In order to determine the constancy of the results of the Brucellergen skin test and of the opsonocytophagic test after the lapse of considerable time, Huddleson, Gould, and associates (210) made a second test five months after the first on a group of individuals, 99 of whom were classified, on the basis of the first test, as susceptible, 105 as infected, and 84 as immune. The comparative results of the two tests are set forth in Table XXV. Of the 105 individuals previously classified as infected, 33, or 32 per cent, were classified by the second test as immune and 3, or 2.8 per cent, as negative. In other words, 3 failed to react to the intradermal test and 33 showed a phagocytosis picture in which 60 per cent or more of the cells were marked. The change from a state of infection to immunity in 32 per cent of the individuals in a period of five months is a common occurrence in subclinical cases of brucellosis. Since the physical examinations of the individuals revealed in many instances nothing suggestive of active infection, it may be

TABLE XXV

Results of second Brucellergen and opsonocytophagic tests on susceptible, infected, and immune individuals five months after first test

SUSCEPTIBLE GROUP				INFECTED GROUP				IMMUNE GROUP			
Number tested	*Negative*	*Negative to infected*	*Negative to immune*	*Number tested*	*Infected to negative*	*Infected to immune*	*Infected*	*Number tested*	*Immune to negative*	*Immune to infected*	*Immune*
99	90	5	4	105	3	33	69	84	–	4	80

After Huddleson, Gould, and associates (210)

assumed that many of those classified as infected in the first test were of the subclinical form.

Of the 84 classified as immune in the first test, four were classified as infected in the retest. The failure of the *Brucella* opsonic test to reclassify 5 per cent of the individuals as immune is possibly due to discrepancies in the test. Of the 187 individuals who had shown a positive reaction to the intradermal test, only 1.6 per cent failed to react to the test after an interval of five months.

Angle and associates (5) have made a very comprehensive survey of the status of 7,122 school children in Kansas City as regards their skin sensitivity to *Brucella.* Of 629 between four and nine years of age, 5.9 per cent were positive. Of the remaining total between ten and nineteen years of age, 9 per cent were positive. In the total group there were 1,117 colored children. Only 2.7 per cent were positive. It was noted that there was a direct correlation between the number of positive reactions in the groups tested and their opportunity to associate with animals or consume raw milk; 79.3 per cent of the positive reactors consumed raw milk. A tuberculin test was made at the same time on these children and it was found that there was no correlation between positive reactions to either allergen.

Other data supporting the value of Brucellergen as a skin test agent for detecting *Brucella* allergy in human beings may be found in Chapter IV, Part Four, pages 157–159, and in Part Three of this chapter (page 255).

PART THREE. THE OPSONOCYTOPHAGIC TEST

While making a study of methods for improving the laboratory diagnosis of brucellosis in human beings, the author and his associates (217) discovered that the neutrophilic leucocytes in whole citrated blood of human beings, who had recovered from brucellosis, phagocytized *Brucella* cells in large numbers in a proper phagocytic system. It was also observed that leucocytes in whole blood from actively infected cases showed a lower degree of phagocytic activity than leucocytes in the blood of individuals after recovery and that the blood of those who had no past or present history of infection showed little if any phagocytosis.

The advantages of using whole blood in a phagocytic system have been pointed out by Shattock and Dudgeon (405), Hektoen (186), and many others. Shattock and Dudgeon employed the term "opsonocytophagic" to define a system that made use of whole citrated blood.

Many investigators have given thought to the interpretation of the results of the opsonocytophagic tests conducted on human beings and animals during or after recovery from many infectious diseases. The interpretations which they have given to the results differ somewhat from those obtained in studies of *Brucella* infection.

For example, a continuous, high phagocytic power of the blood for certain organisms has been interpreted as an indication of the carrier state. Ledingham (263), Hamilton (170), and others have observed that serums of typhoid carriers invariably show a high opsonic index when compared with the serums of normal individuals. Such findings have been considered useful in detecting the typhoid carrier. If the high phagocytic power of the blood for *Brucella* that is found in those who recover from brucellosis and in

many individuals who give no history of the clinical manifestations of the disease is taken as evidence of a carrier state, then there is a large group of individuals in this country who are constantly carrying *Brucella.* The data that have been collected by the author and his associates on blood specimens from human beings do not indicate that a continuous high phagocytosis for *Brucella* signifies a carrier state.

It has been the observation of the author that a positive skin reaction does not have specific significance as a diagnostic measure in doubtful cases of brucellosis, unless the opsonocytophagic test demonstrates the presence of opsonins for the organism. The latter test should be made at the same time the skin test is performed or within seven days after that.

PROCEDURE

The method (217) which has been adopted at Michigan State College for the quantitative estimation of the opsonocytophagic power of blood for *Brucella* is a modification of the Leishman-Veitch technic (265, 460). In the Leishman-Veitch procedure the whole citrated blood of the patient is employed instead of serum mixed with a washed leucocyte suspension.

Preparation of Blood Specimens

Two procedures have been used for measuring immune opsonins quantitatively. One is the well-known and widely used serum dilution system and the other makes use of inhibiting agents, such as inorganic salts. The latter has proved to be simpler and more applicable to the routine examination of a large number of specimens. The method involves the addition of the proper amount of sodium citrate to the blood at the time of collection. The salt serves not only to inhibit clotting, but to retard the action of opsonins in the blood of normal and infected human beings. It has been ob-

served that opsonins are present in lower concentration in the blood of normal and infected individuals than in the blood of those proved to be immune, immunity being determined by failure to become infected when exposed to virulent cultures of *Brucella* or to infective tissues or excretions.

The action of opsonins can be controlled by the addition of sodium citrate in various concentrations. The phagocytic power of blood, determined in an *in vitro* system, is inversely proportionate to the concentration of the sodium citrate present. In other words, by decreasing or increasing the concentration of this salt, one can obtain various degrees of opsonic activity. Such a procedure gives results analogous to those which would be obtained if the opsonin concentration of a serum were measured in various progressive dilutions in the presence of added washed leucocytes.

The blood specimens on which the test is to be made are collected in 5 cc. amounts in glass vials in which has been placed 0.2 cc. of a 20 per cent solution of sodium citrate in physiological salt solution. The final dilution of sodium citrate that obtains in the blood is 0.8 per cent. The test should be conducted on the specimens within six hours after collection. The specimens should be kept in a cool place. The polymorphonuclear cells in blood disintegrate very rapidly when it is kept warm for two or three hours. The specimens should be thoroughly shaken directly before mixing with the bacterial suspension.

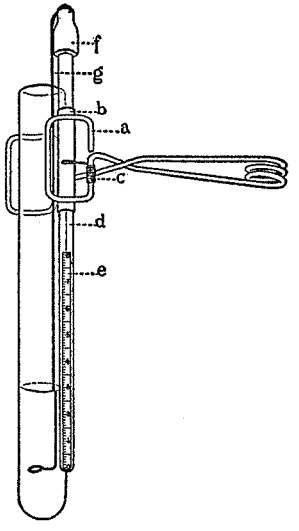
Bacterial Suspension

The importance of employing a known smooth culture of *Brucella* and preparing it in a suspension of the proper concentration for the test cannot be overemphasized. The differences that may obtain in the results of an opsonic test with cultures of *Brucella,* selected without giving attention to their variant characteristics, are emphasized in Table I, page 15. Any one of the three species of

Brucella is suitable, provided the culture does not contain variant cells.

Unless one makes an effort to check the culture employed at close intervals for dissociation, the results of the opsonocytophagic test have little, if any, significance. The culture used for routine testing or for research should be obtained from a laboratory where it has been used and found satisfactory.

Measurement of the bacterial concentration in this test is best done by the Gates apparatus (Figure 35), using a wire loop of 20



After Gates (140)

"To a wire test-tube clamp (a) is soldered a 4 cm. piece of 7 mm. (inside diameter) brass or copper tubing (b) slotted in the middle to receive the free end of a small coiled spring (c) which presses against an 18 cm. length of glass tubing (d), holding the tubing in place, but allowing it to be raised or lowered freely in the tube. A narrow paper centimeter scale (e), reading upward, is sealed within the glass tubing, which is surmounted by a stub of heavy walled rubber tube (f) to hold the end of the No. 20 gauge nichrome wire loop (g). Iron wire may be used, but it rusts and flakes off when repeatedly wet and heated. Nichrome or chromel wire retains its black color and is unaffected by repeated use. The free end of the wire is bent at right angles into a small circle, so that it lies horizontally in the center of the test-tube opposite the zero point on the centimeter scale when the instrument is held in the upright position."

FIGURE 35. THE GATES APPARATUS FOR MEASURING BACTERIAL SUSPENSIONS. ONE-HALF LIFE SIZE

gauge instead of 18, and a 60-watt bulb as a light source. This method of measuring the concentration of dense bacterial suspensions, while it is the best available, is by no means satisfactory. The technic for using the apparatus as described by Gates (140) follows.

In use a measured quantity of the specimen to be estimated is placed in a sterile test-tube, 1.6 by 16 cm., in the clamp. The wire loop, viewed

by looking down into the mouth of the test-tube, is lowered into the suspension and adjusted until it is just beyond the limit of vision through the fluid; i.e., the opacity of the supervening column of suspension is just sufficient to hide the loop. This end-point is more accurately observed than one with the loop faintly visible. The depth of disappearance is then read on the centimeter scale at the bottom of the meniscus, care being taken that the test-tube is held perpendicularly, with the meniscus at the level of the eye. A measured amount of the diluent is then added and mixed by agitation, and the second reading is made. The original volume of the suspension (*vol a*), the amount of diluent added (*vol b* minus *vol a*) and the two observed readings (*a* and *b*) give the necessary data for obtaining the corrected reading (*a* minus *m*) on the suspension. This corrected reading, by comparison with the standard for the given organism, figured by inverse proportion as already demonstrated, gives the concentration of the suspension in millions of organisms per cc. A separate sterile test-tube should be used for each suspension examined. The nichrome wire loop is dried and sterilized in a flame. The rubber cap (f) permits it to be held out at a right angle for this purpose.

The readings and the calculations, on a slide rule, can be made in 2 or 3 minutes when the standard for the given organism is known. Owing to differences in acuity of vision, a certain personal equation is involved in the reading of the end-point, and the standards should be worked out for each observer by comparison of corrected depth of disappearance readings and the corresponding bacterial count. Once the standards are established, suspensions of the same organism can be estimated rapidly. The method should be found useful in vaccine and serological laboratories in which many suspensions have to be standardized.

The bacterial suspension is prepared fresh each day by suspending several platinum loopfuls of the growth from a forty-eight-hour liver agar slant culture in sterile physiological salt solution of pH 7. The turbidity of the suspension should be adjusted to one centimeter by the Gates wire loop or to tube 16 by the McFarland nephelometer.

No factor will affect the results of the phagocytic test so mark-

edly as the lowering of the concentration of the bacterial suspension, or diluting the serum to be tested. Jersild (235) has pointed out that opsonization is a zone phenomenon. A serum that appears to have a low opsonic activity with a given bacterial suspension may have a much higher activity on dilution to 1:2 or 1:3.

The effect of varying the concentration of the bacterial suspension is illustrated by the data presented in Table XXVI. Values

TABLE XXVI

Effect of the concentration of suspended Brucella *cells on phagocytosis*

SOURCE OF BLOOD	TURBIDITY OF ANTIGEN		CELLS SHOWING ACTIVITY			
	Gates	*McFarland*	*Ma*	*Mo*	*S*	*N*
Individuals	0.5 cm.	Not measurable	8	11	6	–
infected with	1.0 cm.	Tube 16	13	10	2	–
Brucella	1.5 cm.	Tube 8	6	15	4	–
	2.0 cm.	Tube 5	–	–	25	–
Individuals	0.5 cm.	Not measurable	25	–	–	–
immune to	1.0 cm.	Tube 16	24	1	–	–
Brucella	1.5 cm.	Tube 8	9	11	5	–
	2.0 cm.	Tube 5	–	6	19	–
Individuals	0.5 cm.	Not measurable	22	3	–	–
immune to	1.0 cm.	Tube 16	21	4	–	–
Brucella	1.5 cm.	Tube 8	–	6	13	6
	2.0 cm.	Tube 5	–	–	25	–

Degree of phagocytosis: Ma = marked; Mo = moderate; S = slight; N = none.

for the various concentrations of bacterial cells are given in terms of the Gates wire loop and the McFarland nephelometer. When the concentration of the bacterial suspension is decreased, the degree of phagocytosis that obtains is markedly lower.

The Test

Into clean small glass vials, such as are used for the agglutination or Kahn test, are placed 0.1 cc. of the whole blood and 0.1 cc.

of the bacterial suspension. After the contents are mixed thoroughly, the vials are placed in a water bath for thirty minutes at 37° C. Continuous agitation during the period of incubation tends to inhibit phagocytosis. Considerable sedimentation of the blood cells takes place during the incubation period. The cells should be resuspended by shaking after the period of incubation. Directly after removing the tubes from the incubator, a small amount of the cell suspension is removed by means of a finely drawn capillary pipette to which is attached a small rubber bulb. A drop of the cell suspension is placed at one end of a thoroughly cleaned and polished glass slide, and drawn across the slide by placing the end edge of another slide at such an angle that the spread becomes thinned out and terminates at or near the opposite end. In a spread of this type, most of the leucocytes may be found near the terminating edge of the spread. The blood film should be dried as rapidly as possible to prevent shrinking of the leucocytes. Rapid drying may be obtained by placing the slides in front of a small electric fan. A small heating unit from an electric heater, if attached to the front of the fan and operated simultaneously, will greatly increase the speed of drying.

In staining spreads, the slides are placed face upward on a suitable rack and the spreads covered with 0.5 cc. of Hastings' stain (Hartman and Leddon Company, Philadelphia). After an exposure of twenty-five seconds, 1 cc. of distilled water adjusted to a pH of 6.4 is added to the stain on the slide. At the end of ten minutes, the spread is gently washed free from stain with distilled water and dried in front of an electric fan.

System of Recording Results

The size of the organism in question and the marked degree of phagocytosis that occurs in cells from immune individuals have necessitated the employment of a different system of recording

phagocytic activity for *Brucella* from that which is commonly used in studies of this nature.

In examining neutrophiles in a blood smear for their phagocytic activity attention should be given to the age of the cell. Old neutrophiles, that is, those in which the nuclei-connecting filaments have disappeared, should not be considered. Old cells do not ingest bacteria.

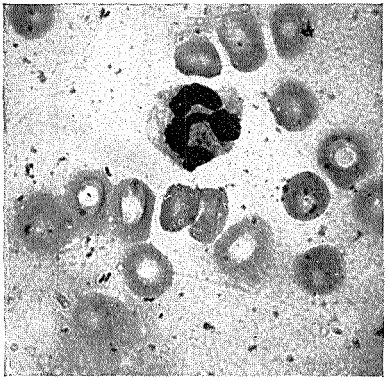
In routine work, a total of 25 cells is counted in different sections of the spread, and each cell is recorded as:

Negative, when no phagocytosis occurs.
Slight, when from one to 20 bacteria are found in the cell.
Moderate, when from 21 to 40 bacteria are found in the cell.
Marked, when the number of bacteria in the cell is above 40.

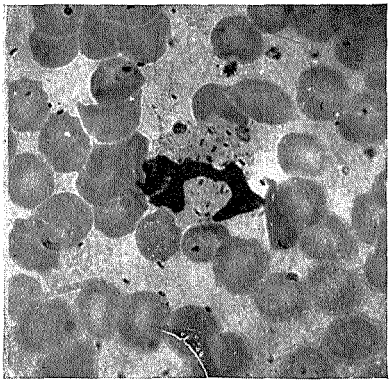
The bacteria are so numerous in those cells showing marked phagocytosis that it is impossible to count them all. Examples of different degrees of phagocytosis of *Brucella* are shown in Figure 36.

The foregoing method of measuring degrees of phagocytosis is admittedly only approximate. It was adopted after making thousands of examinations by different methods.

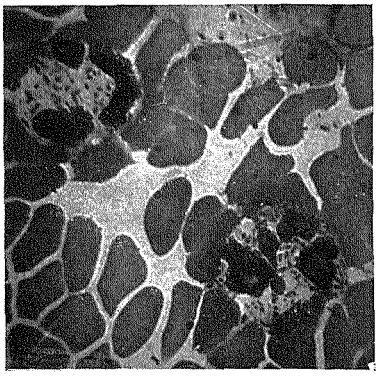
It has been found from a study of the phagocytic test on cases of brucellosis in Malta due to *Br. melitensis* that blood stains such as Hastings' are unsatisfactory for identifying phagocytized bacteria. This is due to the fact that neutrophiles in blood of individuals infected with *Br. melitensis* usually contain a large number of pseudobasophilic granules which when stained cannot always be distinguished from *Brucella* cells. When they are present it is impossible to estimate the degree of phagocytosis with any sort of accuracy. While the author has encountered a similar finding in only one specimen of blood from an individual infected with *Br. abortus,* it would not be surprising to find others. If neutrophiles



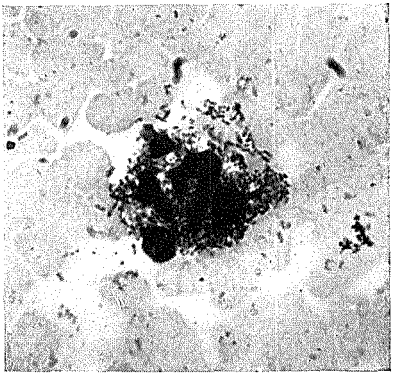
NEGATIVE



SLIGHT



MODERATE



MARKED

FIGURE 36. DIFFERENT DEGREES OF PHAGOCYTOSIS OF BRUCELLA

in the blood of all patients in Malta infected with *Br. melitensis* show bacteria-like staining granules, individuals in the United States infected with *Br. melitensis* probably present a similar picture.

An alternative stain for spreads has been found which will stain the bacteria in neutrophiles and at the same time leave the bacteria-like granules unstained. The procedure for making and applying this stain is as follows:

Fix spreads in chloroform for five to fifteen seconds.

Stain (Calmette, Nègre, and Boquet, 49)

Toluidine blue	0.5 gram
Ethyl alcohol 95 per cent	10.0 cc.
Phenol	3.0 cc.
Distilled water q.s.	100.0 cc.

Dry the spread and stain it with the above mixture for one minute. Wash off excess stain with distilled water. Do not prolong washing. Dry.

The red cells stain green; the nuclei of the leucocytes stain bluish purple; the bacteria stain blue.

Evans (107) has described a method for estimating the phagocytic activity of cells for *Brucella* in thick blood smears, treated before staining with distilled water containing one per cent acetic acid and 5 per cent formalin to dissolve the red cells. Smears thus treated are then stained with Bordet-Gengou's toluidine blue solution. Instead of classifying the number of bacteria in neutrophiles into four groups, Evans correlates the degree of phagocytosis according to the "phagocytic index system" described by Foshay and LeBlanc (134).

The nuclei and bacteria in a cell are so shrunken by the Evans method of fixing and staining that it is not possible to determine with accuracy the degree of phagocytosis. One can only say that a cell shows no, slight, or marked phagocytosis.

In order that the interpretation of the combined agglutination, opsonocytophagic, and allergic tests may be clarified with respect to the status of a given individual toward *Brucella* infection, a system of diagnosis according to the combined results of the three tests is set forth in Table XXVII. If an individual shows a positive intradermal reaction, and the agglutination and opsonocytophagic tests are negative, the intradermal reaction should be considered non-specific.

TABLE XXVII

Proposed system for the diagnosis of brucellosis according to the results of the agglutination, allergic, and opsonocytophagic tests

AGGLUTINATION TEST	ALLERGIC SKIN TEST	OPSONOCYTOPHAGIC POWER OF BLOOD	STATUS TOWARD BRUCELLA
−	−	Zero to 20 per cent of cells, slight	Susceptible
−	+	Zero to 40 per cent of cells, marked	Infected ?
+	+	Zero to 40 per cent of cells, marked	Infected
−	+	60 to 100 per cent of cells, marked	Immune
+	+	60 to 100 per cent of cells, marked	Immune

Clinical laboratories and investigators have encountered instances when the Brucellergen test was negative, although the leucocytes in whole blood showed a considerable degree of phagocytic activity. There are two possible explanations for this. The patient may have opsonins for *Brucella* in the blood because he is actively infected with *Bact. tularense* or has recently recovered from an infection due to this organism; two such cases have been brought to the attention of the author. Or the patient may be one of a small percentage infected with *Brucella* who fail to develop skin allergy.

The author has also found that the results of the opsonocyto-

phagic test in patients with *melitensis* brucellosis often cannot be given the same interpretation as results of the test in patients with *abortus* or *suis* brucellosis. Blood taken during the course of the disease from patients infected with *Br. melitensis,* and sometimes blood from which *Br. melitensis* can be cultured, shows the same phagocytic picture that is presented by blood taken from immune individuals.

DATA SUPPORTING THE VALUE OF THE TEST

Examination of Blood from Cases Diagnosed as Brucellosis

Each of the nineteen cases presented in Table XXVIII showed clinical symptoms similar to those seen in acute and chronic bru-

TABLE XXVIII

Opsonocytophagic power of blood from cases diagnosed as brucellosis

CASE NO.	SEX	DATE OF ONSET*	DATE OF TESTS	AGGLUTINATION TEST	ALLERGIC SKIN TEST†	CELLS SHOWING ACTIVITY			
						Ma	*Mo*	*S*	*N*
1	Male	12–'29	12/19/31	+1:500	Positive	–	8	10	7
2	Male	2–'31	4/13/31	+1:500	None made	–	–	4	21
3	Female	3–'31	5/15/31	+1:500	Positive	–	–	10	15
4	Male	7–'31	12/ 4/31	Negative	Positive	–	–	–	25
5	Male	11/10/31	1/18/32	+1:500	Positive	–	–	5	20
6	Male	1/18/32	2/17/32	+1:500	Positive	6	4	15	–
7	Female	5–'30	4/ 7/32	Negative	Positive	–	–	–	25
8	Male	2/20/32	4/13/32	+1:500	Positive	–	2	3	20
9	Male	4/ 3/32	4/21/32	+1:500	Positive	–	–	21	4
10	Boy	3/10/32	5/15/32	+1:500	Positive	–	4	8	13
11	Female	5/ 1/32	5/16/32	+1:500	Positive	–	3	5	17
12	Male	4/10/32	5/16/32	+1:500	Positive	–	6	6	13
13	Male	3/20/32	5/16/32	+1:500	Positive	1	2	4	18
14	Male	6/ 7/32	6/ 9/32	+1:500	Positive	3	4	14	4
15	Female	6/10/32	6/24/32	+1:500	Positive	–	2	19	4
16	Male	6/ 1/32	6/30/32	+1:500	Positive	–	3	22	–
17	Female	2–'30	7/ 7/32	Negative	Positive	3	4	15	3
18	Girl (3 years)	6/30/32	7/11/32	Negative	Positive	–	–	–	25
19	Infant (17 months)	7/19/32	7/22/32	Negative	Positive	–	–	4	21

Degree of phagocytosis: Ma = marked; Mo = moderate; S = slight; N = none.
Agglutination: + = complete.
* Approximate. † Made with Brucellergen.

cellosis before a laboratory diagnosis of the disease was made. The laboratory diagnosis was based on either a positive blood culture or on the combined results of the agglutination, allergic, and opsonocytophagic tests. In the absence of a positive blood culture and positive agglutination test, the diagnosis was made on the basis of the results of both the allergic test and the opsonocytophagic test. No diagnosis was based on these two tests until sufficient data had been accumulated from previous studies to warrant their application to the diagnosis of brucellosis.

The laboratory tests and the allergic skin test were used in the diagnosis of the disease within a few days after the onset of symptoms in a few cases, while in others the interval varied from six weeks to approximately two years.

Brucella melitensis was isolated from the blood of cases 3, 14, and 15. These were laboratory infections. *Br. abortus* was isolated from the blood of cases 5, 8, 9, and 13. Blood cultures from the remaining twelve cases were negative.

It may be noted from the data presented in Table XXVIII that the opsonocytophagic power of the blood is low or absent in all cases with the exception of No. 6. This individual had a comparatively mild form of the disease as expressed in terms of clinical symptoms. Experience with this test has shown that the phagocytic power rises and falls during the course of the disease. If the invading organism is very active in the body, the phagocytic activity of the cells is likely to be low. On the other hand, if it is not very active, as indicated by clinical symptoms, the phagocytic activity of the cells will be higher.

In view of the fact that many apparently normal and ill individuals may show a positive Brucellergen reaction when the blood culture and the agglutination test are negative, a diagnosis of brucellosis based on a positive allergic skin test combined with the opsonocytophagic test might be questioned. We have given con-

siderable attention to this question in our studies. The contention that a diagnosis of brucellosis can be made on the basis of the two tests in question is supported by data which are to follow concerning the degree of ingestion of the organisms on the part of the cells in blood examined shortly after and long after recovery from the disease, as compared to that which takes place in the blood from patients during the course of the disease. Further support is offered by the fact that those cases in question which respond to specific treatment with Brucellin also show marked phagocytosis of *Brucella* cells.

TABLE XXIX

Opsonocytophagic power of blood from cases during and after recovery from brucellosis

DATE OF ONSET	DATE OF TEST	CELLS SHOWING ACTIVITY *Ma*	*Mo*	*S*	*N*	DATE OF RECOVERY	DATE OF TEST	CELLS SHOWING ACTIVITY *Ma*	*Mo*	*S*	*N*
11/10/31	1/18/32	–	–	5	20	1/30/32	2/ 5/32	25	–	–	–
2/ 1/31	4/13/32	–	–	4	21	4/20/32	4/23/32	23	–	2	–
2/20/32	4/15/32	–	2	3	20	4/26/32	5/15/32	23	2	–	–
3/20/32	5/15/32	–	4	8	13	5/18/32	6/11/32	25	–	–	–
4/10/32	5/16/32	–	6	6	13	7/15/32	7/23/32	25	–	–	–
3/20/32	5/16/32	1	2	4	18	5/26/32	6/11/32	19	6	–	–
6/ 7/32	6/ 9/32	3	4	14	4	6/20/32	7/26/32	25	–	–	–
6/10/32	6/24/32	–	2	19	4	7/ 5/32	7/ 7/32	25	–	–	–

Degree of phagocytosis: Ma = marked; Mo = moderate; S = slight; N = none.

Examination of Blood from Patients During and After Recovery from Brucellosis

The comparative results are illustrated in Table XXIX on eight known cases of brucellosis. The opsonocytophagic test was made during the disease from two days to two months after the onset. The time of making the test on each case after recovery varied from two days to approximately one month. The data clearly show that the opsonocytophagic power of the blood is low during the disease and becomes very marked after recovery.

Examination of Blood from Individuals Known to have had Brucellosis

Table XXX illustrates the results of the opsonocytophagic test and agglutination test on fifteen individuals after recovery from brucellosis. These cases are not included in the group in Table XXIX. The tests were made at intervals varying from thirty-six days to four years after recovery from the disease.

TABLE XXX

Opsonocytophagic power of blood from individuals after recovery from brucellosis

CASE NO.	SEX	PERIOD OF DISEASE	DATE OF TESTS	CELLS SHOWING ACTIVITY				AGGLUTINATION TEST
				Ma	*Mo*	*S*	*N*	
1	Male	1–'30 to 4–'30	2/ 4/31	21	–	–	4	+1:25
2	Male	12–'26 to 4–'27	4/ 1/31	25	–	–	–	+1:25
3	Male	1–'26 to 12–'26	4/17/31	22	1	2	–	–
4	Male	7/ 3/30 to 7/15/30	4/17/31	22	2	–	1	–
5	Male	1–'28 to 4–'28	4/17/31	21	2	1	1	+1:50
6	Female	5/ 5/30 to 5/25/30	4/20/31	22	–	3	–	+1:25
7	Male	5–'30 to 6–'30	4/29/31	24	–	1	–	+1:50
8	Female	5/ 1/32 to 5/31/32	5/31/32	25	–	–	–	+1:500
9	Female	3–'31 to 5/25/31	5/31/31	25	–	–	–	+1:500
10	Male	12/ 6/29 to 1/15/30	4/17/31	25	–	–	–	+1:50
11	Male	4/15/30 to 6/30/30	2/ 8/32	23	2	–	–	–
12	Male	2–'29 to 4–'29	3/31/32	25	–	–	–	+1:25
13	Male	1–'31 to 3–'31	7/ 7/32	25	–	–	–	+1:25
14	Male	3–'30 to 5–'30	7/ 7/32	25	–	–	–	–
15	Male	9–'30 to 12–'30	7/ 7/32	25	–	–	–	P1:25

Degree of phagocytosis: Ma = marked; Mo = moderate; S = slight; N = none.
Agglutination: + = complete; P = incomplete.

The opsonocytophagic power of the blood for *Brucella* is very marked in all cases, regardless of the interval between recovery and the phagocytic examination. It is interesting to note that there is no relation between the *Brucella* agglutination titer of the individual's serum and the ingestion capacity for *Brucella* of the leucocytes in the blood.

Individuals whose blood shows a marked opsonocytophagic power for *Brucella in vitro,* like those cases illustrated in Table

XXVIII, will also show an allergic skin reaction of the same degree of severity as those who are infected with *Brucella.*

In Table XXXI are presented data on seventeen packing-house employees, to support the contention that those who are constantly exposed to *Brucella*-infective materials or to animals infected with

TABLE XXXI

Opsonocytophagic power of blood from a group of packing-house employees after exposure to Brucella*

CASE NO.	AGGLUTINATION TEST	CELLS SHOWING ACTIVITY				CLINICAL EVIDENCE OF INFECTION
		Ma	*Mo*	*S*	*N*	
1	+1:25	22	2	1	–	No
2	+1:500	4	4	17	–	Yes
3	Negative	23	2	–	–	No
4	+1:100	18	7	–	–	No
5	Negative	23	2	–	–	No
6	Negative	–	–	–	25	No
7	Negative	–	–	6	19	No
8	+1:500	6	11	7	1	Yes
9	+1:500	–	3	8	14	Yes
10	+1:50	24	1	–	–	No
11	+1:500	2	11	9	3	Yes
12	+1:500	No test made				Yes
13	Negative	25	–	–	–	No
14	+1:50	22	3	–	–	No
15	Negative	–	–	2	23	No
16	+1:500	No test made				No
17	+1:500	–	16	9	–	Yes

Degree of phagocytosis: Ma = marked; Mo = moderate; S = slight; N = none.
Agglutination: + = complete.
* The examinations recorded were made July 28, 1932. Examinations made November 12, 1931, showed no agglutination and all cells negative. Date of exposure unknown.

Brucella will sooner or later become infected. Blood specimens of these men were examined on November 12, 1931, and reacted negatively to both the agglutination and opsonocytophagic tests. One of the men in this particular group, engaged in the manufacture of sausages, developed clinical brucellosis in June 1932. An examination on July 28, 1932, of the blood of each, including the one, No. 17, who was showing symptoms typical of the dis-

ease, revealed valuable information pertaining to *Brucella* infection and immunity. The results of the agglutination test showed that eleven had been exposed to infection; that is, the organism had passed beyond the epithelial barrier of the skin or mucous membranes. The opsonocytophagic test was conducted on all except two. The results of this test showed that all those reacting to the agglutination test and two others not reacting had been exposed to infection. The blood of those who were showing symptoms of the disease, Nos. 2, 8, 9, 11, and 17, had low phagocytic power for *Brucella.* The phagocytic power of the cells was either marked or negative in those showing no symptoms of the disease. *Br. suis* was isolated from the blood of No. 11.

Examination of Blood from Large Groups of Individuals

The groups in question were veterinarians, packing-house employees, college students enrolled in bacteriology courses, hospital patients, and inmates of a state prison.

The results of the opsonocytophagic test and agglutination test for *Brucella* in these groups are presented in Table XXXII. Most of the twenty veterinarians examined had been engaged in cattle practice. The opsonocytophagic power of the blood of nineteen was very marked for *Brucella.* None gave a history of having clinical symptoms characteristic of brucellosis. Many of them, however, report the appearance of eruptions on their arms after removing retained placentae from aborting cows. This sign indicates hypersensitiveness to *Brucella* (see Figure 15, Skin eruptions due to *Brucella* allergy, page 100). The skin test is always positive in such cases.

Blood specimens were taken from 176 men and women in three packing houses and one stockyard in Michigan. Most of the men were engaged in work which necessitated the handling of fresh pork or beef. The women were employed in wrapping cured ba-

TABLE XXXII

Opsonocytophagic power of blood from certain groups of persons; also estimated percentage exposed to Brucella *infection*

GROUP	NUMBER TESTED	DATE OF TEST	CELLS SHOWING ACTIVITY			AGGLUTINATION TEST	PER CENT EXPOSED TO INFECTION
			All cells marked	*Few cells slight*	*All cells negative*		
Veterinarians	20	6/20/31	19	–	1	From negative to 1:500	95.0
Packing-house employees	176	11/12/31	40	47	89	From negative to 1:500	22.7
College students	29	5/10/31	5	9	15	From negative to incomplete 1:25	17.2
Hospital patients	240	1931–32	30	45	165	From negative to 1:50	12.0
Men in state prison	133	7/22/32	14	26	97	All negative except one, 1:500	10.5

con for market. The stockyard employees came in contact with live animals only. The results of the opsonocytophagic test alone indicate that 40, or 22.7 per cent, of the employees have at some period in the past been infected with *Brucella.* Of the total examined, three were showing clinical symptoms of brucellosis at the time the blood specimens were collected.

The twenty-nine college students were in two separate groups, one of which was examined about one year after the other. Of the total number examined, the blood of five, or 17.2 per cent, showed the degree of phagocytosis observed in those who have at one time been infected with *Brucella.*

The hospital patients from whom blood specimens were examined constituted a rather miscellaneous group of males and females. They represented cases of infectious and organic disease, injuries, and blood donors. Of the total number examined, the blood of thirty, or 12 per cent, showed the degree of phagocytosis observed in those who have at one time been infected with *Brucella.*

The prison inmates examined were of two groups, namely, kitchen personnel and patients in the tuberculosis hospital. The latter group may be divided into diagnosed cases and suspects. Of those examined, the blood of fourteen, or 10.5 per cent, showed a marked degree of phagocytosis for *Brucella.* These men came from all walks of life and should represent a cross-section of what one would expect to find from an examination of the blood of the general population. Patients examined in the hospitals and students also represent a cross-section of the general population.

Meyer and associates (300) have summarized in Table XXXIII the results of phagocytic tests made on 1,285 individuals. They are of the opinion that the presence of *Brucella* opsonins in the blood of an individual is an indication of latent infection.

Keller, Pharris, and Gaub (244), who have made a very thorough

TABLE XXXIII

Phagocytic tests on persons exposed to Brucella

OBSERVER	PLACE	NO. OF PERSONS TESTED	PHAGOCYTIC TEST	ESTIMATED PER CENT OF LATENT INFECTION	OCCUPATIONAL GROUP
I. F. Huddleson, H. W. Johnson, and E. E. Hamann	Michigan	20	19	95.00	Veterinarians
		176	40	22.70	Packing-house employees
		29	5	17.20	College students
		240	30	12.50	Hospital patients
		133	14	10.50	State prisoners (men)
K. F. Meyer, B. Eddie, L. Veazie, and B. Stewart	California	36	33	91.60	Hospital patients
		27	17	62.90	Laboratory workers
		67	3	4.47	Clinic patients
		42	4	9.50	Medical students
		27	–	–	Dental students
		100	74	74.00	Veterinarians
		161	108	67.08	Butchers, slaughterers
		111	55	49.50	Wool-workers, skin-tanners, etc.
		40	19	47.50	Truck drivers, teamsters
		30	17	56.60	Stockmen, laborers
		24	13	54.16	Mechanics, sales department
		22	10	45.45	Meat inspectors, technicians
	Total	1,285	461	35.87	

After Meyer and associates (300)

study of the *Brucella* diagnostic tests according to procedures recommended by the author, summarize their findings as follows:

"(1) In all of a series of eight patients, who had had brucellosis for periods varying from four weeks to fifteen months, the skin test performed with the nucleoprotein of *Brucella* was positive. In one recovered patient the agglutination test was negative, in one case of seventeen months' duration the agglutination titer was 1/20, in two patients the titer was 1/80, in two cases the titer was 1/160, and in the remaining two cases the titer ranged from 1/640 to 1/5,120. In six patients in this group the opsonocytophagic test showed marked phagocytosis in a majority of the polymorphonuclear leukocytes examined, indicating immunity according to Huddleson, Johnson, and Hamann. In two of the patients one of whom had been ill for four weeks and one who had chronic brucellosis of seventeen months' duration, the opsonocytophagic test showed phagocytosis in a majority of the leukocytes examined, but not to a degree sufficient to indicate that immunity had been established.

"(2) In a series of seven individuals, three laboratory technicians working with *Brucella* and four veterinarians, the skin test was positive in six instances. In one veterinarian who had received experimentally one injection of *Brucella* vaccine, the allergic skin test was negative. In three members of this group, the agglutination titer was 1/40; in one it was 1/80; in one it was 1/320; and in two of the veterinarians it was negative. In five individuals a majority of the leukocytes showed marked phagocytosis; in one person phagocytosis was demonstrated, but not to a marked degree; and in one person a majority of the leukocytes showed no phagocytosis. The opsonocytophagic test in the vaccinated individual showed marked phagocytosis in a majority of the leukocytes examined. In each of the persons in this group the agglutination test with *P. tularensis* was negative.

"(3) The skin test was positive in sixty or 4.8 per cent of 1,247

apparently well individuals in two institutions. Agglutination tests were performed on all of these individuals and in twelve or 1.0 per cent of this group titers ranged from 1/20 to 1/160. In eight instances the titer was 1/20, in one case the titer was 1/40, in two it was 1/80, and in one individual it was 1/160. In five instances in this group, the agglutination titer was found to vary from 1/20 to 1/80 in persons with negative *Brucella* skin tests.

"(4) In 155 persons showing positive *Brucella* skin tests, 12 or 7.8 per cent were found to have agglutination tests varying in titer from 1/20 to 1/160. This finding suggests the greater efficiency of the skin test in determining infected individuals.

"(5) In classifying the immunity status by means of the opsonocytophagic test in 155 persons with positive *Brucella* skin tests, it was found that 51.6 per cent were infected with no immunity, 8.4 per cent were partially immune, and 40.0 per cent were immune.

"(6) The specificity of these tests has been reported previously in a group of patients in the wards of the Vanderbilt University Hospital."

Examination of Blood of Students During an Epidemic of Melitensis *Brucellosis*

During a small epidemic of brucellosis among students due to *Br. melitensis,* the author (219) studied the comparative value of the diagnostic tests in 41 clinical cases and 49 others associated with them. The results of the tests are presented in Table XXXIV.

The results of the blood cultures, the skin test, and phagocytosis test for each case are grouped together according to their maximum agglutination titers. Of the 4 clinical cases whose blood sera were negative to the agglutination test, 3 showed a positive blood culture on the same date. The blood examinations in 2 of the 4 cases were made ten days and seven days after onset and in one, three days before the onset. The blood culture was positive in 12 other clinical

TABLE XXXIV

Results of the blood culture test, the skin test, and the phagocytosis test in 41 clinical and 49 subclinical cases, grouped by their maximum agglutination titer

	CLINICAL CASES				SUBCLINICAL CASES			
	Neg.	*1:25*	*1:50*	*1:100 or higher*	*Neg.*	*1:25*	*1:50*	*1:100 or higher*
Agglutination titer	4	12	7	18	26	8	6	9
Blood culture								
Negative	1	–	–	5	25	8	5	8
Positive	3	12	7	13	1	–	1	1
Brucellergen test								
Negative	–	–	–	–	1	1	1	–
Positive	4	12	7	18	25	7	5	9
Phagocytosis								
Low or moderate	3	10	6	14	16	6	4	7
Marked	1	2	1	4	–	1	1	2
Negative	–	–	–	–	10	1	1	–

cases which at the time showed a maximum agglutination titer of 1:25.

The Brucellergen skin test was positive in all clinical cases. The size of the local reaction varied from 2 to 6 cm. The results of the phagocytosis test were confusing in 8 of the 41 clinical cases in that the phagocytic picture was similar to that observed in immune individuals. It is obvious from what is now known of the limitations of each of the laboratory tests that one cannot place too much reliance on any one test to confirm the diagnosis of brucellosis. The results of all the available tests must be carefully analyzed in terms of the past and present state of health of the patient before a diagnosis of the disease can be arrived at. And this should be the task of the attending physician rather than the diagnostic laboratory.

THE OPSONOCYTOPHAGIC TEST APPLIED TO THE BLOOD OF CATTLE

After considerable preliminary study it was found that the phagocytic system applicable to the study of human blood was not satisfactory for cow's blood (218). This is chiefly because the blood of

cattle, when mixed with a heavy suspension of *Brucella* in 0.85 per cent salt solution, agglutinates the bacteria rapidly into large clumps during the incubation period. Clumping of the bacterial cells occurs in the presence of whole blood regardless of whether the blood comes from infected or non-infected animals. Rapid bacterial clumping makes it impossible to obtain an accurate estimation of the phagocytic power of the polymorphonuclear cells, or to obtain results on repeated examinations of the same blood samples that check with a close degree of accuracy.

It was evident, therefore, that before a satisfactory phagocytic system could be employed to determine the phagocytic power of cow's blood for *Brucella* it would be necessary to find a means of retarding the clumping of the bacterial cells during the incubation period. The difficulty was finally solved by suspending the bacterial cells in distilled water containing the proper amount of barium chloride and sodium chloride.

For cattle the same procedure may be used for measuring the phagocytic power of the blood that was found satisfactory for human beings, with the following modifications.

The blood specimens are collected in 5 cc. amounts in vials containing 0.4 cc. of a 12.5 per cent sodium citrate solution. The final dilution of sodium citrate that obtains in the blood is 1.0 per cent.

The bacterial suspension is prepared in distilled water containing 0.6 per cent sodium chloride and 0.5 per cent barium chloride. The stock solution should be kept tightly stoppered. The turbidity of the bacterial suspension should read one cm. on the Gates wire loop apparatus.

The opsonocytophagic power of blood from the cow is determined and the results of the test given the same interpretation as those obtained with human blood.

PART FOUR. THE BLOOD PICTURE IN BRUCELLOSIS IN HUMAN BEINGS AND IN BOVINE ANIMALS

In Human Beings

There are scattered reports in the literature to the effect that the blood picture in brucellosis is characterized by a leucopenia with a relative lymphocytosis, a slight to moderate secondary anemia, and a color index of less than one.

Rainsford (374) has studied the blood picture in a large number of cases of brucellosis in Malta. The findings on twelve patients which he studied are presented in Table XXXV. His discussion of the results follows.

"It will be seen that at an early stage of the disease and in the acute stage a leucopenia is commonly present; it will also be noticed that a low mononuclear count was present in practically all cases during the acute stages: these two facts alone are most helpful in diagnosis. When a patient first comes under observation with high fever and indefinite physical signs, it is difficult for the clinician to decide what laboratory investigations to ask for; the blood-count can in these circumstances afford some indication. If a leucopenia is present it helps to rule out at once either pneumonia or that very large group of diseases, hidden pyogenic infections. The field for investigation consequently becomes narrowed down to a very considerable degree. In Malta, the differential diagnosis usually lies between enteric, undulant, sandfly and malarial fever: the low mononuclear count helps to exclude malaria; also if the case is one of malaria, the parasites will nearly always be found when the differential count is being made. Enteric, undulant and sandfly fevers all give low leucocyte counts: the leucopenia also helps to rule out coliform infections of the urogenital tract, as these generally give rise to a leucocytosis. Sandfly fever is frequently accompanied by pain in one or both loins, and a differ-

TABLE XXXV

Blood picture in twelve cases of brucellosis in Malta

CASE NO.	DATE OF ONSET	DATES OF BLOOD COUNT	BLOOD PICTURE SHOWING NUMBER OF CELLS PER C.MM.					REMARKS
			Total W.B.C.	*Poly-morphs*	*Lympho-cytes*	*Mono-nuclears*	*Eosino-philes*	
1	6/24/31	8/17/31	6,520	3,160	2,940	420	–	Very acute case that improved slowly; acute stage
		9/24/31	7,000	2,730	3,780	420	–	Showing signs of improvement
2	8/ 6/31	9/18/31	10,400	4,576	5,408	416	–	Acute case in acute stage
		9/24/31	7,200	2,664	4,104	432	–	Commencing to improve
3	9/18/31	2/ 2/32	10,400	3,952	6,136	312	–	Convalescent
4	7/21/31	8/15/31	10,200	4,488	5,151	306	255	Very acute
		9/23/31	10,600	5,406	4,717	424	53	Improving
		1/22/32	13,200	9,900	3,036	264	–	Relapsed and now chronic; very ill and febrile
5	1/ 5/32	3/ 1/32	8,400	3,780	4,200	336	84	Convalescent; pulmonary type originally
6	1/31/32	2/16/32	5,600	2,668	2,596	336	–	Very acute case that was cured quickly; acute stage
		3/11/32	5,600	2,520	2,800	280	–	
		3/21/32	5,200	3,328	1,664	156	52	
7	3/22/32	4/ 5/32	9,800	6,664	2,940	98	98	Acute case
		4/17/32	8,600	4,902	3,440	258	–	Showing signs of improvement

TABLE XXXV (*cont.*)

CASE NO.	DATE OF ONSET	DATES OF BLOOD COUNT	BLOOD PICTURE SHOWING NUMBER OF CELLS PER C.MM. *Total W.B.C.*	*Poly-morphs*	*Lympho-cytes*	*Mono-nuclears*	*Eosino-philes*	REMARKS
8	2/ 9/32	2/16/32	5,800	3,190	2,494	116	–	Very acute case that did not improve on treatment
		2/29/32	6,400	4,544	1,600	256	–	
		3/11/32	5,600	2,856	2,632	112	–	
		3/28/32	10,400	7,592	2,496	312	–	After sodium nucleinate showing slight signs of improvement
		4/ 8/32	5,600	3,696	1,568	336	–	
9	4/18/32	4/28/32	6,800	3,944	2,662	194	–	Very acute case that showed little signs of improvement while under treatment; acute stage
		5/17/32	5,600	3,136	2,352	112	–	
		6/ 1/32	6,200	3,534	2,418	248	–	Very slightly improved
10	5/10/32	5/19/32	4,400	3,036	1,188	176	–	Extremely mild abortive type of case; acute stage
		6/ 1/32	5,800	3,074	2,668	58	–	Afebrile
11	5/20/32	5/26/32	11,000	6,930	3,630	440	–	Mild case that recovered rapidly
		6/16/32	10,400	5,616	4,472	312	–	Acute stage
		6/23/32	6,000	3,050	2,442	442	66	Almost convalescent
12	5/22/32	6/23/32	11,190	5,824	4,480	886	–	Acute case that had not improved up to time of leaving hospital on fifty-third day after onset

After Rainsford (374)

ential diagnosis between sandfly fever and coliform infections of the kidneys is greatly assisted by the knowledge of whether a leucocytosis is present or otherwise. If, therefore, the clinician acquires early information of the presence of a leucopenia, it usually means that he obtains information concerning the agglutination reactions to enteric and *Brucella* groups early, as one leads to another; it will also probably mean that a blood-culture is taken at a time when a positive result is most likely. It is of utmost importance to receive early information of the agglutination reactions, for if at a later date a rise in titer is noted a definite diagnosis can be made. This fact, however, concerns the enteric group of diseases more than the *Brucella* group.

"From a study of the table it will be seen that a rise in the total white-cell count and in the mononuclears was usually accompanied by an improvement in the condition of the patient; the most obstinate cases to treatment failed to show any improvement in their leucocyte count. It is to be noted, however, that in some cases, although the leucopenia persisted, the mononuclear count rose and that these cases improved: the blood-count is consequently of some assistance in prognosis. It is only rational to assume that the first line of defence in a disease characterized by bacteriaemia will be that offered by the reticuloendothelial system. If, therefore, it is considered that the large mononuclear cells in the peripheral blood are a unit of this system, then it becomes easier to understand why an increase in those cells should be accompanied by an improvement in the clinical condition.

"It has only been possible to obtain the blood-picture in one chronic case (No. 4); this case at first improved and was discharged cured; after leaving hospital he relapsed and was re-admitted; it will be seen that in the chronic stage his blood condition showed a leucocytosis, but that the mononuclears were still well below the normal number; blood-cultures taken during the chronic

stage all proved sterile. It is, therefore, probable that pathologically this stage is characterized by a local invasion of some tissue with local necrosis and caseation as seen in experimental infections in animals; this fact might account for the leucocytosis and polymorphonuclear increase.

"Convalescent cases generally show a relative lymphocytosis like that which follows most infections."

Munger (322, 324), in the author's laboratory, has studied the blood picture in 32 cases of *melitensis* brucellosis occurring on the Island of Malta. Her findings follow.

"SIZE OF RED BLOOD CELLS. There are no references pertaining to the size of the red blood cells in brucellosis. A study of their size in these cases revealed that there is a marked variation in the blood of brucellosis patients from the normal. In 35 per cent of the patients studied it was found that an increased number of the cells were smaller than normal, and in 19 per cent there was a tendency of the cells to be larger than normal. The average red cell measurement formula was of a 39–37–24 ratio instead of the 33–34–33 normal ratio. The red cells of some patients varied in size from 3 to 10 microns, although the normal variation is from 6 to 10 microns. This variation in size is probably effected by an accompanying splenic and liver pathology.

"THE WHITE BLOOD CELLS. The white blood cell count on the average is lower than normal with a relative and absolute monocytosis. There is a quantitative increase in the non-filamented neutrophiles."

One interesting point that has not been noted before is that many of the mature small lymphocytes are much larger than normal. These have been termed "pathologic lymphocytes" or "large mature lymphocytes." These cells have all the appearance of small lym-

phocytes, the ratio of the nucleus to cytoplasm being similar to that of the normal small lymphocyte. The pathologic lymphocytes are about 12 to 14 microns in diameter. The small lymphocytes usually measure from 8 to 10 microns. As many as 30 to 80 per cent of the lymphocytes may appear as this type of cell in about 40 per cent of brucellosis patients.

Sabin (395) by supervital staining has found, in cases of brucellosis, an increase in the type of monocyte which is similar morphologically to the type of monocytes associated with various forms of hepatic involvement. These appear to be similar to the atypical monocytes found in catarrhal jaundice (395). In disease associated with liver pathology, Isaacs (230) has described "a cell averaging 15 by 13 microns, with an oval nucleus, rather dense chromatin (lymphoid in character), foamy blue-staining cytoplasm (monocytoid), but with absence of the minute, red staining granules of the monocyte. There is no perinuclear clear zone, as in the lymphocyte. Occasional inclusion granules are found in the cytoplasm. The margin is wavy." The "liver damage cell" of Isaacs was present consistently in all of the cases of brucellosis examined.

Another interesting observation that has been noted in patients infected with *Brucella melitensis* is a marked basophilia of the granules of the neutrophiles. This phenomenon may be associated with the temperature elevation that the patients experience. It is believed that this is characteristic of *melitensis* infection since it has rarely been encountered in *suis* and *abortus* infections. The granules are similar in size to the *Brucella* bacteria and stain similarly.

In Bovine Animals

Bell and Irwin (19) studied the blood cell changes in two groups of cows following induced *Brucella* infection. One group was susceptible and one resistant to infection. Both groups showed a significant drop in the average number of leukocytes per cubic milli-

meter of blood following exposure. Then both groups following infection showed a definite rise in the polymorphonuclear cells.

A marked increase in the monocytes was noted in the susceptible group. All changes noted, however, had no great significance as a means of determining infection.

VII

ERADICATION OR CONTROL OF SOURCES OF BRUCELLOSIS INFECTION

Brucellosis in man rarely if ever spreads to other human beings although it is conceivable that it is contagious from man to man under certain conditions; certainly it is the part of wisdom to take suitable precautions against such a possibility. The control or prevention of brucellosis hominis is a problem in animal hygiene and veterinary medicine, and this is fortunate since veterinary medicine is better organized for the control of this disease than human medicine.

The ideal solution of the brucellosis problem would be the eradication of *Brucella* by destroying all sources or reservoirs of the various species of the organism. These reservoirs are, so far as we know, the infected domesticated animals, especially the goat, cow, pig, and to a lesser degree, the importance of which cannot now be appraised, the sheep, horse, and barnyard fowl. It is doubtful whether the proved existence of *Brucella* in the dog, water buffalo, elk, and various species of fowl has any practical significance so far as the problem of control is concerned. The program of eradication may involve any geographical area or political unit depending upon economic and other circumstances, and may be considered a success in the limited section where the program is carried out. However, it must be recognized that the brucellosis-free island thus created may be entirely surrounded by the dangerous water of infection. The "splendid isolation" thus created must be maintained at the price of an eternal vigilance, the armaments of which are furnished by modern sanitary science with its scientific under-

Note: This chapter has been contributed by Ward Giltner.

standing of infection and its socially acceptable quarantine and isolation measures. Where more or less complete eradication is not practicable, compromise measures must be provided.

BRUCELLOSIS BOVIS

Fortunately, especially in this country, there is wide experience in animal disease control. It will suffice to call attention to the modes of attack that have proved effective against three types of disease: exotic plague; a well-established geographically limited epizootic disease (Texas fever); a disease that was probably imported but which is rapidly becoming panzootic (tuberculosis). There is little or nothing in the procedures employed to eradicate the first two types of disease that seems applicable to a well-considered campaign against brucellosis. However, a consideration of tuberculosis readily suggests that we have here an animal disease that resembles brucellosis in many respects.

There are significant differences between tuberculosis and brucellosis so that, in spite of the many points of similarity, we may not hope that an exact duplicate of the plan for attacking the former disease will be applicable to the latter. Birch (23) has placed in parallel columns the resemblances and the differences between the two diseases. Most students of the problem of animal disease eradication will accept his picture as reasonably accurate. Italics have been added by the writer to indicate the points of the contrast; obviously there is more difference than resemblance.

TUBERCULOSIS	BRUCELLOSIS
1. Specific infectious disease acquired by contact.	1. Specific infectious disease acquired by contact.
2. Chronic disease, usually spreads slowly, many months between date of exposure and time when animal becomes spreader.	2. Chronic disease, exceedingly erratic, spreads rapidly or slowly, spreaders sometimes develop in one month from date of exposure, many appear in two or three months.

TUBERCULOSIS	BRUCELLOSIS
3. Animals less than one year old highly susceptible, readily acquire permanent infection, and break down in later life.	3. *Animals less than one year old only slightly susceptible, rarely acquire permanent infection.*
4. Pregnancy exerts no influence on probabilities of spread.	4. *Pregnancy, which may terminate at any time, multiplies probabilities of spread.*
5. No period of special danger of spread.	5. *Special danger of spread at time of parturition.*
6. Tends to become progressively worse in the individual. Recovery not a factor as it relates to official measures.	6. *Destroys the value of many animals in a short time. Majority of those which remain gradually become tolerant of the infection and assume the outward appearances of immunity but remain as carriers and potential spreaders. A few actually recover and cease to be carriers.*
7. Recently infected animals least dangerous to their associates.	7. *Recently infected animals most dangerous to their associates.*
8. Because of relatively low and decreasing prevalence, reactors can be destroyed and the owners indemnified. Disposal of reactors is therefore a standardized procedure.	8. *Because of a relatively high prevalence, which forbids indemnities, and to the high value as individuals of selected chronic reactors, handling of reactors is the most difficult individual problem which sanitary officials must face.*
9. Immunity, tolerance and recovery play no part in the disposal of reactors.	9. *The chronic reactor which usually is a tolerant individual, with behavior suggesting true immunity, often has a higher value than a clean cow when badly infected herds require replacements.*
10. There is a reasonably accurate test to identify infected animals, the tuberculin test.	10. There is an equally accurate test to identify infected animals, the agglutination test.
11. The only effective method of control consists in establishing and protecting a clean herd through periodic testing.	11. The only effective method of control consists in establishing and protecting a clean herd through periodic testing.

TUBERCULOSIS	BRUCELLOSIS
12. "Once a reactor always a reactor" is sound policy in using the tuberculin test though numerous false reactions occur.	12. *Temporary and threatening reactions are an important factor in using the agglutination test.*
13. One test interferes in greater or less degree with those made subsequently in the same animal.	13. *One test does not interfere with subsequent ones in the same animal.*
14. Making all tests official may be accomplished fairly well through control of tuberculin.	14. *Making all tests official probably cannot be accomplished, though an official standard can be set, and maintained.*
15. There is need to make all tests official because one test modifies the accuracy of subsequent ones.	15. *There is no need to make all tests official, but there is need for official tests.*
16. When doubt exists as to the accuracy of a test a purchaser, or a state receiving the tested animal, cannot always apply repeated retests.	16. *An unlimited number of retests may be applied, and the same blood sample may be tested in several different laboratories.*
17. No material is readily available which may be used to produce reaction in clean cattle which a purchaser may wish to reject.	17. *Material is readily available which will cause a clean animal to react in approximately two weeks.*

Exception may be taken to item 11. The writer would not hold so rigidly to the thought that the only effective method of control in either disease is periodic testing. This may not be the only method in so far as Bang's disease is concerned since there is reason to hope for aid from vaccination.

To summarize, therefore, brucellosis differs from tuberculosis, and, in consequence, measures to control or eradicate the former disease must differ from those that have proved practicable in the eradication of the latter disease in these respects: animals under one year of age have more resistance to *Brucella* infection, a fact which has stimulated an effort to increase and prolong this resistance by vaccination. The spread of brucellosis is effected most commonly at the time of parturition or abortion and recovery from an attack of the disease occurs in some cases and leaves the animal with some immunity. Brucellosis is perhaps three times as preva-

lent as was tuberculosis at the time its eradication was begun. Practically there was more dispute as to the interpretation of the diagnostic test than was the case with the tuberculin test as its use developed. There is less to interfere with repeated and multiple tests in the use of the agglutination test for brucellosis than in the use of tuberculin for tuberculosis.

A thorough study of the so-called area plan of cattle tuberculosis eradication in the United States and of the problems of brucellosis, especially of cattle, convinces one that this plan with appropriate modifications might be adapted to brucellosis eradication more hopefully than any other procedure of animal disease eradication yet tested.

The area plan for the control or eradication of cattle tuberculosis in the United States is under the supervision of the Bureau of Animal Industry of the United States Department of Agriculture. In cooperation with the respective state departments of agriculture, and, in turn, with the county boards of supervisors, full-time veterinarians and part-time veterinary practitioners give the tuberculin test to all the cattle in a prescribed area. The reactors are slaughtered under government supervision and disposal of the carcass is made in accordance with the federal meat inspection regulations. The owner is reimbursed on the basis of an appraisal (not in excess of a fixed maximum for grade and registered cattle respectively) and the salvage of the carcass. Retests are then made on the remaining cattle at stated intervals until no reactors appear. The premises are disinfected and only tested cattle are permitted to be purchased. When an area (county, more or less) is freed of reactors to the point of less than 0.5 per cent, it is quarantined against outside infection. Finally, when a whole state attains this status it is designated as a modified accredited area. At present the District of Columbia and every state, except California, are accredited.

The tuberculosis eradication campaign was born only after a

long and tedious gestation period and painful delivery; it has encountered natural difficulties inherent in the disease and the ignorance of those fighting it; it has met the stubborn opposition of a small but irritated minority; it has had its day in court and on the battlefield; it has encountered difficulties in getting appropriations for inaugurating the work in some areas and in maintaining the appropriations for continuing the work after it has got under way. There is evidence that the success of the area plan has been fostered by the peculiar economics of the dairy situation. Not only has the dairyman realized that a tuberculous cow is as a rule not a good producer, is an actual or potential menace to his other cattle and hogs (to say nothing of his family), is a factor in lessening sales due to the public attitude toward tuberculous milk; but he has realized that in the interests of maintaining prices for dairy products the slaughter of very considerable numbers of milch cows may be more of a blessing than a blow to the industry.

Regulative Measures

According to the committee report of 1931 of the U. S. Livestock Sanitary Association (381), the question of the relationship of brucellosis in cattle and other animals to public health has held a prominent position. Considerable national publicity at one time indicated that Bang's disease was an important factor in public health. Careful research work has gone a long way toward changing opinions with regard to the seriousness of Bang's disease in human health. At the present time, there is sufficient information to indicate that *Br. abortus* in milk is responsible for brucellosis in human beings. The exact extent to which this disease in cattle is responsible for infecting human beings is not definitely determined and final decision on this particular point must await further reliable findings. It is apparent that the livestock sanitary officials minimize the effects that the bovine type of this disease has on the

human family, while the health officials tend to regard the bovine infection with more concern.

Some cities have passed or contemplate passing ordinances prohibiting the use of milk coming from infected cattle. Some ordinances tend to be more drastic than necessary, and as a result place an unwarranted burden on the herd owners.

Movements have been started in some states to provide funds to eradicate this disease and to indemnify owners whose reacting animals are condemned and disposed of. Herd owners are inclined to support such a movement. Livestock sanitary officials at this time are favorably disposed toward taxation which will pay the indemnity on reactors to the abortion test. The amount of infection is so high, when compared with the amount of existing tuberculosis at its peak, that if operating costs and indemnity were paid, in sums commensurate with those expended for tuberculosis eradication, appropriations to cover such costs and indemnity would have to be stupendous. To eradicate Bang's disease from the herd, the owner must assume considerable responsibility relative to the execution of the sanitary program, since progress cannot be made unless there is a cooperative attitude toward this program. It is true that some animals which react to tests for Bang's disease may possess considerable economic value, and, if possible, valuable breeding animals that are infected may be kept under strict isolation.

During recent years, some of the states have passed regulations providing that only cattle negative to the abortion tests may be exhibited at state fairs. Some of the big national exhibitions of cattle have likewise adopted similar rules. Following the example of state and national fairs, regional and county fairs have likewise passed similar regulations. Such regulations compel the owner of pure-bred livestock to make an early endeavor to rid an infected herd of this disease.

Some livestock owners seem to possess a keen appreciation of the

devastating effects of the disease. Their interest is aroused to the point where they possess the desire to undertake a clean-out program. They appreciate at this stage that a pure-bred animal in the future will have greatly reduced value unless it can be ascertained to be free of Bang's disease.

The legal basis for the control of Bang's disease in Michigan is found in the following regulations (366):

REGULATIONS FOR THE CONTROL OF BANG'S DISEASE

By virtue of the authority conferred upon the Commissioner of Agriculture under the provisions of Act 181 of the Public Acts of 1919, as amended, the following rules and regulations relating to the Abortion Disease of cattle are established to become effective on and after July 1, 1930.

1. Abortion Disease, Bovine Infectious Abortion or Bang Bacillus Disease shall mean the disease wherein an animal is infected with the Bang bacillus (*Brucella Abortus*), irrespective of the occurrence or absence of an abortion.

2. An animal shall be considered infected with Bang bacillus (*Brucella Abortus*) if it gives a positive reaction to any test for Bovine Infectious Abortion recognized and approved by the Michigan Department of Agriculture; or if the Bang bacillus (*Brucella Abortus*) has been found in the body or its secretions or discharges.

3. Animals infected with Bovine Infectious Abortion as herein defined shall not be sold, traded, given away nor removed from the premises except under permit from the Michigan Department of Agriculture. Permits for the removal of infected animals will be issued only under the condition that their destination shall be immediate slaughter or that they be placed in infected herds.

4. In each and every instance where animals defined herein as infected are sold, traded, given away, removed to other premises, or offered for sale the owner or his agent must represent such animals as infected. Before any cattle which have been vaccinated with living Bang bacilli are sold or moved from the premises of the owner, said owner or his agent, shall notify the prospective purchaser, or owner, or owners of

cattle, with which such cattle will come in contact, that such cattle have been so treated, giving the date of treatment.

5. All dairy or breeding cattle over six months of age entering the State of Michigan in any manner, except cattle from herds officially certified to by the Livestock Sanitary officials of the state of origin as free from Bovine Infectious Abortion, must pass an agglutination blood test approved by the Livestock Sanitary official of the state of origin within thirty days prior to importation, provided such test shall not be recognized if made within five days before or fifteen days after calving. The date of test and results must be shown on properly executed certificates. Copy of such certificates should be attached to the waybill and another copy forwarded immediately to the State Veterinarian at Lansing, Michigan. Provided, further, that this section shall not be held to apply to cattle moved into the State of Michigan for feeding and grazing purposes and kept segregated from dairy and breeding cattle during the feeding period; nor shall this section apply to cattle moved into the State of Michigan for exhibition purposes but in the event that exhibition cattle are sold to remain in the State of Michigan, or are retained by the owner in Michigan longer than sixty (60) days, such cattle shall be subject to an agglutination blood test approved by the Michigan Department of Agriculture. Cattle infected with Bovine Infectious Abortion as herein defined shall not be brought into Michigan except for immediate slaughter or under permit from the Michigan Department of Agriculture for entry into specified infected herds.

6. It shall be the duty of any person making any blood tests, or other diagnostic tests for Bovine Infectious Abortion, to report such tests in writing to the Department of Agriculture at Lansing, Michigan, within five days after the completion of such tests. Each report shall be signed by the person who shall have made the test and shall contain a complete statement of the actual results of the test; the name and address of the owner or person in control of the animals tested; and the identification of each animal tested by means of ear tag numbers, registration numbers or tattoo numbers.

7. No person shall inject or otherwise administer to any livestock any substance containing, or purported to contain, living Bang bacilli (*Brucella Abortus*) except upon specific written permission to do so from an authorized representative of the Michigan Department of Agriculture.

The holder of a permit to administer vaccine for the treatment of Bovine Infectious Abortion shall submit a report to the Department of Agriculture, Lansing, Michigan, within ten days showing a record of each animal so treated, the name and address of the owner or person in control of the animals treated and the name and address of the manufacturer of the vaccine used.

8. Any person, firm, or corporation selling or delivering any product containing or purported to contain, living Bang bacilli for the purpose of vaccination and Bang bacilli antigen for the purpose of testing, within the State of Michigan shall within five days report such sale or delivery to the Commissioner of Agriculture, Lansing, Michigan, specifying in such report the date of sale, the name and address of the purchaser, or consignee, and the quantity of the product sold or delivered.

Since the inception of the eradication programs by the various states, many hundreds of herds of cattle have been freed from Bang's disease. In 1934 the United States Bureau of Animal Industry in cooperation with the various states instituted a program for the reduction of cattle. This program called for the elimination of diseased cattle, especially those infected with Bang's disease. From July 1934 to January 1, 1942, 51,432,162 cattle were tested by the agglutination test in cooperation with the various state animal disease control laboratories. The number of cattle so tested that were found to be infected and were eliminated and slaughtered was 2,239,805. The removal of such a large number of sources of infection during the past eight years has thus far had little, if any, effect in reducing the incidence of brucellosis in human beings.

Control by Vaccination

Br. abortus vaccines, both killed and living, have been studied for many years by many investigators in an attempt to control Bang's disease. As early as 1906 Bang employed killed bacteria for the prevention of the disease. His results, however, were far from encouraging. Buck and Creech (40) also failed to obtain protection

in animals that were repeatedly treated with killed vaccine. One of the chief fallacies in the use of killed and living vaccines was that many investigators were thinking of the symptoms of a disease, that is, premature expulsion of the fetus, rather than of the prevention of infection. So for many years the idea behind the employment of vaccines was to prevent abortion rather than to prevent infection. It has only been in recent years that the nature of the disease in the cow has been fully understood. It is now known that prevention of infection is just as important, if not more so, than the prevention of the symptoms of the disease. It is known that infection in the udder, which occurs in an infected animal, is just as important economically as the loss of the calf by premature expulsion.

Failure to understand the nature of the disease in past years led to the injection of living virulent cultures into non-pregnant animals. After inoculation, it was observed that a large percentage of the animals so treated carried their calves to maturity, even when they had become infected. As the result of early work of this nature, promiscuous injection of cattle with virulent cultures took place throughout the United States and various other countries. Many manufacturers of biologicals even went so far as to advertise the fact that great pains were taken to keep the organism used in the vaccine alive and virulent. For a time not only was *Br. abortus* used in the preparation of the vaccine, but certain individuals and manufacturers of biologicals resorted to the use of living cultures of *Br. suis*. There is no doubt that as a result of the wide use of virulent cultures in the preparation of vaccines thousands of cattle became infected. This has served to increase the incidence of infection in dairy cattle in European countries as well as in the United States.

The question one may ask is, why did so few of the animals inoculated with virulent cultures fail to abort? Since we have be-

gun to understand the nature of the disease in a herd of cattle it is easy to understand why so few abortions resulted. We know today that a large percentage of animals that become infected naturally while non-pregnant do not abort. Since this is the course of the disease in non-pregnant animals infected naturally, it would be reasonable to expect a similar occurrence in non-pregnant animals injected with living organisms that are virulent.

In 1921 a search was begun at the Michigan State College for a culture of *Brucella* of low virulence for experimental animals and cattle. After considerable study, a culture was found that failed to produce a permanent infection or gross changes in the organs of the guinea pig even when injected intraperitoneally in large doses. A suspension of this organism in saline solution in a living state, when inoculated into both pregnant and non-pregnant cattle, failed to produce infection. The effects of this culture on a very large number of guinea pigs, on cattle, and on human beings have been studied since 1921. In no instance has infection been established or noticeable anatomical changes produced in the organs of the inoculated animals.

The culture in its original state gave rise to agglutinins and in some instances to a high titer. This was considered an objectionable feature. The control or elimination of the disease at the present time is based upon the detection of specific agglutinins in a certain titer. If cattle are injected with this vaccine in a state which will increase specific agglutinins, the presence of these agglutinins as a result of vaccination will complicate the control or the elimination of the disease in infected animals even though those which have been injected with the vaccine are not infected. A successful attempt was made in 1929 to prepare the organism in a dissociated state so that on injection no noticeable agglutination titer would result.

During the first four years of study, the data indicated that this

type of vaccine gave some degree of protection against infection (299). Since that time the vaccine was used on animals in several herds shortly after infection had appeared. In such herds there was no noticeable difference in the incidence of infection between the treated and control animals. It was concluded, therefore, that a vaccine made from a dissociated culture of *Brucella* produced little, if any, immunity in susceptible animals.

As early as 1924 Buck and Creech (40) obtained data from experiments which indicated that the inoculation of non-pregnant heifers of breeding age with *Br. abortus* in the living state seldom, if ever, resulted in permanent infection.

In 1925 Buck (38) began a series of experiments designed to determine the efficacy of suspensions of live cultures of *Br. abortus* to produce sufficient immunity in calves to protect against infection when they became mature. Several cultures of different degrees of virulence were used in the experiments. The final results seemed to indicate that one culture of moderate virulence, No. 19, had possibilities. The 3 calves treated with this culture resisted infection on artificial exposure.

The study begun by Buck was continued by Cotton, Buck, and Smith (66) and the results of two experiments were summarized in a report in 1934. In one experiment, 6 heifers near breeding age were treated with strain 19. In another 9 were treated. After breeding, they were exposed, along with controls, to infection by way of the conjunctiva. In the first experiment one treated and all 8 controls became infected. In the second experiment, none of the treated but 7 of 11 controls became infected. Two of the treated animals aborted, but no evidence of *Brucella* infection could be found. The results of these studies led Cotton and his associates to suggest that calves between the ages of four and six months be treated with strain 19 in order to avoid a prolonged serum agglutination titer.

Since 1938 there have appeared numerous reports pertaining to

the efficacy of strain 19 vaccine in the prevention of brucellosis in cattle in field and experimental herds. Hardenbergh (172) vaccinated 143 calves, leaving 73 controls. They were maintained in a herd under natural conditions of exposure. After reaching maturity, 3 or 2.4 per cent of the vaccinated calves and 4 or 6.2 per cent of the controls became infected. Mills (311), Thomsen (441), Tompkins (443), and Haring and Traum (178) likewise vaccinated a large number of calves that were maintained under natural conditions. All report (see Table XXXVI) encouraging results in the protection against infection after reaching breeding age.

Birch, Gilman, and Stone (27) have conducted a well-controlled experiment with strain 19 on calves to determine its immunizing value and the duration of the immunity. The animals, after breeding, were exposed to infection by being placed in quarters with aborting cows. Of 33 vaccinated animals and 23 controls, 10 of the former and 17 of the latter became definitely infected. Twenty-eight of the vaccinated and 14 of the controls were observed through a second pregnancy. During this period 7 vaccinated and 7 controls became infected.

The Bureau of Animal Industry of the United States Department of Agriculture has been conducting extensive studies of strain 19 in calves in privately owned herds since 1936. Mohler (315) has summarized the results in the following paragraphs:

"Of the calves vaccinated, 8,182 have now dropped calves involving three pregnancies, of which 5,673 were first, 2,026 were second and 483 were third pregnancies.

"There were 7,872, or 96.2 per cent, normal parturitions in these herds. Of the latter number, 6,526, or 82.9 per cent, calved normally and also were negative on post-parturition test; 399, or 5.1 per cent, calved normally but were positive to the post-parturition test; and 947, or 12 per cent, calved normally and were suspicious to the post-parturition test.

TABLE XXXVI

Summary of results of vaccination with BAI Strain 19 as reported by several investigators

OBSERVER	METHOD OF EXPOSURE	VACCINATED		CONTROLS	
		Total	*Infected*	*Total*	*Infected*
Cotton *et al.* (66)	Artificial	15	1 (6.6%)	19	15 (78%)
Hardenbergh (172)	Natural	143	3 (2.4%)	73	4 (6.2%)
Mills (311)	Natural	142	12 (8.5%)	46	16 (34%)
Thomsen (441)	Natural	266	9 (3.3%)	135	34 (25.5%)
Tompkins (443)	Natural	24	4 (16.6%)	32	9 (28.1%)
Tompkins (443)	Natural	222[a]	3 (1.3%)	–	–
Birch *et al.* (27)	Natural	35[a]	10 (28.5%)[e]	23[a]	17 (73.9%)
Mohler *et al.* (315)	Natural	8,182[b]	128 (1.6%)	–	–
Haring and Traum (178)	Natural	2,872[c]	169 (5.9%)[d]	1,763	245 (13.9%)[d]

[a] First parturition. [b] Report covers part of 3 parturitions. [c] Parturitions. [d] Abortions. [e] Spreaders and reactors.

"On the other hand 310, or 3.8 per cent, abortions occurred in these groups, of which 182, or 58.7 per cent, of the aborting animals were negative to the post-parturition test and 99, or 31.9 per cent, were positive to this test, while 29, or 9.3 per cent, of the aborting animals were pronounced suspicious. Consequently, on the basis of the blood agglutination test, only 128, or 1.6 per cent, of the abortions occurring in this group of 8,182 animals involved in the three pregnancies could be attributed to brucellosis."

Haring and Traum (178) have collected considerable data on the immunizing value of strain 19 in cows and heifers as well as young calves in privately owned herds. The results are based on 2,872 pregnancies in 1,956 animals over a period varying from one to six years. One hundred and sixty-nine of the pregnancies terminated in abortions. They estimate that less than 15 per cent of these abortions were due to brucellosis. In one herd in which 44 per cent of the animals were infected, vaccination was practiced on the non-infected for a period of six years. At the end of this time, when all of the original infected animals had been removed, the remaining vaccinated ones were free from brucellosis. This would indicate that the disease can be kept under control by vaccination and actually eliminated by proper herd management.

Rabstein and Welch (373) have studied the effects of strain 19 vaccination on animals from the standpoint of the persistence of the agglutinin response as well as its immunizing value. A summary of their results follows:

"The project covered three age groups of which 642 were vaccinated between 3 and 8 months (group I), 89 between 9 and 12 (group II), and 65 between 13 and 21 months of age (group III). Out of 796 bled prior to vaccination only four were positive and nine suspicious. No difference was noted in the rate at which these and other animals lost their titer following vaccination. All of the

796 animals vaccinated were positive in a dilution of at least 1:200 two weeks following vaccination with the exception of two which remained negative even after being repeatedly vaccinated. Vaccinated calves showing a blood titer of at least 1:200 were in direct association with negative susceptible animals and in no instance did the latter show any change in their negative status.

"There was a direct relationship between the age of the animal and the time of vaccination and the length of time that a positive blood reaction was retained. As an example, nine months following vaccination, 91 per cent of group I, 50.5 per cent of group II, and 20 per cent of group III were negative to the agglutination test. At 18 months following vaccination, group I showed 1.4 per cent positive and 3.4 per cent suspicious, while group III had 20 per cent positive and 40 per cent suspicious.

"Of the pregnancies recorded on animals vaccinated in this experiment, 172 have had one calf, 90 have had two, 48 have had three, 26 have had four, and 8 have had five calves each. Out of the total number of pregnancies, ten, or 1.5 per cent, terminated in abortions of which five appeared to be due to *Br. abortus* infection.

"After an average period of about four years under the calfhood-vaccination plan, the total number of adult animals in the eleven experimental herds increased from 596 to 686. Three of the herds are now completely negative, having replaced their reactors with vaccinated animals of their own raising. Exclusive of young stock, there are now 334 vaccinated animals, or 48.9 per cent of the total number in these herds. This group of 334 animals exceeds the 216 reactors formerly in the herds at the beginning of the experiment, but since the owners retained their reactors until unprofitable, a residue of 55 old reactors still remains."

From the studies that have thus far been made with strain 19 as an immunizing agent against bovine brucellosis, it is reasonable to con-

clude that when it is used on calves between the ages of four and eight months sufficient immunity is developed to protect at least 90 per cent against natural brucellosis during the first pregnancy; that a high degree of protection remains during the second and third pregnancies; that the treatment of calves, non-pregnant heifers, and cows does not lead to the establishment of the injected organisms in the animal body, thus producing a carrier state; that calves and young heifers show an agglutinin titer for only a few months after vaccination, thus eliminating confusion in the use of the agglutination test as a means of detecting active infection.

The proper and continued use of strain 19 vaccine should serve a useful purpose in preventing the spread of infection in infected herds and in preventing its occurrence in those free from brucellosis.

It may play as useful a role in the control of brucellosis (Bang's disease) as the slaughtering of infected cattle.

McEwen (277) in England also has been investigating the possibility of using a live culture of *Br. abortus* of low virulence for immunizing adult animals as well as those under breeding age. In 1937 there appeared the first comprehensive report of his studies in this direction in which a sufficient number of controls were left to give the results significance. In one herd he treated 109 animals, leaving 98 as controls. Four of the former and 5 of the latter became infected. During the second year there were left 90 treated and 73 controls for observation. This time 2 of the treated and 14 of the controls became infected. Continuing the observation for a third year, there were 38 treated and 29 controls left of which none of the treated and 7 of the controls became infected.

BRUCELLOSIS CAPRINUS

This is primarily a goat and sheep disease, but there has come to our attention evidence that the microbe is invading the cattle of France. In the United States, *melitensis* infection has appeared as

a result of the famous S.S. *Joshua Nicholson*–S.S. *St. Andrew* importation of milch goats and in the southwestern states along the Mexican border.

In the Mediterranean countries, the disease is not being successfully combated by vigorous application of diagnostic methods and segregation or slaughter of the reactors. In the first place, the diagnostic procedure, the agglutination test, has severe limitations in caprine brucellosis and no adequate alternative diagnostic method is available. Furthermore, the people of these lands would not submit to the stamping-out methods so complacently as American stock owners. Vaccination of goats is being attempted, but this procedure must be considered as being in the experimental stages. In the meantime, pasteurization of goat's milk should offer a considerable degree of protection to man. Here, again, long-established practices and lack of adaptability interfere with the successful introduction of new methods. Pasteurization of milk would not offer protection against contact infection. This can be expected only with the suppression of the disease. In view of the observations of Taylor, Lisbonne, Vidal, and Hazemann (430) that *melitensis* infection in both goats and sheep is self-limiting, the application of isolation methods to its control in these animals would seem worthy of trial.

Europe needs to be very vigilant in quarantining against the introduction of the infected milch goat into *melitensis*-free territory. Already this infection has made some progress among sheep and cattle in France. The United States appears to have successfully guarded against further importations of *melitensis* infection. Special attention should be given this matter in the southwestern states bordering on Mexico. In the absence of adequate statistical information relative to the prevalence of the disease in the goats of these states, it is premature to state dogmatically what mode of attack is to be recommended. Efforts should be made to prevent the

interstate shipment of infected goats and the isolation and proper disposal of them should be considered.

BRUCELLOSIS SUIS

The swine brucellosis problem in the Americas and Europe appears to be one which involves only *Br. suis*. No one has yet been able to demonstrate natural infection in swine due to *Br. abortus*. The epizootiological surveys that have been made show that swine brucellosis is prevalent in many sections of the United States, South America, and Central Europe. The control of the disease in swine is not only of considerable economic importance to swine breeders, but presents an economic problem to the dairy industry and a health problem to those interested in safeguarding human health.

In only a few instances has any effort been made to control or eradicate the disease from individual herds of hogs or from those in a wide area.

Data obtained by Huddleson, Johnson, and Hamann (216) in a study of the natural course of brucellosis in three naturally infected herds of hogs in Michigan indicate that it is for the most part a self-limiting disease. Many animals found infected by the application of the serum agglutination test become negative to the test within a ninety-day period. Segregation of those found positive to the test rapidly places the disease under control.

Thomsen (439) has succeeded in completely eliminating swine brucellosis from Denmark by the application of the serum agglutination test and slaughter of the reactors.

APPENDIX

CASE REPORTS

APPENDIX

CASE REPORTS

CASES STUDIED IN IOWA*

Intermittent Type

CASE 1. Male, aged 40, farmer and hog raiser. Late in December 1926, the patient noted that he was unusually tired in the evening and that his appetite was somewhat impaired. During January 1927, the weakness increased. There were sleeplessness, more marked anorexia, occasional feverishness, and irregular night sweats. He was also troubled at times by backache and complained of a stiffness of the neck. Symptoms progressively became worse. Early in February he consulted his physician and an elevation of temperature was found. Pyorrhea was noted, but otherwise the physical examination was negative. Dental examination, with x-ray, was advised. Several apical abscesses were revealed and the teeth concerned were extracted. One week later the physician was called to the patient's home. He found an obviously ill man, with a moderately high fever and distressing joint pains. A pyogenic septicemia was considered and a blood culture was taken. Blood for a Widal test was sent to the State Hygiene Laboratory to rule out typhoid and this, examined routinely for brucellosis, was found to agglutinate *Br. abortus* in a 1:320 dilution. Two later tests were also positive. The blood culture was discarded after seventy-two hours' incubation, at which time the subcultures showed no growth. Throughout February and March the patient was bedfast. So profuse were the night sweats that quite regularly the bed linen would have to be changed between 1 A.M. and 2 A.M. His wife, who nursed him, reported that through his illness he was restless and quite irritable. There was a gradual loss of weight. The fever was somewhat irregular, varying from normal to 101° F. in the morning and from 101° F. to 103° F. in the evening. An unusual feature of this case was a definite arthritis, with effusion into the knee joints. There was uneventful convalescence which covered a period of two months, following which the patient gradually returned to work.

* Hardy and associates (175).

Twenty months later he reported that he had been enjoying good health.

CASE 2. Male, aged 38, farmer. About the middle of August 1929, the patient noted that in the evening he would be unusually tired, without appetite, and frequently suffering from headache. These symptoms persisted, and one month later he consulted an oculist. Lenses were prescribed, but the frontal headache persisted. He then consulted his physician who accurately diagnosed his ailment. Two blood tests showed agglutination for *Br. abortus* in a 1:2,560 dilution. Early in November, when we saw him, he was still ambulatory. His chief complaints at that time were profuse night sweats, rigors, of which he had had ten, and diffuse lower abdominal pain. He had moderate headache, some joint discomfort described as stiffness, and constipation. We found his temperature at noon to be 102.5° F. (unusually high for that hour, he explained, because he had had to do quite strenuous work that morning). Ordinarily the temperature was normal until noon, reaching a maximum of 102° F. to 105° F. in the early evening.

The patient's own disability and our advice were at first not sufficient wholly to restrict his activity. He became strictly bedfast only when a unilateral orchitis and epididymitis developed a few days later. Throughout October he continued to be quite ill. During November he improved rapidly, but a condition developed that was diagnosed as tenosynovitis of the right hand. At the end of the month there was still a low-grade fever and moderate weakness. The appetite was very good and weight was being regained. By January the patient considered that he had fully recovered.

Ambulatory Type

CASE 3. Male, aged 38, farmer. There was an insidious onset during April 1929. During the first six weeks of illness the patient thought he had "chronic flu." Because of the persistence of the symptoms, he applied to his physician at the end of this period. He reported that he had a moderate weakness, though in the morning he ordinarily felt fairly strong, but in the afternoon he was able to do little. He complained of general aching, some headache, chiefly behind the eyes, but also in the lower occipital region and back of the neck. He had no definite joint

pains, but complained of muscular soreness and stiffness. "I could scarcely move," was his own description. Night sweats had occurred, but these were not profuse. These symptoms varied somewhat in severity and persisted through the summer. His appetite was never good, and he was troubled with constipation. More than twenty pounds of weight were lost. A non-productive cough persisted throughout the illness. The fever occurred only in the afternoon and evening and was rarely above 101° F., but reached a maximum of 103° F. This patient also noted that the more active his exercise, the higher the temperature rose. Except for the coarse râles, and a moderate tenderness in the upper abdomen, the physician reported no abnormal physical findings. Laboratory tests showed a blood serum agglutination for *Br. abortus* in a 1:320 dilution in June, and in a 1:80 dilution in November. Throughout his illness of seven months' duration, the patient continued to do the necessary work on his farm. He obtained extra help only during the more strenuous season of harvest and threshing.

Case 4. Male, aged 13, doctor's son. This illness began insidiously during October 1928. The lad's parents first noticed that he was less eager to play in the afternoon and was not interested in the evening meal. The boy complained of some headache, which he described as pain in the eyes and back of the neck. An evening temperature of 99.5° F. to 100° F. was found. There were no other abnormal physical findings. The father restricted the boy's activity for one week, but throughout the remainder of the mild illness, which lasted one month, he lived normally. His blood serum gave an agglutination for *Br. abortus* in a 1:160 dilution.

Undulant Type

Case 5. Male, aged 43, farmer. The patient's illness began about December 15, 1928. He noted marked weakness, a moderate anorexia, general aching, particularly in the lumbar and cervical regions, and some fever. By January first he felt that he had recovered from an attack of "la grippe." Shortly after the new year the same symptoms reappeared, this time more severe, and the physician was consulted for the first time. During January and February he had at least four attacks of fever, with apyrexial intervals, in which he did not feel ill. His case was diagnosed

as typhoid and "typhoid flu," but on analysis of a blood specimen sent to the laboratory, by the third physician consulted, the diagnosis of brucellosis was established. The titer was 1:1,280. The patient was admitted to the university hospital on March first during an apyrexial period. Five days after admission the fever reappeared and increased daily for four days, reaching a maximum of 101.8° F. Following this it gradually subsided, reaching normal four days later. During this febrile period the patient's only complaint was constipation. He noted some feverishness, did not enjoy reading, and was less interested in his food. He did not, however, appear ill and no notable physical abnormality was detected throughout his illness. On March 15 the patient was discharged, and from that time his progress was followed by correspondence. Letters of March 24 and April 25 reported recurrences of fever. The note of the latter date, after describing the early symptoms of these attacks, read as follows: "I had almost forgotten how I did feel when I was sick, but it all came back, the ache and pain in the limbs, the headache and backache, the soreness across my bowels, and constipation. I didn't have any chill this time, but my fever broke about 2 A.M. today, and I sure did sweat." The patient considered that his symptoms were most severe during what proved to be his last febrile period. A later letter, on June first, stated that he had no fever, but was still weak, even though doing light work.

CASE 6. Male, aged 38, farmer. This patient was unable to give any date of onset, but stated that during the spring months of 1927 he noted that he tired easily and had headache which gradually became more frequent and severe. Early in June he first consulted his physician, making an office call when he had a temperature of 103.2° F. His symptoms at that time were marked weakness, profuse night sweats, rigors, anorexia, and constipation. Shortly after this the patient came to the hospital. His temperature was found to be remitting, normal or about normal in the morning and from 102° F. to 103° F. in the evening. The physical examination was negative except for slight abdominal tenderness. The agglutination test was positive for brucellosis. The patient did not consider himself sufficiently ill to remain in hospital, and after ten days insisted on being discharged. The fever gradually subsided and with this his symptoms disappeared. Two relapses occurred, the first

after six weeks, with a duration of two weeks, the second after four months lasting one week only. During the relapses his symptoms were mild in degree though similar in nature to those in the original attack.

Atypical Cases

CASE 7. Male, aged 26, a packing-house employee and laborer. His serum agglutinated *Br. abortus* in diagnostic dilution. The onset of his acute illness was preceded by a definite complaint for a period of almost one month of lassitude, headaches, and drowsiness. During this time he continued to work. For three days before a physician was called, the patient was quite ill. During that period he had marked prostration and complained of some headache, backache, and acute pain in the back of the neck. The physician reported that the temperature was regularly remitting, but observation showed a daily increase of one degree until 104° F. was reached. It was then sustained for ten days at a high level. During this period the patient was acutely ill. Early in his acute illness he had one rigor. Throughout he had marked constipation. Delirium and coma rapidly developed and a fatal outcome seemed certain. Unusual in the course of this illness was the rapid enlargement of the spleen. At the time of the first consultation the physician reported that the organ was not palpable. Four days later it could just be felt, and one week after this its lower margin had reached the umbilicus. After the ten days with high fever the temperature dropped almost by crisis. The spleen decreased in size as rapidly as it had increased, and convalescence proceeded uneventfully.

CASE 8. Male, aged 26, farmer. The family history for tuberculosis was entirely negative. The onset was very insidious, the patient stating that he "had not been feeling fit at all." In January 1930, he first consulted his physician, at which time his major complaints were progressively increasing weakness, fever, cough, and night sweats. Additional inquiry revealed also that he had spells of chilliness, two rigors, severe pain in the back of the neck, anorexia, moderate irritability, sleeplessness, and very profuse sweats, and had lost weight. During the following months the condition, which was not definitely diagnosed, did not improve and the family requested a consultation. A diagnosis of "tuberculosis in its worst form" was made; the patient, his wife, and parents were acutely

distressed by the outcome, but acting on the advice of the consultant, the young man prepared to sell his farm, stock, and equipment. The family physician had, however, after long delay sent us a blood specimen and this we found to agglutinate *Br. abortus* in a dilution of 1:640. This young man came to the university hospital for further study. His cough persisted and he had mucoid or mucopurulent sputum. Moist râles, diffusely scattered, were heard chiefly at the bases, posteriorly. The spleen was easily palpable. X-ray of the chest was entirely negative. The patient looked well, and after a short rest in bed felt so well that he could not be persuaded to remain in the hospital.

The prominent features of this case are the symptoms and findings of pulmonary tuberculosis, but the rigors, pain in the back of the neck, and the palpable spleen make such a clinical diagnosis questionable. The course of the infection supports the diagnosis indicated by the laboratory findings.

CASE 9. Male, aged 40, preacher. The patient became suddenly ill, feeling weak and somewhat feverish. His temperature was taken and found to be elevated and a physician was called. Early in the infection a rigor occurred, and from the first there was a cough. Only later did general aching appear. Sweating was never marked. Physical examination at first was essentially negative. A few days later, auscultation revealed moist râles over the bases, posteriorly. His physician detected slight dulness on percussion, and a suggestion of bronchial breathing. Pneumonia was suspected. Brucellosis being also considered as a possibility, blood was sent to the laboratory and the first specimen agglutinated *Br. abortus* in a 1:640 dilution; the second, four weeks later, in a 1:5,120 dilution. Two blood cultures remained sterile, but by guinea-pig inoculation *Br. suis* was isolated from the feces.

CASE 10. Male, aged 36, farmer. When the patient first presented himself, his only complaint was swollen and painful testes. In spite of a negative history, a gonorrheal epididymitis was diagnosed. A detailed history later revealed that for four months prior to the onset of this symptom he had had an increasing lassitude and weakness. Subsequently the sweating characteristic of brucellosis developed, and this led to the correct diagnosis. The tests on the blood serum were made show-

ing agglutination for *Br. abortus* in titers of 1:640 and 1:1,280 respectively. *Br. suis* was isolated from the blood stream.

CASE 11. (Reported by I.F.H.) Female, aged 49, housewife. The patient lived on a farm and drank raw milk. Animals in the herd had been found infected with Bang's disease. No other member of the family had been ill. The patient had visited her physician, Dr. S. V. Barnum of Lansing, Michigan, several times during 1936–1937. She was admitted to the University of Michigan Hospital April 28, 1937. The report of the examining physician follows.

"She entered with a chief complaint of weakness and malaise. She was very apathetic and very negativistic and it was difficult to get a good history from the patient. She states that she has had these symptoms for the past eight years. They became worse in September 1936. During the past winter the patient had a productive cough, raising about one-half cupful of yellowish sputum daily, never blood streaked. For three weeks, she had drenching night sweats which have ceased. There have been no chills. However, the patient has been running an afternoon temperature of 99° F. to 100° F. since the fall of 1936. Shortness of breath on exertion and occasional twinges of chest pain have been present. She had lost ten pounds during the winter. However, she has gained that back at the present time. There has been some pounding of the heart. Tremor of the hands is occasionally noted when nervous. At times she is constipated and complains of aching in the rectum after defecation. Definite slowing of mental processes and memory difficulty have been noted by the patient. There has been some nocturia and she states that she has had some difficulty in swallowing, choking both on liquids and solids. The patient was sent in with the diagnosis of hyperthyroidism and had been taking 10 to 15 drops of Lugol's solution daily since October 1936 without relief of symptoms. On close questioning, it was found that some of the cattle on this patient's farm were infected with Bang's disease.

"*Summary of physical examination.* Examination revealed a well-developed and well-nourished adult female, apathetic, negativistic in reaction, stating that she was sick all over. Lips were dry. Breath was foul. Mouth was dry. Slight dulness was heard at the left apex posteriorly with decreased tactile fremitus, vocal resonance, and occasional

post-tussive râles heard at this area. The heart was of normal size. Rate and rhythm normal. No irregularities or murmurs. Blood pressure 130/75. Abdomen was slightly tender throughout. No masses felt. No viscera palpated. Descending colon felt and rather spastic. No costovertebral tenderness.

"*Summary of laboratory findings.* During the course of hospitalization, frequent urinalyses were made and these were negative with the exception of occasional red blood cells and 0–10 white blood cells per high-power field. Routine Kahn examination for syphilis was negative. On admission, the blood was within normal limits, white count slightly elevated to 11,850. This was checked within a few days, found to be normal, and remained normal during the remainder of her course in the hospital. Routine stool examination negative. One sputum examination revealed no spirochetes or acid-fast bacilli. A basal metabolic rate May 6, 1937, was plus 25. On May 10, sedimentation rate 0.44 mm. per minute. Basal metabolic rate May 17 was plus 29. Sedimentation rate on May 27 was 0.35 mm. June 1, basal metabolic rate was plus 22. On May 31, a blood cholesterol was 170 mg. per 100 cc. Blood agglutinations were negative for *E. typhi, S. paratyphi* A and B, *Br. abortus,* and *Br. melitensis,* the phagocytic index revealing the following polymorphonuclear leucocytic phagocytic activity toward *Br. abortus:* negative 64 per cent, slight 20 per cent, moderate 12 per cent, marked 4 per cent. The patient was given intradermally 0.1 cc. of Brucellergen and in twenty-four hours there was erythema of about 3 by 3.5 cm. which increased in forty-eight hours to 4 by 4.5 cm. This gave a marked rise in temperature which gradually subsided. It was felt that the skin test indicated brucellosis.

"*Summary of X-rays and consultations.* The patient was seen by the Department of Gynecology and a relaxed pelvic floor with cystocele and rectocele, beginning descensus of the uterus, and uterine fibroids were found. Phase angle was performed, being .83 and definite in the hyperthyroid range. The Department of Otology found no foci of infection. The Department of Psychotherapy made a diagnosis of typical anxiety hysteria on the basis of which all her neurotic manifestations could be explained. She was suffering from an extreme degree of abulia and the ataxis type of astasia abasia. The patient was seen by the Department of Surgery where it was felt that no hyperthyroidism existed

and that the symptoms were due to an effort syndrome. Barium enema was negative by X-ray. Chest X-ray negative.

"*Summary of course in the hospital.* During the course of hospitalization, the patient ran almost a daily temperature elevation 99° to 99.2° F. with the exception of three days following Brucellergen when temperature soared to 103.5° F. Throughout her hospitalization, the patient received no Lugol's solution and repeated basal metabolic studies were taken to determine whether or not the patient had hyperthyroidism. All these studies revealed the basal metabolic rate to be well above the upper limit for normal. However, it was felt that clinically this patient did not have hyperthyroidism. She ran a persistent tachycardia, varying between 120 and 85 and averaging between 90 and 100. During the last week or so of hospitalization, the patient was given Lugol's solution and showed a basal metabolic rate of plus 22 after lugolization. This was the lowest basal metabolic rate the patient had reported. However, it was still felt that the patient did not have hyperthyroidism but that the symptoms were on the basis of her anxiety hysteria. She lost six pounds while in the hospital. Her mental outlook improved markedly and the patient seemed to have an insight into her disease. She began to appear more normal, stating that she felt very much rested and was anxious to get back into her home again. The problem in her case was to decide whether or not hyperthyroidism was present and we came to the conclusion that in spite of the elevated metabolic rate, there was no hyperthyroidism present in this patient.

"*Final diagnosis.*

1. Anxiety hysteria
2. Undulant fever
3. Spastic constipation
4. Relaxed pelvic floor with cystocele and rectocele
5. Uterine fibroids
6. Descensus uteri, early.

"Discharge June 1937."

The patient was returned to Dr. Barnum, and on July 22, 1937, the senior author (I.F.H.) was asked to confirm the diagnosis. The blood examination on the date mentioned revealed an agglutination titer of 1:25. The opsonic test showed 6 cells marked, 2 moderate, 11 slight, and 6 negative of 25 cells examined. Blood culture was negative.

The patient was then given four intramuscular injections of Brucellin at intervals of three days. There was a noticeable abatement of symptoms after the second injection. Following the fourth injection all clinical manifestations of the disease disappeared. An opsonic test made August 20, 1937, showed 24 cells marked and 1 moderate out of 25 examined. Up to September first, 1937, the patient had had no return of symptoms of the disease.

CASE 12. Male, aged 33, farmer, entered the State University of Iowa Hospital, February 8, 1932, with a provisional diagnosis of "intestinal flu" and complaining of soreness over the entire abdomen since October first, 1931. There had been nausea, dull pain in the hips and back, chills, continuous fever as high as 103° F., obstinate constipation, nocturia, weakness, tiredness, severe sweats at night, and loss of forty pounds in weight. The patient had stayed in bed but three days. He had had no fever from about November 10 to December 15. On December 15 he began to have severe pain just to the left of the sacrum, aggravated by standing but somewhat relieved by walking. The fever, sweats, and above symptoms also returned.

The chief complaint while on the medical service was severe pain in the right lumbar region and hip. Physical examination revealed an evident loss of weight, spleen just palpable, moderate tenderness in the left sciatic notch, W.B.C. 5,400. The agglutination test was positive for brucellosis in a 1:320 dilution; blood cultures were negative; and x-ray examination showed some evidence of osteoarthritis of the hip.

On March 5 he was transferred to the orthopedic department because of the severe pain which was not relieved by ordinary means. X-ray revealed destructive changes in the interarticular facets of the lower lumbar vertebrae on the right, especially the fourth and fifth, and a distortion of the psoas shadow. At operation the fourth and fifth vertebrae were found to be eroded and fused. A cavity was located just anterior to this which contained 200 cc. of thick pus and from which *Br. abortus* was isolated. A sinus remained, which drained intermittently until about April 1, 1933. He then felt quite well except for a little remaining soreness.

About January first, 1934, the patient again developed a low-grade fever between two and six o'clock in the afternoon, the highest being

100.5° F. There were shifting pains radiating to both groins which at times were severe enough to require morphine for relief. He returned to the orthopedic department on February 15 and a left psoas abscess was drained. The brucellosis agglutination test was negative at this time but unfortunately other laboratory tests were not carried out.

CASE 13. Male, aged 27, farmer. The patient entered the State University of Iowa Hospital, September 15, 1932, complaining of weakness, drowsiness during the day, insomnia, loss of strength and appetite, loss of weight, and constipation. He felt perfectly well until the spring of 1932 when he began to feel weak, but he continued to work on the farm until late August. At this time the fatigue became more marked and vomiting began. The vomiting spells were always preceded by a frontal headache. This was quite severe and was accompanied by stiffness of the neck, but no fever or chills. The headache occurred daily. The patient thinks he lost thirty pounds in weight.

The physical examination was negative save for a temperature of 99.2° F. Patient returned home with a probable diagnosis of brain tumor.

During September 1932 the patient was taken to the Mayo Clinic, and a diagnosis of left frontal tumor was made. At operation no pathology was recognized. Following this there occurred an elevation of temperature with signs of meningeal irritation, including bilateral choked discs of 2 to 4 diopters. He was discharged about the middle of October with a diagnosis of some inflammatory condition rather than tumor formation.

He returned to the Mayo Clinic in November 1932, and remained until the latter part of January 1933. On November 28 the fundi were negative except for a mild thickening of the retina around the discs. On December first, 1933, the spinal fluid showed one plus xanthochromia, 221 small lymphocytes, 21 large lymphocytes. Five taps taken between this date and December 9, 1932, had shown variable amounts of blood. On January 5, 1933, the spinal fluid was yellow and contained 63 lymphocytes. Numerous W.B.C. counts varied between 6,100 and 8,000.

The blood agglutination test was positive in a dilution of 1:320 for brucellosis. On January 4, 1933, both discs were again choked, 3 diopters each. On January 14 an exploration was done and a meningoencephali-

tis was disclosed in the right frontomotor area. Specimens from the area were inoculated into guinea pigs and from these microorganisms of the *Brucella* group were isolated.

During December 1932, he had had transient attacks of weakness of the left arm and leg. After his return home on March 8, 1933, he had his worst attack of paralysis involving the left arm and leg so that they could not be used for three weeks. There were no convulsions. There was gradual improvement until the patient could walk for a considerable distance but the left hand continued to be "stiff and slow."

Fatal Cases

Case 14. Male, aged 21, packing-house employee. Our first contact with this patient was during a survey conducted in a packing plant for evidence of brucellosis among the employees. The patient considered himself well at that time, but his serum agglutinated *Br. abortus* in a 1:2,560 dilution. One month later (November 26, 1928) he stopped work and consulted his physician because of profound weakness. During December and January he passed through a moderately severe course of brucellosis, with the usual night sweats, anorexia, and restlessness, and in addition two attacks of anginal pain in the left chest, side, and arm (January 14, 1929, and January 24, 1929). Evidence of myocardial failure, but without constant signs of valvular lesions, appeared and soon the patient died (February 21, 1929). Blood received on December 10, 1928, agglutinated *Br. abortus* in a dilution of 1:2,560. Culture medium inoculated with blood and incubated four days was sent to us, and from this *Br. suis* was isolated.

Necropsy. Three hours after death a necropsy was performed by Dr. Woodward of Mason City, Iowa, and from him the following notes were obtained.

Height 5 feet 8 inches; weight 140 pounds; below the knees there was edema. The serous cavities contained clear fluid as follows: abdominal, 2 liters; pleural, 1 liter on each side; pericardial, 300 cubic centimeters; the lower lobe of right lung showed fibrous adhesions to the chest wall. There was marked anthracosis in lungs and bronchial lymph glands. The trachea and bronchi contained mucopurulent material. The heart was hypertrophied to twice its usual size, and weighed 597 grams. When the heart was removed, an abscess in the interior mediastinum was opened, it was the size of a hen's egg and contained a bloody pus.

The aorta had an erosion one centimeter in diameter and the anterior cusps were entirely destroyed. There was a mass three centimeters in diameter occupying the sinus behind the valve and connecting with the abscess in the mediastinum. The liver was markedly enlarged and of the nutmeg type. The spleen was enlarged, but on section no unusual pathological change was noted. Other gross abnormalities were not noted. From a culture of heart's blood which was sent us, the *Br. suis* was isolated. The content of the abscess cavity was not examined culturally.

Histological examination. Portions of the various organs were preserved and also sent for examination. Sections were prepared and stained. One set was sent to the Hygienic Laboratory (now the National Institute of Health), Washington, D. C., and the detailed report made by Passed Assistant Surgeon R. D. Lillie is presented here in full.

A. *Pancreas.* Islets numerous and some quite large. No focal lesions.

B. *Peribronchial lymph gland.* Moderate amount of coal pigment, marked reticuloendothelial hyperplasia, with relatively few free macrophages, some of which contain phagocytosed red corpuscles. Germinal centers are inconspicuous and made up largely of small lymphoid cells. Moderate numbers of leucocytes seen among the reticulum cells.

C. *Lymph glands.* (1) Reticuloendothelial hyperplasia is even more numerous and more of the macrophages contain red corpuscles. The swelling and vacuolation, close packing, and necrosis of these cells seen in typhoid are not noted here, and fixed reticuloendothelial cells greatly predominate.

(2) Again even more marked hyperplasia of the reticuloendothelium along the course of the sinuses, with moderate numbers of lymphocytes, polymorphonuclears, and macrophages in the meshes of the fixed tissue cells. Around these occupying about the middle third of the lobules are zones of swollen liver cells with more eosinophilic vacuolated cytoplasm, the vacuoles being fine to medium in size. The centers of the lobules are occupied by a more or less confused mass of vacuolated oxyphil liver cells with karyolytic nuclei, or no nuclear staining whatever, between which are surviving endothelial cells containing yellowish-brown granular pigment and not infrequently dilated blood-filled capillaries. The periportal connective tissue shows moderate lymphocyte infiltration.

D. *Spleen.* The follicles are of moderate size. A few of these show

centers of large swollen reticulum cells with cloudy appearing oxyphil cytoplasm which appears very finely granular with a wide aperture immersion lens on oblique illumination. The pulp contains a considerable amount of blood, a few leucocytes and macrophages, and moderate numbers of lymphoid cells.

E. *Kidney*. The glomeruli present occasional patches of swollen granular parietal capsular epithelium. The cortical convoluted tubules show granular oxyphil or finely reticular cytoplasm, often with distinct rodded border toward the lumen. Their lumina contain granular debris, irregular oxyphil masses, and some more compact hyaline cast-like masses. The coarse limbs or Henle's loops show probably a little more cellular swelling and more debris in the lumen. The collecting tubules of the cortex and the medulla present relatively normal epithelium and contain hyaline and less often granular, elongated, rather compact masses. A small area in the pyramid shows centrally more or less broken down polymorphonuclear leucocytes, about this a zone of mixed polymorphonuclear and large, rounded, or stellate cells with large vesicular leptochromatic nuclei and rather broad lightly eosinophil cytoplasm. The last grade over into fibroblasts toward the periphery. Here considerable numbers of lymphocytes are seen and the whole lesion is surrounded by a zone of intense congestion and interstitial hemorrhage.

Anatomical findings. Reticuloendothelial hyperplasia of lymph glands; centrolobular necrosis and degeneration of liver; nephrosis, acute toxic.

Case 15. Female, aged 57, housewife. In the past and present history of the patient and in the family history there were no significant data. When seen June 20, 1928, the following history was obtained.

The patient last felt well in December 1927, but from that date she noted weakness which progressively became more severe. In January 1928, she was in bed for one week with a febrile illness considered by her as "flu." Recovery from this was slow and incomplete. Through February and March she continued with her housework, but complained of general aching and weakness. From April first the symptoms were moderately severe, though she did not become bedfast until June first. Her symptoms were weakness, progressively increasing, and spells of chilliness, particularly in the afternoon, so severe that she would go to bed with a hot soapstone and would still be cold. Several

rigors occurred. She sweat profusely, usually after midnight, the bed linen becoming "wet clear to the mattress." General aching, varying in severity, mild headache, backache, and marked anorexia, with distressing and persistent nausea and occasional vomiting, occurred. The latter was the prominent symptom throughout the last part of her illness. There was a hacking cough, with glairy mucoid sputum and loss of weight, estimated at 40 pounds in a woman weighing normally 180 pounds. The temperature during June was remittent in character, rarely above 102° F.

The striking feature of the physical examination was the weakness of the patient, who readily became exhausted by talking. A few fine râles were heard scattered over the lung bases posteriorly. The spleen or liver could not be palpated. Superficial glands showed no enlargement.

The latter course of this illness showed no new features. The nausea and vomiting could not be controlled; the patient progressively became worse and died August 15, 1928.

During June and July three blood specimens were received and *Br. abortus* was agglutinated twice in a 1:640 dilution and once in 1:1,280 dilution. No hemocultures were taken.

The urine was found to contain a small number of pus cells and some albumin.

Necropsy. A partial necropsy was allowed, and this was performed by Dr. Nyquist of Eldora, Iowa. He reported that the striking feature was the complete absence of any notable gross pathological changes in the organs of the abdominal and chest cavities. This was confirmed by an examination in the gross of the organs sent for study.

Histological examination. Sections were prepared and sent for confirmation of our observations to the Hygienic Laboratory (now the National Institute of Health), Washington, D. C. Their report in part follows (findings by Passed Assistant Surgeon R. D. Lillie).

A. *Small intestine.* Moderate postmortem autolysis of mucosa.

B. *Pancreas.* Patchy interstitial fibrosis with areas of infiltration chiefly with lymphocytes, and a few plasma cells and macrophages; chronic interstitial pancreatitis.

C. *Spleen.* Pulp space contains largely laked blood. Moderate numbers of free large mononuclear cells in sinuses. Follicles small, made up largely of small lymphoid cells. No focal lesions.

D. *Muscle.* Plainly striated, much interstitial fat.

E. *Gall bladder.* Mucosa autolyzed; stroma shows patches of infiltration with lymphocytes and in places plasma cells. The serosal layer appears thickened and fibrous.

F. *Duodenum.* No focal lesions or ulceration. Mucosa shows autolysis of villus epithelium and many lymphoid cells in the stroma.

G. *Lungs.* New small patches of scarring and coal pigmentation.

H. *Heart.* Considerable epicardial fat. Fibers show moderate lipochrome pigmentation at the poles of the nuclei, clear-cut cross striation, well-defined fibrillae, and a moderate amount of transverse fragmentation.

I. *Fat tissue.* No pathology.

J. *Liver.* The centers of the lobules showed dilated capillaries filled with laked blood, between which are compressed liver cells distended by large vacuoles. This zone is often surrounded by a zone of much distended, coarsely vacuolated liver cells. This vacuolation is probably due to fatty infiltration. There are no focal necroses. The periportal connective tissue contains a moderate number of lymphocytes.

K. *Colon.* No ulcers or focal lesions. Moderate autolysis of mucosa.

L. *Kidney.* Considering the degree of autolysis in other tissue and the hemolysis of the blood in the kidney capillaries, the convoluted tubules appear very well preserved. Lesions of the glomeruli or vessels are not noted.

Anatomical findings. Chronic interstitial pancreatitis; chronic cholecystitis; fatty infiltration of liver; passive congestion of liver; fragmentary myocardial degeneration.

Comments. Without knowledge of the bacteriological, clinical, serological, and gross anatomical findings we hesitate to make an interpretation of the findings in these two cases [Nos. 14 and 15]. The presumed fatty infiltration or degeneration in the centers of the liver lobules in the two cases rests purely on morphology, the ordinary microchemical methods not being applicable, as the material is already stained and mounted in balsam.

CASE 16. Male, aged 27, farmer. Prior to the present illness the patient had enjoyed good health. Onset began insidiously about the end of March 1929. He first noted that he readily became tired and drowsy

and with difficulty continued his work. He remained ambulatory until early June, but for most of the period could undertake no work. He was seen June 19, 1929, and his symptoms were as follows: marked weakness; rigors, nightly for two weeks; profuse night sweats; severe general aching; intermittent headache, worse during ambulatory period; mild lumbar backache, stiff neck—"required rubbing every night"; anorexia and constipation, with severe spells of nausea and vomiting; restlessness and irritability; for two weeks a severe cough, which persisted and became the prominent feature of the latter part of the disease.

We have no record of the physical findings.

The blood specimens collected twice during June both agglutinated *Br. abortus* in a 1:320 dilution.

Br. abortus and *suis* were isolated from the blood cultures. The fact that both porcine and bovine varieties were isolated was of particular interest.

Toward the end of July the symptoms and signs of lung abscess developed. Early in August an operation for drainage was performed. The patient gradually grew weaker and shortly after he died.

Whether one or both of the varieties of *Brucella* found by culture were primarily involved in the production of the lung abscess, or whether the infection reduced the general and local resistance to other organisms which brought about the tissue destruction cannot be determined.

CASE 17. Female, aged 27, housewife. This patient had enjoyed exceptionally good health prior to the onset of the present illness. Her husband had suffered from a fever during January and February 1927, which clinically had been diagnosed as typhoid fever, but the characteristics were found to be those of brucellosis. Blood serum obtained during April agglutinated *Br. abortus* in a 1:100 dilution, but caused no agglutination of *E. typhi* or *S. paratyphi.* Throughout his illness he had been nursed by his wife. During his convalescence, on March 12, she suddenly became acutely ill. The evening meal had been enjoyed with friends, but shortly afterwards she was taken home to bed with severe headache, prostration, and fever. Before midnight the physician was called and a temperature between 103° F. and 104° F. was found.

For ten days the patient was cared for at her home. Her complaints were extreme weakness, marked general aching, spells of chilliness, complete anorexia, frequent nausea, and occasional vomiting. On March 21, the tenth day of illness, the patient was admitted to the hospital. The physician's admission note was as follows: "The high temperature is not characteristic of influenza, but the absence of all symptoms and findings of typhoid make it the only diagnosis available at this time." There was no diarrhea, tympanites, or notable constipation, and the patient lacked the dull toxic appearance so characteristic of typhoid. During the subsequent course the patient was remarkably free from discomfort, complaining only of extreme exhaustion and feverishness. The temperature was sustained and continued to rise. The pulse became very rapid, but the respiration was never embarrassed.

Physical examination throughout was essentially negative. Late in the disease râles appeared. The spleen was not palpated.

The illness progressed, delirium and coma appeared, and there were involuntary passages of urine and feces. The patient died on the twenty-first day of the disease, with death attributed to a myocardial failure.

Blood smears were examined by us. There was obviously a marked leucopenia and the differential count showed polymorphonuclears, 21 per cent; small lymphocytes, 10 per cent; and large mononuclears, 69 per cent.

Three blood specimens, collected on the eleventh, fifteenth, and nineteenth days of the disease, were sent for Widal tests. The first two were dried specimens, the last whole wet blood. The first specimen showed no agglutination of *Br. abortus,* the second showed microscopically some clumping in the 1:40 and 1:80 serum dilutions, while the third showed complete agglutination in the 1:80 serum dilution. This record of increasing agglutinations gives strong evidence of the specific nature of the infection, even though the final titer is not high.

The temperature chart of this case is shown as the first of the two malignant types illustrated in Figure 16.

No postmortem examination was allowed.

There is here clinical evidence of an overwhelming septicemia, with no evidence of any localization.

The possible source of this infection gives added interest to this case.

The family used pasteurized milk. The husband was frequently away and acquired his infection from some undetermined source, while the wife as nurse may have acquired her infection from the excreta of her husband.

CASE 18. (Reported by Sanders, 397, and by Hansmann and Schenken, 171.) Male, aged 24, pressman. The patient entered the State University of Iowa Hospital October 7, 1931, complaining of headache and spells with stiff neck, stupor, weakness, and difficulty in walking. On Christmas Eve, 1930, while at a show, his left foot seemed to be asleep, and the tingling spread from the foot up the leg to the thigh, the left half of the body and neck, and also one-half of the tongue. He walked to a physician's office but could not talk. Before the doctor arrived the condition cleared up, lasting thirty minutes in all. Headache began about ten minutes later, was very severe over the temples, and was associated with pain in the neck for several hours. Several such attacks followed (every two or three weeks) involving the right side of the body as well as the left. The patient was delirious on four occasions and unconscious for a variable period of time. The condition was always relieved by lumbar puncture.

In September 1930 he developed a high temperature (105° F.), was nauseated, vomited, had severe headache, and became unconscious. During a lumbar puncture the patient suddenly developed a convulsion which caused the needle to be broken off. He was sent to the hospital chiefly for the removal of the needle. It was stated that the weight varied, losing with the attacks and gaining during the interval. The appetite was always good. A low-grade temperature was found to be persistent.

The eyes were found to be prominent, spleen palpable, station and gait unsteady, and reflexes disturbed. The blood serum agglutinated *Br. abortus* in a 1:1,280 dilution. X-ray showed a broken needle measuring about 5 cm. in length in the midline between the fourth and the fifth lumbar vertebrae. The needle was removed and postoperative course was uneventful. In the hospital the seizures continued and became more frequent. The eye grounds revealed evidence of increased intracranial pressure. Spinal fluid culture yielded *Br. suis*. The fluid was quite tur-

bid. On November first, 1931, the patient again became delirious, developed a divergent squint, and a stiff neck. The spinal fluid was found to be quite bloody and *Br. suis* was again isolated. The patient died on November 3, 1931.

Necropsy. When the meninges were exposed, grayish-white tubercles were noted in the leptomeninges overlying the superior surface of both cerebral hemispheres. On removal of the brain a large blood clot was discovered involving the base and covering the medulla, pons, cerebral peduncles, and the optic chiasma. After fixation a mycotic aneurysm was found which had ruptured. *Br. suis* was isolated from the "tubercles," blood clot, and lymph nodes, but the heart's blood showed no growth.

CASE 19. (Reported by Dr. C. P. Carson of Denver, Colorado.) Female, aged 40, housewife. The patient, a resident of Colorado, was admitted to the hospital September 28, 1937. A diagnosis of brucellosis was established October 7, 1937. The patient died October 27, 1937.

Necropsy. The body was slightly warm with no rigor mortis present. There was general edema over the entire body, most marked in the lower extremities. There were no identifying scars on body.

A. *Abdomen.* The peritoneum was shiny and free from exudate. The intestines were all moderately distended with gas. There were adhesions of omentum over the entire peritoneal wall and intestines. There were 2,000 cc. of clear, straw-colored fluid in the abdominal cavity.

B. *Liver.* Normal in size and consistency.

C. *Pancreas.* No pathology.

D. *Gall bladder.* No pathology.

E. *Spleen.* The spleen was of normal size. The cut surface presented a dark red color. The pulp scraped away with moderate ease.

F. *Stomach.* The gastrointestinal tract showed no abnormalities other than slight congestion of the vessels.

G. *Kidneys.* Both kidneys were normal in size. The capsules stripped fairly easily. The cut surface showed a pink color with slight congestion.

H. *Uterus.* The right uterine tube was enlarged to about 2 cm. diameter. The lumen was patent, but sealed at the fimbriated end. The lumen contained about 2 cc. of sero-purulent fluid. There was a walled-off abscessed area, containing about 75 cc. of pus, with a slight greenish tinge of color, under the right broad ligament.

I. *Thorax.* There was nothing of note found in either the pleural cavity or the lungs.

J. *Heart.* The pericardium contained no adhesions. There was a slight increase of fluid, about 50 cc. The heart was of the normal size and the muscle was of fairly good consistency. There were no valvular lesions.

K. *Kidneys.* The kidneys showed some slightly increased interstitial fibrosis. The tubular epithelium showed slight cloudy swelling. The glomeruli showed no noteworthy changes.

L. *Uterus.* The right uterine tube walls were of increased thickness, edematous, and considerably infiltrated with round cells. The folds of mucosa were markedly infiltrated with round cells and showed free pus cells in the lumen. A section of tissue from the abscessed wall of the right broad ligament showed typical abscess formation.

M. *Spleen.* There was no histopathology demonstrable in sections of the spleen.

CASE DUE TO *BRUCELLA SUIS*

CASE 20. (Keefer, 243.) *A.S.* (Med. No. 48513); Aged 19; Male; White; Laboratory technician. Admitted October 18, 1922; Discharged December 10, 1922.

Complaint.—Evening fever and chills for three weeks.

F.H.—Unessential.

P.H.—The patient has always enjoyed excellent health until his present indisposition. He has never had any serious illness. No history of typhoid fever, pneumonia, malaria or rheumatic fever. One year prior to his present illness he had an acute maxillary sinusitis which was promptly and completely relieved by proper treatment.

His habits have been exemplary. He has not been out of Baltimore City for several months, and then he was not out of the state but attended a National Guard Encampment. He has never drunk goat's milk. He has not been exposed, so far as he knows, to typhoid fever, tuberculosis or malaria. He has been working as a technician in the histological laboratory of the Medical School but no bacterial cultures have been used in his work, and no goats have been kept in the laboratory. He has always been very fond of milk and has been in the habit of eating considerable quantities of cheese.

Present illness.—Four weeks before entering the hospital the patient began to suffer from frontal headaches which came on most frequently

in the late afternoon and early evening. The headaches were soon followed by and associated with an evening fever and chilly sensations. His headaches became less severe and seemed to be relieved somewhat by wearing glasses which corrected a slight myopia.

Three days before admission to the clinic, he developed pain and distress in the epigastrium which was accompanied by nausea but no vomiting, and his temperature, taken that evening, was 102° F. With these abnormal sensations he had chilliness, moderate prostration, restlessness, insomnia and headache; upon several occasions he had definite chills and drenching sweats. These symptoms were always worse at night but during the day he felt tired and had aching in his joints, particularly the hip and knee joints. He did not give up his work in spite of his indisposition until he entered the ward.

P.E.—The temperature was 99.2° F. (38° C.). Pulse 72. Respirations 20 per minute. Blood pressure 120/65. There was evidence of recent loss of weight, but the skin and mucous membranes were of fairly good color and no abnormal eruptions were present. The bones and joints showed nothing abnormal. The lymph glands were not enlarged. Examination of the head and neck revealed nothing abnormal except several carious teeth. The tonsils were not enlarged.

The heart and lungs were clear. The abdomen was soft and the spleen was not felt. Examination of the genitalia, reflexes and nervous system showed nothing striking. A rectal examination was negative.

LABORATORY EXAMINATIONS

Blood: R.B.C.	4,348,000		
Hb.	68%	(Sahli)—(normal reading 85%)	
C.I.	0.8%		
W.B.C.	12,000		

Differential formula—300 cells

P.M.N.	55%	6,060
P.M.B.	0.3%	40
P.M.E.	0.7%	80
S.L. & L.L.	34%	4,620
L.M. & Trans.	10%	1,200
	100%	12,000

Stained smear showed slight pallor of all the red blood cells. No abnormalities were seen except the increase in lymphocytes and the slight increase in the large mononuclear-transitional group. No malarial parasites were seen in fresh or in stained preparations.

Blood Wassermann.—Negative.

Blood culture.—Taken the day patient entered was sterile after four days' incubation.

Urine.—Clear; Sp.G. 1,020; no albumin, casts, bile, or blood. Test for Urobilin was positive.

X-ray of chest.—Lungs clear, heart and aorta not enlarged.

X-ray of sinuses.—Clear.

Widal.—Negative.

Urine culture.—Negative.

Stool culture.—Normal intestinal flora.

A very careful search for foci of infection, including sinuses, teeth, gastrointestinal and urogenital tracts, revealed nothing abnormal except several carious teeth.

Summary.—A young man who had been perfectly well until three weeks before his admission to the hospital. The onset of his sickness was gradual, with evening fever, chills, headache and malaise, and was followed by loss of flesh, insomnia, arthralgias and weakness. Upon admission to the hospital the only significant features revealed by the physical examination were slight evidence of loss of weight, slight pallor, several carious teeth, and slight fever.

Laboratory examinations revealed nothing of great importance at first, with the exception of the blood picture which showed a moderate secondary anemia with a slight leucocytosis and an increase in the lymphocytic, large mononuclear, and transitional elements.

The diagnosis of Malta fever became evident on recovery of the organism from the second blood culture.

Blood picture.—[During hospitalization a number of blood examinations were made.] The striking features are a slight grade of secondary anemia with normally appearing red blood cells and a white blood cell count which varies from six to twelve thousand with a relative and absolute increase in the lymphocytes as high as 52 per cent with an increase in the large mononuclear-transitional group as high as 22 per cent. . . .

COURSE IN THE HOSPITAL

Symptomatic.—The patient's symptoms at all times during his stay in the hospital were mild and consisted chiefly of headaches, attacks of restlessness and irritability. These were most prominent during the height of the fever. He had numerous drenching night-sweats and occasionally definite chilly sensations. He had definite arthralgias, but never any swelling or tenderness of the joints. *He was entirely free from neuralgic pains* and never had any signs of any of the complications of this disease, such as orchitis, parotitis or neuritis.

He had moderate anorexia and constipation and lost several pounds of weight, felt weak and worn out. Outside of these rather generalized symptoms indicative of a low-grade infection, he was not very much disturbed.

Fever.— . . . the fever was remittent in character with several waves of rise and fall. One of the remarkable things is that the temperature became normal and the patient improved markedly long before his blood stream was free from organisms.

Recovery was complete. The patient, when seen in September 1923, had gained thirty pounds in weight and had never had a recurrence of fever or symptoms.

[The case was dismissed from the hospital, asymptomatic, on December 10, 1922. The organism was cultured from the patient's blood on repeated examinations up to January first, 1923. This is certain evidence that a patient may be asymptomatic and yet continue to carry the organism for a considerable period of time.]

CASES TREATED WITH BRUCELLIN

CASE 21. (Huddleson and Johnson, 214.) Male, aged 22, veterinary student. Physician: Dr. Olin, College Hospital, Michigan State College.

Approximate date of onset: June 3, 1932.

Probable source of infection: Laboratory.

Brief description of symptoms and signs: Fever, sweats, chill, joints ache, sensitive skin, headaches over entire head, enlarged left cervical lymph node, loss of appetite, depressed mental attitude.

Titer of agglutination test: 6/9/32, + 1:1,000.

Opsonocytophagic activity of citrated blood before treatment: 6/9/32.

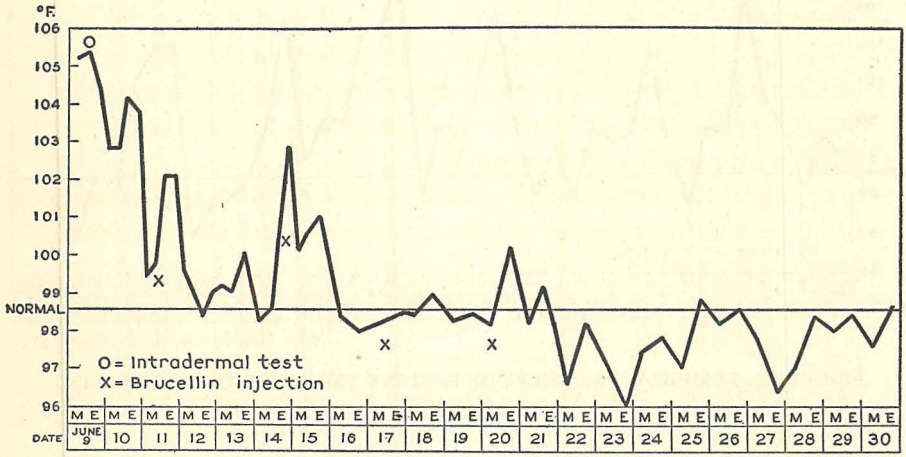
of 25 cells examined, 3 showed marked phagocytosis, 4 moderate phagocytosis, 14 slight, and 3 negative.

Intradermal test: 6/9/32, positive.

Blood culture: 6/9/32, positive, *Br. melitensis* isolated aerobically.

Dates of injection of Brucellin: 6/11/32, 1 cc.; 6/14/32, 1 cc.; 6/17/32, 1 cc.; 6/20/32, 1 cc. Reaction from each injection moderately severe.

Results: All objective and subjective symptoms disappeared about June 22, 1932. Opsonocytophagic activity of citrated blood: 6/25/32, of 25 cells examined, all showed marked phagocytosis. Opsonocytophagic activity of citrated blood: 7/25/32, results same as 6/25/32. Agglutination test: 7/26/32, + 1:500. See Figure 37 for record of temperature.



After Huddleson and Johnson (214)

FIGURE 37. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 21

CASE 22. (Huddleson and Johnson, 214.) Male, aged 42, farmer. Physician: Dr. Coffey, University Hospital, Ann Arbor, Michigan.

Approximate date of onset: April, 1931.

Probable source of infection: Unknown.

Brief description of symptoms and signs: Weakness, ease of fatigue, generalized aching periodically.

Titer of agglutination test: 6/29/32, + 1:640.

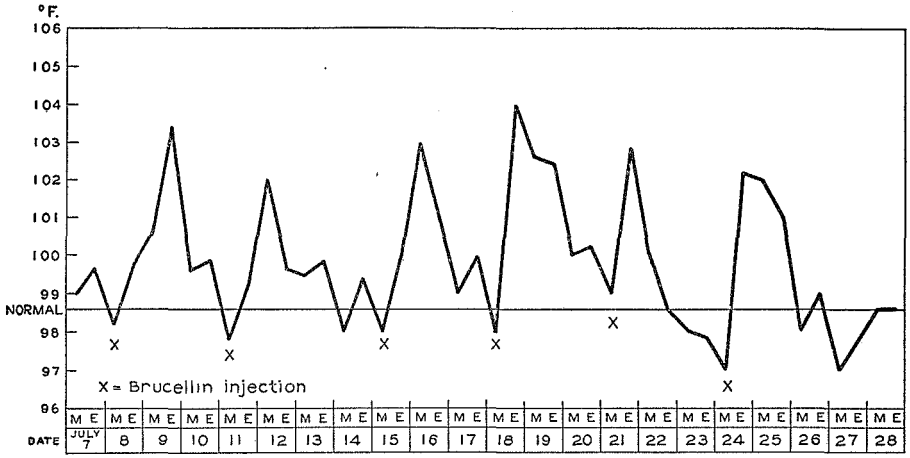
Opsonocytophagic activity of citrated blood: Test made at the Uni-

versity Hospital before treatment: of 100 cells examined, none showed marked phagocytosis, 2 were moderate, 22 were slight, and 76 were negative.

Intradermal test: None made.

Blood culture: 6/29/32, negative.

Dates of injection of Brucellin: 7/8/32, 0.5 cc.; 7/11/32, 0.5 cc.; 7/15/32, 0.8 cc.; 7/18/32, 1.0 cc.; 7/21/32, 1.0 cc.; 7/24/32, 1.0 cc. Reac-



After Huddleson and Johnson (214)

FIGURE 38. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 22

tions from injections would usually begin with a rise in temperature about five hours following injections and would reach a peak of 103 to 105° in about twenty hours. Following this the temperature would drop to normal within twenty-four hours. The only subjective symptoms were generalized aching pains, often accompanied by headache.

Results: All objective and subjective symptoms disappeared about 7/27/32. Opsonocytophagic activity of citrated blood: 7/27/32, of 100 cells examined, 46 were marked, 24 moderate, 20 slight, and 10 negative. For record of temperature see Figure 38.

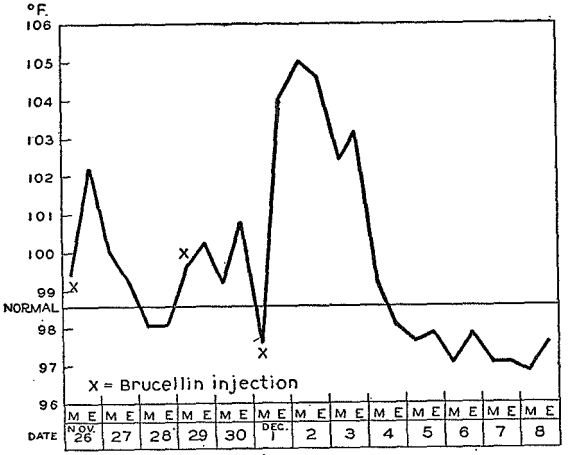
CASE 23. (Huddleson and Johnson, 214.) Male, aged 35, manager of milk receiving station. Physician: Dr. Fillinger, Ovid, Michigan.

Approximate date of onset: November 19, 1932.

Probable source of infection: Either composite grade A raw milk or contact with cows.

Brief description of symptoms and signs: Fever, chills, basal headaches, aches and pains all over as if having influenza, tender spots on arms and legs.

Titer of agglutination test: 11/25/32, + 1:100.



After Huddleson and Johnson (214)

FIGURE 39. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 23

Opsonocytophagic activity of citrated blood before treatment: 11/25/32, of 25 cells examined, 10 showed marked phagocytosis, 7 moderate and 8 slight.

Intradermal test: 11/25/32, positive.

Blood culture: 11/25/32, negative.

Dates of injection of Brucellin: 11/26/32, 1 cc.; 11/29/32, 0.5 cc.; 12/1/32, 1.1 cc. Very marked reaction beginning four hours after 1 cc. on 11/26/32 and lasting for twenty-four hours. Very mild reaction after second injection, beginning four hours after injection and lasting twenty-four hours. A very severe reaction after 1.1 cc. injection beginning four hours after injection and lasting three days.

Results: All objective and subjective symptoms disappeared after 12/

4/32. Opsonocytophagic test: 12/8/32, of 25 cells examined, 20 showed marked phagocytosis and 5 moderate. For record of temperature see Figure 39.

CASES IN MALTA TREATED WITH BRUCELLIN

CASE 24. Female, aged 18, domestic servant. Physician: Professor P. Xuereb, Valletta, Malta.

Approximate date of onset: October 7, 1937.

Probable source of infection: Goat's milk.

Brief description of symptoms and signs: Fever, slight frontal headache and backache, severe pain in left hip, constipation, enlarged spleen.

Titer of agglutination test: October 24, 1937, + 1:500.

Intradermal Brucellergen test: October 25, 1937, 4 +

Blood culture: October 7, 1937, *Br. melitensis* isolated.

Dates of injection of Brucellin: 10/26/37, 1 cc.; 10/30/37, 1 cc.; 11/8/37, 1.5 cc.; 11/10/37, 1.5 cc.

Results: There was a noticeable drop in temperature after the second injection. Brucellin was discontinued until the temperature again became elevated. Two injections of 1.5 cc. each were then given. A marked systemic reaction followed the first with complete disappearance of all symptoms and signs of the disease. The patient was kept under observation for twelve days after the temperature returned to normal. See Figure 40.

CASE 25. Female, aged 17, no occupation. Physician: Professor P. Xuereb, Valletta, Malta.

Approximate date of onset: August 26, 1937.

Probable source of infection: Goat's milk.

Brief description of symptoms and signs: Fever, rigors, occipital headache, backache, slight cough, slight dyspnea, loss of appetite, constipation, enlarged spleen, albumin in urine.

Titer of agglutination test: 10/23/37, + 1:500.

Intradermal test: 10/23/37, + 3 x 6 cm.

Blood culture: 10/23/37, *Br. melitensis* isolated.

Dates of injection of Brucellin: 10/25/37, 9 A.M., 0.6 cc.; 10/28/37, 5 P.M., 1 cc.; 11/1/37, 4 P.M., 1.5 cc.

Results: Systemic reactions were obtained from the first two and fourth

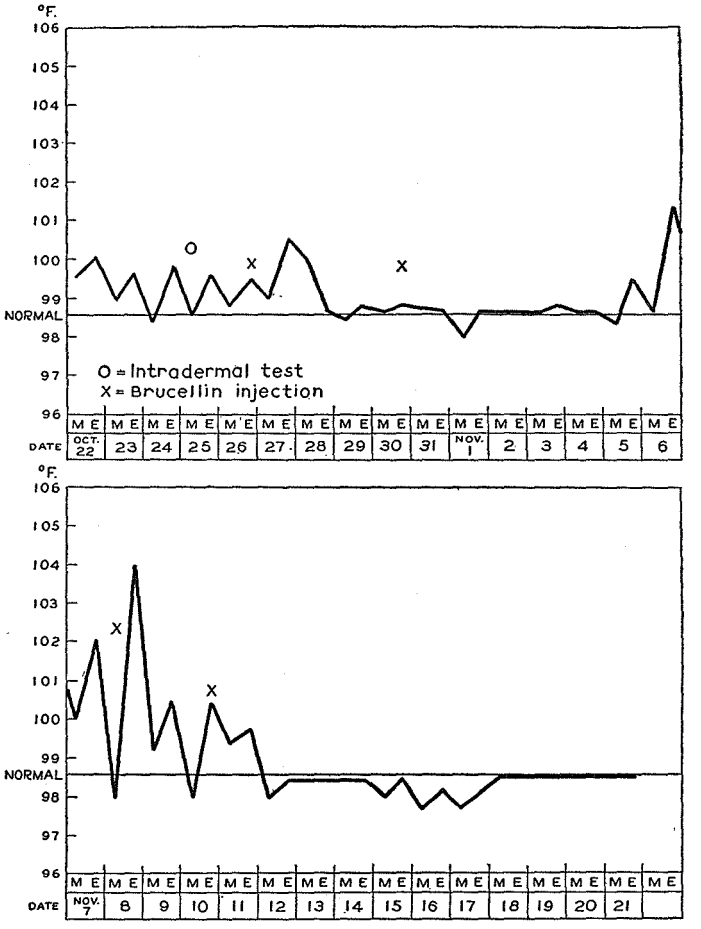


FIGURE 40. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 24

injections. All symptoms and signs of disease disappeared after fourth injection. Duration of disease after beginning treatment, eleven days. See Figure 41 for record of temperature.

CASE 26. Male, aged 34, cab driver. Physician: Professor E. H. Ferro, Valletta, Malta.

Approximate date of onset: September 15, 1937.

Probable source of infection: Goat's milk.

Brief description of symptoms and signs: High fever, sweats, chills, frontal headache, slight backache, slight cough, slight dyspnea, poor appetite, constipation, enlarged spleen, mental attitude dull, albumin in urine.

Titer of agglutination test: 10/25/37, + 1:500.

Intradermal test: 10/25/37, + 5 x 8 cm.

Blood culture: 10/25/37, *Br. melitensis* isolated.

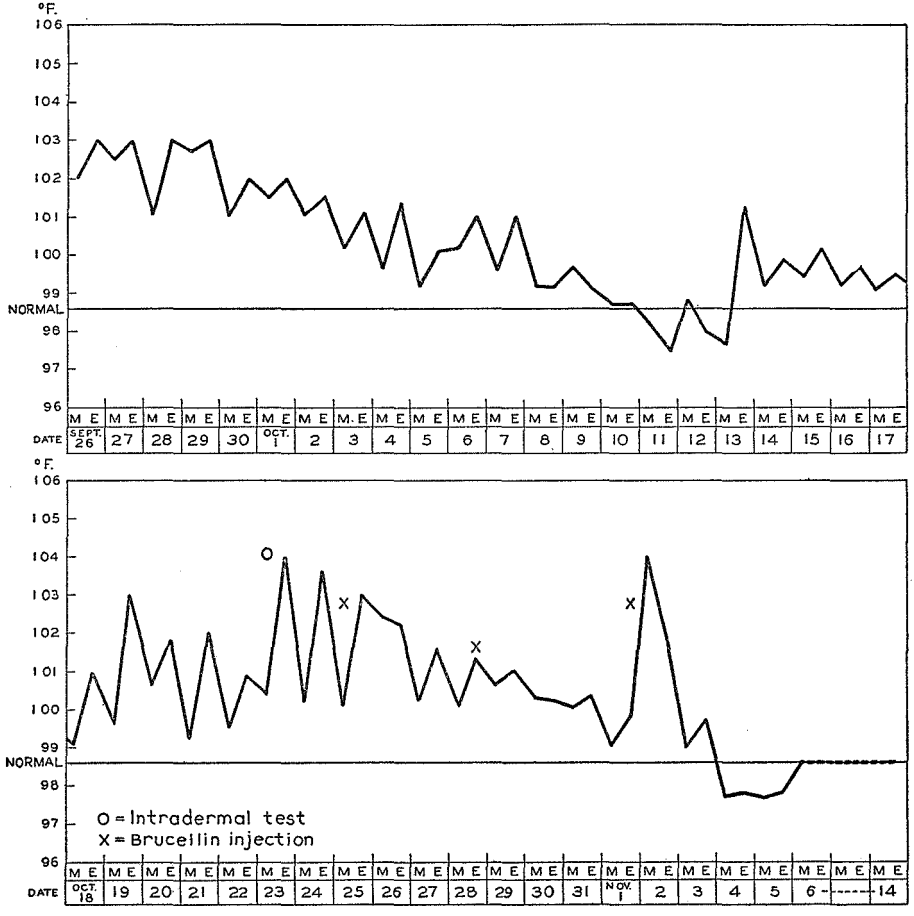


FIGURE 41. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 25

Dates of injection of Brucellin: 10/28/37, 5 P.M., 1 CC.

Results: Systemic reaction to the injection was followed by the rapid disappearance of all signs and symptoms of the disease. The duration of disease after beginning treatment was three days. See Figure 42 for temperature record.

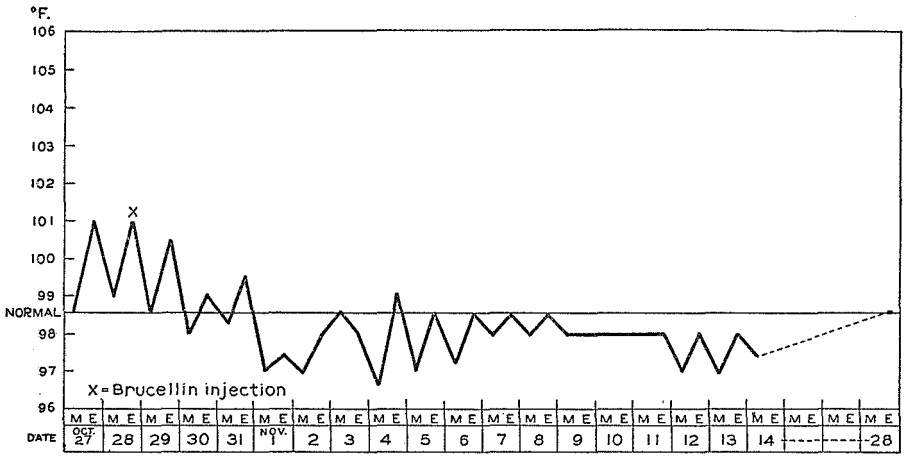


FIGURE 42. TEMPERATURE CURVE OF PATIENT TREATED WITH BRUCELLIN: CASE 26

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