OBSERVATIONS ON THE BRUCELLA ABORTUS
INFECTION IN THE BOVINE

DISSERTATION
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INTRODUCTION

Farmers and veterinarians alike have adopted the basic principle, whenever possible, that it is wiser and more economical to eradicate infectious diseases of animals rather than tolerate the economic losses they incur. This is especially true when such diseases have been found to be transmissible to man. Such drastic control methods often have encountered active and sometimes violent opposition which gradually subsides when the wisdom of the program becomes more apparent.

We can all be proud that this is the safest country in the world in which to raise livestock. This would not be true, were it not for eradication procedures adopted in the control of such destructive diseases as foot-and-mouth disease, Texas fever, contagious pleuropneumonia, tuberculosis and brucellosis. Contagious pleuropneumonia never was permitted to gain a foothold. Foot-and-mouth disease has been stamped out several times and is not now present within our borders. Tuberculosis is a relatively minor problem with the attention now directed toward the eradication of the disease from a small percentage of animals, and the prevention of its reappearance in our herds by periodic retesting. The incidence of bovine brucellosis has declined from approximately 10 percent in 1934 to 2.7 percent in 1955.
Such results have been brought about through years of research which have provided us with information as to the basic nature of such diseases, the finding of satisfactory methods of control and then applying the methods in active and vigorous disease control programs.

It was the purpose of these experiments to add, in a small way, to the already vast knowledge of information so that this country will continue to be recognized as the safest place in the world to raise livestock.
REVIEW OF THE LITERATURE

General Information

Definition. Brucellosis is a specific infectious disease of animals and man, caused by micro-organisms or bacteria of the genus Brucella. The three known types of this genus are: (1) *Brucella abortus* - most commonly causing the disease in cattle; (2) *Brucella suis* - most commonly causing the disease in swine; (3) *Brucella melitensis* - most commonly causing the disease in goats.

In cattle, the disease is also known as Bang's disease and contagious abortion; in swine, as contagious abortion. In man, brucellosis is known as undulant fever or sometimes when caused by *Br. melitensis*, as Malta or Mediterranean fever.

History. *Br. melitensis* was the first species of the genus Brucella to be identified. It was isolated by Bruce (27) in 1887. This same species had been isolated from the spleen of patients who had died of a disease named "Mediterranean or gastric fever" by Marston (169) as early as 1859. The causative organism was named *Streptococcus melitensis* by Hughes (130) in 1892, and *Micrococcus melitensis* by Bruce (26) in 1893. Early workers did not recognize the bacillary form of the organism. The chief host of *Br. melitensis* is the milk goat. This fact was discovered by Zammit (260), 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 the aborted fetuses of sheep and goats in France, Italy, and Argentina.

*B. abortus* was first isolated and described as a Bacillus, by Bang (2), 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. *B. 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.

The earliest recorded evidence of brucellosis in cattle in the United States, appeared in "The Cultivator" in 1843, Evans (70). At this time, many losses from abortion in cattle in the Eastern United States were reported. Evans suggested that *B. abortus* may have been brought to this country in cattle, since abortion rates of 50 to 60 percent were observed in some parts of Great Britain as early as 1567. Epidemics of abortion occurred in cattle in the region of the Mississippi River according to writings by Jennings in 1864, Huddleson (117). However,
the nature and the common cause of these outbreaks were not known until several research workers in Europe studied the problem.

In the United States, \textit{Br. abortus} was first isolated from cows which aborted, at the Illinois Experiment Station, by MacNeal and Kerr (160).

\textit{Br. suis} was first isolated by Traum (247), in 1914, from fetuses expelled prematurely from sows. Although this species of Brucella resembles \textit{Br. abortus}, culturally and antigenically, it differs markedly in one respect, in that it does not require an increased CO\textsubscript{2} tension for primary isolation. The hog appears to be the chief host for \textit{Br. suis}.

\textit{Br. suis} has been isolated from hogs in the United States, Hungary, Denmark, Switzerland, Brazil, Argentina, and Japan. The Danish strains (117) differ from those isolated in the United States in that they produce little, if any, H\textsubscript{2}S when grown on a suitable solid culture medium. \textit{Br. suis} has been cultured from naturally infected horses, fowl, cattle, dogs and human beings.

\textbf{Infection in the Cow.} \textit{Br. abortus} tends to localize in the pregnant uterus, udder and lymph glands of the affected cow. In the gravid uterus, the placenta is attacked and the resulting inflammatory changes when severe may cause premature expulsion of the fetus. The true cause
of the act of abortion is not clearly understood. It has been suspected that the act of abortion may be due to allergic manifestations, due to the multiplication of the micro-organisms in the uterus. *Br. abortus* has been found in the placenta, in the vaginal discharge and in the milk of blood test positive cows following normal parturition by Edgington and King (62,63,65), by Cotton (49), Gwatkin (93), and others. Cotton (49), Schroeder and Cotton (224), and Giltner and Bandeen (83) found that the organism usually disappears from the uterus of infected cows within a period of 60 days following parturition, but may remain in udder and lymph nodes for many years. Isolation of *Br. abortus* from cow's milk was accomplished for the first time by Schroeder and Cotton (225) and the organism was found in the milk from apparently healthy cows, as well as from cows that aborted. Udder infections of five years duration were observed. Their observations have been confirmed by many investigators including Edgington and King (62,63,64,65), Gwatkin (93), and Green (89).

**Infection in the Bull.** The possibility of the spread of *Br. abortus* by the bull was suggested by Bang (2). Infection in bulls, as indicated by the blood test, was observed by Rettger and White (211). Isolation of *Br. abortus* from the testicles of 5 of 37 blood test positive bulls was reported by Buck *et al.* (31). However, Hadley and
Lothe (96), and King (143) were unable to infect heifers by natural service to blood test positive bulls. Two of the bulls used in King's experiment were excreting *Br. abortus* in their semen. Thomsen (246) infected 5 of 15 heifers by breeding them to a bull immediately following the introduction of ground infected placental tissue into the preputial sac.

It appears that natural service in infected herds is not a principal means of spreading Brucella infection. However, the addition of bulls from positive herds to negative herds is a possible source of Brucella infection (200).

Spread of infection by artificial insemination with semen from infected bulls has been demonstrated by Seit (228), Bendixen and Blom (6), and Manthei et al. (166). Often, infected bulls show no physical evidence of the disease. However, orchitis sometimes results from the localization of Brucella in the testes (Gilman (80)).

**Infection in Animals Other Than Cattle.** Domestic animals, other than cattle, that may suffer with brucellosis include swine, goats, sheep, horses and even poultry. Wild animals such as moose, deer, elk, buffalo, and rabbits are known to be susceptible. Experimental animals that may readily be infected include guinea pigs, rabbits, mice, white rats, and monkeys.
Brucellosis in swine was first diagnosed in the United States by Traum (247). Many subsequent reports show that the disease is widespread and often causes serious losses from abortion. Brucella organisms commonly found in swine (Br. suis) differ from those usually found in cattle (Br. abortus) by being more pathogenic for man and guinea pigs, not requiring an increased CO₂ tension in the surrounding atmosphere for growth on culture mediums and by susceptibility to certain dyes (Huddleson (117)). Br. suis has been isolated from cow's milk by Hasseltine (107) and others. A good review of swine brucellosis has been written by Hutchings (132).

Brucellosis in horses appears to be fairly common, and was first reported in 1924. McNutt and Murray (180) isolated the organisms from an aborted fetus of a mare; however, abortion is not a common symptom of equine brucellosis. Supraspinous bursitis is a common characteristic of the disease in horses, as shown by Rinjard and Helger (213), Fitch et al. (73), and others. Karlson and Boyd (136) examined five horses that reacted to the blood agglutination test for brucellosis. Br. abortus was isolated from the feces of two, an abscess of the supraspinous bursa of one, lesions of the sternum of one, and lesions of the ribs of the fifth horse. White and Swett (255) and Fitch and Dodge (76) presented evidence showing
that infected horses may be a source of infection in cattle.

Although it has been shown that Brucella is often isolated from supraspinous bursitis lesions, no one was able to reproduce the disease until Roderick, Kimball, McLeod, and Frank proved that a mixture of Brucella and Actinomyces bovis was needed to reproduce the condition (193).

Sheep, dogs, and cats possess a high degree of resistance to brucellosis (Boyd (21)). However, dogs may temporarily shed Br. abortus in their urine and feces following the ingestion of infected milk or aborted material, and a few cases of abortion in bitches have been reported by Morse (188). Rats may also serve as carriers of infection following the ingestion of infected materials according to Fitch and Bishop (72). Recently, Buddle and Boyles (33) reported the recovery of a Brucella mutant from the semen and lesions in the genitalia of forty-five rams, from diseased fetal membranes, aborted fetal lambs, and the colostrum of naturally infected ewes. The CO₂ sensitive non-smooth stabilized mutant behaved in the dye sensitivity, and in other tests in a fashion not incompatible with the organism being a variety of Br. melitensis adapted to sheep in the New Zealand environment.

Susceptibility to Infection. Rettger et al. (211) have shown that usually calves up to eight months of age
are resistant to infection. Resistance in non-vaccinated heifers then gradually decreases as they reach sexual maturity. While it is generally agreed that susceptibility is greatest during pregnancy, nevertheless, it has been found by Edgington and Donham (61) and more recently by Manthei (162) that unbred non-vaccinated heifers are susceptible to infection. According to Huddleson (117), about 70 percent of the non-vaccinated pregnant cows abort following initial infection, subsequently some of these cows are sterile but those that conceive have lower abortion rate.

It appears that nutrition has little or no effect on resistance to brucellosis. Hart et al. (105) found that the resistance of a group of cattle fed a highly nutritious diet plus minerals, cod liver oil, and iodized salt, was no greater than the resistance of a control group fed a ration low in protein and mineral content. More recently Berman et al. (12) reported that there was no difference in the abortion or infection rates of cattle fed trace minerals and a control group. During the post-exposure period, differences in the agglutinin reactions were not observed in the two groups. King and Venzke (139) found that wheat germ oil had no effect on the blood agglutination titer or on the course of disease.

Public Health Aspects of Brucellosis. Probably no in-
fectious disease of man and animals has been more generally misunderstood and misrepresented than brucellosis.

Scientific evidence indicates that rarely, if ever, does human contract brucellosis from another human. It appears, therefore, that in the light of present knowledge the prevention and control of brucellosis in man is directly dependent upon its control and eradication in domestic animals. A major effort needs to be directed to bring the disease under control in animals which may act as reservoirs of infection and a means of transmission to man.

There is overwhelming evidence that brucellosis in man occurred in the United States and other countries for many years prior to its diagnosis. Since 1905, when the first reasonably authentic case of human brucellosis was reported in the United States, the number of reported cases has increased to around 7,000 annually (231). Most students of public health reports think that the data do not fully indicate the incidence of human brucellosis.

There is a great lack of uniformity in the performance of the common diagnostic tests for brucellosis and even greater divergence in their interpretation. In the field of veterinary medicine a coordinated effort has been made to standardize the antigens and techniques used in the agglutination test and to interpret the results in terms of clinical and epidemiological significance. Although
these standards may be far from perfect, the success of the brucellosis control program in cattle attests to their value and reliability. Unfortunately, in the field of human medicine there has been no such standardization of tests or coordination of thinking in the diagnosis of the disease. Cultural techniques likewise are diversified and frequently inadequate. This lack of uniformity in conducting tests and reporting results renders much of the literature pertaining to human brucellosis difficult to interpret. Until recent years, Brucella could be recovered only irregularly from patients with brucellosis. These two facts are probably in a great part responsible for the current widespread confusion and non-critical clinical approach to the diagnosis of brucellosis (172).

**Distribution.** Brucellosis is worldwide in distribution. Bovine brucellosis occurs in all areas where cattle are maintained. It is common in all the United States except in areas where an intensive program of eradication is in operation. It is most common in areas where large herds are maintained and where there is frequent transfer or importation of cattle. It is less common in areas where herds are small and self-contained and where additions are only rarely made.

The extent of infection in cattle in the United States is indicated by the records of the Agricultural Research
Service, U.S.D.A. According to a review by Knapp (145),
the National average was 9.83 percent in 1934, and 6.68
percent in 1940. Kuttler (147) reported that for the
period, January to October, 1955, 2.7 percent of the
12,905,520 blood tests made in the United States were
positive. However, many herds concerned had been under
test for some time. Consequently, the percentage re­
ported is undoubtedly lower than the percentage for the
cattle population as a whole.

Swine brucellosis occurs throughout the United States,
wherever hogs are raised. It is most common in corn-hog
belt states in the Middle West. Purebred herds where
individual animals are kept for a period of years are
most commonly infected.

Brucellosis of goats in the United States is con­
fined principally to southwestern states.

Brucellosis in man is increasingly common, or at
least it is more frequently diagnosed in most states.
The infection in man tends to parallel the infection in
domestic animals.

Transmission

The routes of infection are the vagina, mouth, skin
and eye. Bang (2) showed that infection could be produced
by placing cultures in the vaginas of pregnant cows.
Rettger et al. (210) infected open heifers by swabbing the
vulva with cultures. Birch and Gilman (14) demonstrated that infection can be readily induced by feeding infected grain. Cotton and Buck (50) found that living *Br. abortus* organisms can pass through the unbroken skin, as well as the abraded skin. Cattle were infected experimentally by way of conjunctiva by Schroeder (223).

In infected herds, non-vaccinated negative animals may become infected by contact with aborted material, licking infected cows, and eating feed contaminated by discharges from infected cows or by persons carrying infectious material on shoes and clothing. Dust and insects may also serve as carriers of *Br. abortus*. It is possible that transmission may occur during milking.

Probable sources of new infection in Connecticut herds were investigated by Plastridge *et al.*, (201). The addition of recently infected cows (negative at the time of purchase) from untested or infected herds was the most common source of infection. The addition of bulls from infected herds, contact with animals from neighboring herds through inadequate or broken pasture fences, and the association with infected horses or swine were also apparent sources of infection. Circumstantial evidence indicates that in some instances, infection was carried to negative herds by persons coming directly from infected herds.

It was formerly believed that the bull was a chief means of spread of brucellosis, but careful work has dis-
proved this assumption. Many bulls acquire brucellosis, but only a small percentage of these actually become spreaders of the organisms. When the infection localizes in the testicles or adjacent parts of the genital tract, Brucella organisms are eliminated in the seminal fluid and the animal becomes a dangerous spreader.

Manthei (165, 166) was able to transmit brucellosis to susceptible cattle by intrauterine insemination with semen containing virulent Br. abortus. The intracervical method of insemination failed to produce infection in 12 susceptible cattle. Bendixen and Blom (6) reported a similar spread of the disease by artificial insemination under field conditions in Denmark.

There is no conclusive evidence that a bull disseminating virulent Br. abortus in the semen is capable of transmitting infection to susceptible cattle by natural service. The greatest danger in this method of breeding is contamination of premises with semen, urine, and feces. In swine, the condition is somewhat different. Brucellosis is readily transmitted by the boar to sows by natural service.

Resistance of the Organism
Outside the Animal Body

Brucella organisms are rather sensitive to sunlight and are readily killed by common disinfectants and by standard pasteurization. They are believed to live only a short time in pastures and barnyards, unless they become covered
with manure or other protective material. According to Smith et al. (231) the resistance of the bacillus to certain natural influences is as follows: It lived $4\frac{3}{4}$ hours exposed to direct sunlight, 5 days when dried in burlap sacking and kept in an ordinary room; thirty days when dried in burlap sacking and kept in an unheated cellar; thirty-seven days when dried slowly in soil; four days in bovine urine; one hundred and twenty days in bovine feces dried very slowly in a dark cupboard; and in an aborted fetus during cool weather, seventy-five days.

In trials conducted by Cameron (36), Br. abortus survived one hundred and twenty days in manure, seventy-seven days in water, and sixty-six days in wet soil when the test materials were kept at room temperature. In similar studies made by Kuzdas and Morse (148), Br. abortus survived ten days in water and twenty-nine days in manure and soil kept at 25° C. However, in manure and soil stored continuously at freezing or near freezing temperatures, Br. abortus survived for periods up to eight hundred days.

Huddleson et al. (129) were able to culture Br. suis from hog spleens which had been held for a period of thirty days at -10° F. Positive cultures were also obtained at the end of forty days from hog spleens kept in meat-curing brine.

When phenolized anti-hog cholera serum and blood virus were inoculated with Br. suis and stored in a cold room,
positive cultures were obtained from the former after
twelve weeks and from the latter after five months (128).

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 (38).

Thompson (244) found that when ice cream was made from milk naturally infected with Br. abortus and stored at 32° F., the organism remained viable for thirty days. Carpenter and Boak (38) inoculated butter with Br. abortus, and stored it at 8° C. The organism remained viable for one hundred and forty-two days. Br. abortus remained viable in Roquefort cheese for two months. Boak and Carpenter (20) studied the thermal death point of Bacterium abortus in milk and found that Br. melitensis and Br. abortus are killed at 140° F. in 15 minutes but not in 10 minutes; that Br. suis to be completely destroyed required 20 minutes at 140° F. or 15 minutes at 142° F.; and that at 145° F. all three species are destroyed in 10 minutes. Murray et al. (191), using a standard pasteurizing unit, reported that a temperature of 143.6° to 145.4° F. applied 3 minutes was sufficient to kill both Br. abortus and Br. suis in milk.

Natural Course of the Disease

Brucellosis tends to be chronic in all species, but ranges according to the species, resistance of the indivi-
dual, and the Brucella type (abortus, suis, melitensis) from a mild and transitory febrile attack to a severe, recurrent fever with localizations, general clinical signs, and sometimes septicaemia, terminating in death.

In cattle, the bovine type predominates. The young (less than 1 year old) as a rule do not acquire permanent infection or show visible signs. In bulls with localizations in the testicles, permanent infection tending toward sterility is the rule. According to a report of the Special Committee of the United States Livestock Sanitary Association (231), brucellosis in sexually mature cows may follow one of four courses, depending on individual susceptibility.

1. The most frequent course is chronic in nature with outward appearance of recovery but with permanent positive blood agglutination titer and continued intermittent shedding of Brucella organisms.

2. Semi-acute form involving permanent positive blood agglutination titer, shedding of Brucella organisms and clinical signs (chronic metritis, arthritis, low milk production) that tend to destroy the economic value of the animals in a relatively short time.

3. Slight and transient blood agglutination reaction, usually suspicious, the only manifestation.

4. Chronic course, as a rule with months or years in which there is positive blood agglutination reac-
tion with clinical signs (abortion, retained placenta, metritis) and actual shedding of Brucella organisms, followed by complete recovery. This type is relatively infrequent.

In horses the bovine type predominates. The course is generally chronic with occasional acute arthritis, supraspinous bursitis and atlanto-occipital bursitis the chief chronic manifestations. Abortion is not a prominent clinical sign in mares.

In goats the caprine type (Br. melitensis) predominates. The course is usually chronic and follows the same general pattern as does the bovine type in cattle.

In swine, the Br. suis type predominates. The course is usually chronic with occasional acute arthritis, abortion, lameness, posterior paralysis, abscessation, and sterility. Transient blood agglutination titers are the most common.

In man, the course is most severe when the disease is caused by the caprine type, less severe when caused by the suis type and least severe when caused by the bovine type. Apparent recovery is the rule, but regardless of the type prolonged incapacity is frequent with death the exception. Abortions in women are infrequent.
**Period of Incubation**

The period of incubation is the interval of time between the entrance of infection into the animal body and the appearance of clinical signs of the disease.

The period of incubation in brucellosis in animals is quite variable. The minimum incubation period, when abortion is the first clinical sign observed, is about thirty days.

Some cows abort before developing a positive reaction to the blood test, but much more frequently they show a positive reaction to the test before aborting. Some infected cows never abort.

McEwen *et al.* (177) found that the time of the first appearance of a significant blood agglutination titer (1:40 or higher) after exposure to infection was directly proportional to the size of the dose of organisms. Exposure to a large dose of organisms elicits a significant titer in 14 to 28 days. When the exposing dose is less than 1,000,000 organisms, significant titers do not appear until 65 to 156 days.

Experiments conducted by Thomsen (245) showed that the younger the fetus at the time of infection, the longer the incubation period. Individuals in a group of nineteen heifers were exposed orally and by way of the conjunctiva at intervals ranging from ten days to seven months after breeding. Sixteen of the nineteen heifers aborted. The
average interval required for the development of a positive blood agglutination titer decreased from 207 days for the two heifers exposed 21 days after service to 53 days for the single heifer exposed seven months after service. These findings show that a single negative blood test in an animal from an infected herd is of limited value.

It is apparent from this work that in bovine brucellosis the incubation period depends upon three factors, (1) the virulence and number of invading Brucella organisms, (2) the resistance of the animal, and (3) the stage of gestation at the time of exposure.

Clinical Signs

No characteristic clinical signs set brucellosis apart from other diseases that cause abortion. Abortion, death and expulsion of the premature fetus is the most prominent sign. It attains special significance when it occurs repeatedly in the same herd.

When Br. abortus invades the gravid uterus, fetal membranes, 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. According to Huddleston (117), the anatomical changes interfere with the proper nourishment of the fetus, but they are not sufficient to cause death in utero in fetuses expelled at the sixth
month of gestation or thereafter. Huddleson (117) believes this premature expulsion of the fetus may be due to the contraction of smooth muscle fibers of the gravid uterus set in motion by the toxic effects of the endoantigen of the Brucella cells.

One common sequel to abortion is retention of the fetal membranes. Many animals which are infected, although they do not abort, become sterile.

Boyd et al. (68) and others have observed the occurrence of bursitis in Brucella infected cattle. The knee joint is one of the most frequently affected. Humphreys and Moore (131) examined the serous fluid from hygromata of 42 cattle. *Br. abortus* was recovered from 27 of the swellings. Thirty-six of the animals were positive and six were negative to the Brucella agglutination test.

Little and Orcutt (157) were the first to show that agglutinins which appear in the blood of newborn calves come from the ingestion of colostrum containing antibodies and not as a result of infection. The agglutinins do not persist in the blood of the calf longer than 12 weeks on the average.

*Br. abortus* may be demonstrated by cultural methods in the milk, from vaginal swabs, and from fetal stomach contents after abortion (61, 62, 63, 64, 65, 9, 10, 85). Milk from infected quarters will also show the presence of specific agglutinins. Fitch et al. (74) have made a sys-
tematic study of the occurrence of *Br. abortus* in the blood stream of seven heifers 9 to 10 months of age. They were able to recover the organism more often soon after exposure and at the time of the first appearance of agglutinins in the blood.

In swine, the most common clinical sign of brucellosis is abortion or the birth of weak pigs. Sows that abort once will usually farrow normal litters thereafter. Sterility is often a manifestation of infection with *Br. suis*. A persistent but scanty discharge from the uterus may follow abortion as a result of metritis. The testicles of boars, when infected, become swollen. One or both may be involved. Usually such infections are chronic. Bone involvement in chronic swine brucellosis is not uncommon. In one survey of 62 experimentally infected hogs, thirteen or 20.7 percent, showed lesions in the spine (spondylitis) from which *Br. suis* was recovered (23). When lesions are not extensive, a staggering gait or posterior paralysis is seen.

The symptomatology in man is extremely varied. According to Huddleson (117), the most common symptoms are weakness, sweating, headache, anorexia, chilliness and nervousness. Fever, loss of weight and leucopenia are the signs usually associated with brucellosis in man.
Tissue Changes

Brucellosis produces quite different manifestations in the several species of animals which it invades. The disease commonly causes abortion in cattle and swine, but rarely in mares and women. Likewise, the tissue changes caused by Brucella infections are extremely variable in the several different hosts.

In cattle, there may be no visible gross tissue changes on post mortem examination. In other cases, an autopsy may reveal placentitis, mastitis, orchitis, lymphadenitis, and hygromas of the knee.

Hagan (97) described the intercotyledonary portion of the chorion of Brucella infected cattle as being opaque, thickened, and leather-like in appearance. Smith (233) found that Br. abortus multiplied within the epithelial cells of the chorion.

The invasion of the bovine udder by Brucella produces an acute to subacute, and chronic inflammation in varying degrees (213). 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. Ridala (212) found epithelioid cells in the inflammatory foci of udders of cattle infected with Br. abortus. Included with these cells were giant-cells of the Langhans type, and sometimes necrosis, chiefly in the center of the foci, but these foci of epithelioid cells
differed from tuberculous foci in that there was no caseation.

In swine, the above changes have been described but with the following addition: spondylitis and abscess formation in many other parts of the body.

In horses, the disease is commonly manifested by pus formation in the conditions commonly known as "fistula of the withers", "poll evil", and arthritis.

In man, the changes are dependent upon the tissue invaded and the type of organism involved. Localizations occur in the spleen, bone, joints, ovaries, testicles and other tissues.

**Diagnosis**

Serologic tests on blood and milk, allergic tests, and bacteriologic examinations of milk and aborted materials have been used in the diagnosis of brucellosis in cattle. Of the several procedures available, the blood serum agglutination test is now generally recognized as the most accurate procedure for use in routine control programs.

**Tube Agglutination Test.** The test is based on the fact that the blood of man and animals affected with certain infectious diseases, acquires the ability to agglutinate, or clump suspensions of the causative bacterium. This principle was first used in brucellosis by Wright
and Semple (259) in the diagnosis of brucellosis in man, and in the diagnosis of brucellosis in cattle by Grinsted (92). Surface (241) was the first to use the test in the United States and suggested that reactions in serum dilutions of 1 - 100 or above, indicate infection.

Moore (187) regarded the compliment fixation test as impractical. Mohler and Traum (184) suggested the use of the agglutination test alone, because it was less expensive. Both tests were used in the first work in Connecticut by Rettger et al. (211), but the complement fixation test was discontinued in 1930.

In the approved procedure for the tube agglutination test, the antigen in concentrated form (4.5 percent suspension of cells) is supplied by the Agricultural Research Service, U.S.D.A. The antigen is standardized so that the desired concentration for use in the test is obtained by adding one part of concentrated antigen to 100 parts of phenolized saline solution. The diluter is prepared by dissolving 0.5 percent of phenol and 0.85 percent of sodium chloride in distilled water. The density of the diluted antigen corresponds to about 1 on the McFarland nephelometer scale. Four dilutions of serum are prepared by placing 0.08, 0.04, 0.02, and 0.01 ml. of serum, respectively, in each of four test tubes, and adding 2 ml. of antigen. Tube test readings are made after 40 to 48 hours.
incubation at 37° C. In some states the 1:25 dilution of serum is omitted.

The interpretation of reactions adopted by the 1932 Conference of Official Research Workers in Animal Diseases of North America and later by the Bureau of Animal Industry is as follows:

<table>
<thead>
<tr>
<th>1:25</th>
<th>1:50</th>
<th>1:100</th>
<th>1:200</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- or P</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>+</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>Suspicious</td>
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<tr>
<td>+</td>
<td>+</td>
<td>P</td>
<td>-</td>
<td>Suspicious</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>P or +</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Agglutination: - none; P partial; + Positive

More recently, Goode et al. (85) found that of all the isolations made of Brucella organisms from both vaccinated and non-vaccinated cattle, 98.04 percent were from cows within herds which contained one or more animals with sero-agglutinin titers of P 1:400 or higher.

No isolations were made from the milk of vaccinated cows with sero-agglutinin titers in dilutions below 1:100. In view of these findings, they offered the following alternate interpretation of the test in classifying calf-vaccinated cattle and it has now been adopted by most states in the United States:
### Calfhood Vaccinated Animal

*(4 to 8 months at time of vaccination)*

<table>
<thead>
<tr>
<th>1/50</th>
<th>1/100</th>
<th>1/200</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
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<td>Negative</td>
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<tr>
<td>+</td>
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<td>-</td>
<td>Negative</td>
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<tr>
<td>+</td>
<td>P</td>
<td>-</td>
<td>Suspect</td>
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<td>+</td>
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<td>Suspect</td>
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<tr>
<td>+</td>
<td>+</td>
<td>P</td>
<td>Suspect</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Rapid or Plate Agglutination Test.** A rapid method for performing the agglutination test was first described by Huddleson and Carlson (127). The antigen is prepared by adding glycerine, distilled water containing 12 percent sodium chloride, 0.5 percent phenol, 1:25,000 suspension of crystal violet and brilliant green to killed smooth strain of *Br. abortus* (strain 1119), Huddleson (117). The density is adjusted so that the antigen will give results similar to those obtained by the tube test. The plate test is made by placing 0.08, 0.04, 0.02, and 0.01 ml. amounts of serum on ruled squares of a glass plate and mixing one drop of antigen (0.03 ml.) with each amount of serum. The plate is slowly tilted back and forth for two or three minutes, and then placed over an illuminated box. The light is turned off, except during observation, to prevent drying. The plate is taken up and gently ro-
tated after five minutes and then replaced in the box. At the end of eight minutes the serum-antigen mixtures are observed for agglutination, or clumping of the antigen.

The tube and plate tests have been compared by many investigators, including Damon (54), Graham and Thorp (86), Gwatkin (94), and Donham and Fitch (56). In general, agreement of 90 - 95 percent occurred when the two tests were applied to negative and positive samples. Most disagreement occurred when suspicious samples were examined. Factors which control agglutination such as concentration of the serum-antigen mixtures, temperature, and the time interval between mixing the antigen and serum, and reading the results are not as well controlled in the plate test as in the tube test. Although the plate test is generally considered less reliable than the tube test Schubert (226) found, following the study of 99 human patients with proved or presumptive brucellosis, the plate test using 5.0 percent antigen could be used as a satisfactory substitute for the tube test.

Factors Influencing the Blood Serum Agglutination Test. In the early work on brucellosis, each laboratory made its own antigen. As a result, discrepancies occurred in the test, due chiefly to the use of cultures which were either too sensitive or not sensitive enough, and to differences in the technique of making the test (Huddleson (117)).
These faults were largely overcome when the Bureau of Animal Industry, U.S.D.A., began supplying antigen to state laboratories about 1939, along with detailed instructions for its use.

The presence of hemoglobin in the serum interferes with the tube agglutination test. Hemolysis occurs when blood samples are frozen, exposed to summer temperatures for several days during transit, or when contaminated with micro-organisms, water or disinfectants when drawn.

The possibility of reactions to the brucellosis test due to infection with Pasteurella multocida has been investigated. Mallman (161) found that anti-Pasteurella serum prepared in rabbits agglutinated Br. abortus antigen, and Dachena (88) reported that blood serum from cattle suffering from hemorrhagic septicemia contained antibodies which agglutinated Br. abortus antigen. Starr and Snider (238) found that calves infected with live Pasteurella cells sometimes exhibited a titer of 1:50 with Brucella antigen. Kitselman (144), and Starr and Snider (238) found no increase in Brucella agglutinins in cattle following injections with hemorrhagic septicemia bacterin, and Priestly (203) concluded that there is no cross agglutination between Pasteurella and Brucella. Recently Hollister (112) reported that he had noted a rise in titer of 16 of 30 head of cattle injected with Bacterin Formula #I. None of the agglutination reactions were complete in dilu-
tions above 1:100. He did not state the titers of these animals prior to the injections of the Bacterin. When these same animals were reinjected with the same biological four and ten months later, there were no significant changes noted in the blood titers of the cows. Berman (7) stated that he was able to produce anamnestic agglutinins at diagnostically significant titers in strain 19 vaccinated cows. He used commercial bacterins containing Pasteurella multocida and bacterins prepared in his own laboratory. He demonstrated cross reactions between Brucella and Pasteurella serotypes C and D. Evidence of cross reactions between Brucella and Pasteurella species and some of the Vibrio species has been reported by Mallman (161), Eisele et al.,(69) and McCullough et al.,(170).

Morse et al,(190) reported that Vibrio fetus and Brucella were antigenically related, and observed that antiserums prepared in rabbits against seven Vibrio fetus strains gave traces of agglutination with antigens of one or more strains of Brucella in the 1:10 serum dilutions. Plastridge (198) reported a year later that suspicious reactions to the Br. abortus agglutination test in cattle seldom result from Vibrio fetus infection.

Advanced gestation does not appear to affect the agglutination test, as indicated by retests on positive reactors (Plastridge et al.,(200)). However, in infected
herds it is not unusual for an animal to react negatively before calving and positively after calving for the reason that she became infected during the late stages of gestation.

Hess (110) was able to differentiate nonspecific Brucella-agglutinating substance from the specific agglutinating substance in bovine serum by heating the serum for 10 minutes in a 70° C. water bath. Later, in other studies, he reported the isolation of these Brucella-agglutinating substances (111). He found that the specific Brucella agglutinins were associated with the gamma globulin fraction of the serum. The nonspecific substances were associated with a serum fraction containing isoagglutinins, cold insoluble globulins, and certain factors concerned with the clotting of blood.

Roepke (216) has studied the differences between the specific and nonspecific Brucella-agglutinating substances in bovine serum. Nonspecific substance can be adsorbed with a variety of organisms while the specific agglutinins are adsorbed by Brucella only. Heat treatment of the serum at 70° C. for 10 minutes will inactivate the nonspecific substance. Specific agglutinins are inactivated only after a heat treatment of 90° C. for 10 minutes. Up to 38 percent of nonspecific substance was eluted from *Br. abortus* with distilled water, but only 2 percent of the specific aggluti-
nins were eluted by the same technique. The specific agglutinins were found to be associated with the gamma globulin fraction of bovine serum. The nonspecific substance was found to have no association with the gamma globulin fraction.

To date no form of chemotherapy has been found effective in causing a positive reaction to react negatively.

**Agglutination Test on Milk Whey.** Brucella agglutinins were first found in the milk of infected cows by McFadyean and Stockman (179). However, owing to the opacity of the fluid, tests on milk were regarded as of little value. This difficulty was later overcome by removing the cream and then coagulating the milk with rennet and applying the test to the whey. Coolige (45) considered agglutination tests on milk of value in detecting udder infection. Smith et al., (232) observed a high titer of milk when *Br. abortus* organisms were present and concluded that the udder tissue participates in agglutinin production. Titers of 1:80 on milk from individual quarters were usually associated with udder infection, although *Br. abortus* was occasionally isolated from milk with a lower titer, in studies by Gilman (81).

Little and Plastridge (158) considered a positive reaction in whey dilution of even 1:5 in herd milk indicative
of herd infection. For individual animals agglutination in the 1:20 dilution was considered as positive.

More recently Cameron and Kendrick (37) presented data comparing the efficiency of a whey plate test with the conventional blood test in diagnosing brucellosis. The results indicated that the whey test was at least equally as efficient as the blood test in detecting infection.

The findings of Graham and Thorp (87) and Huddleson (117), and others, that Br. abortus may be present in milk with negative or low titers, and that the milk of blood test positive cows may be negative, had led to the general opinion that tests on milk are of limited value.

**Brucella Stained Antigen Milk Tests.** There are at present several Brucella-stained antigen milk tests. The first and most widely used is known as the "A.B.R." (Abortus Bang Ring) test. This test is conducted by mixing 2 drops of antigen (a deeply hematoxylin stained suspension of Brucella abortus cells) to 2 ml. of milk, allowing the mixture to incubate at room temperature for 60 - 90 minutes, and then observing the color. A uniform blue color in the skim portion of the milk with a white or grey cream line ring is interpreted as negative. A blue cream layer with a lighter blue or white skim portion indicates the presence of agglutinins. Positive ring tests have been
obtained when the pooled milk represented milk from one infected cow and from five to fifteen noninfected cows (Roepke (214)).

The Brucella abortus ring test antigen has, to a large extent, been nonspecific and many of the so-called positive ring test reactions have, in reality, been a combination of a ring test reaction and a Schern-Gorlisch (221) reaction. The Schern-Gorlisch reaction is a test for heat treatment of milk. The test is carried out by adding 1 ml. of milk to 1 drop of a 2 percent suspension of red blood cells or 1 drop of a 1 percent suspension of bone charcoal, after which the sample is shaken and incubated at 37° C. for two hours. In the instance of raw milk, the blood cells or bone charcoal apparently adhere to the fat droplets and are carried up to form a red or black ring at the top. The ability of these to adhere during the rising is probably dependent on the amount of fat, as well as the size of the fat droplets, and the quality of the protein layer surrounding the fat droplets. These factors vary from animal to animal, and for this reason, the Schern-Gorlisch reaction functions best on composite samples of several animals as the individual variations are thereby somewhat eliminated.

The ring test was first described by Fleischhauer (78). As a result of his investigations, Fleischhauer
concluded that, in the testing of herd samples, only reactions which occur within twenty minutes following incubation could be considered as positive. The majority of all herd samples would show ring formation forty minutes after incubation. These reactions were considered as being nonspecific or Schern-Gorlisch reactions.

Smitmanns (234) concluded that reactions on herd samples should be considered positive if the reaction started within twenty-five minutes following incubation and if, after two hours' incubation, there was a 2 to 4 mm. wide blue-violet ring sharply defined from the underlying white milk.

Fleischhauer and Herman (79) found that, in the investigation of composite samples, the time of the beginning of the reaction and not the end was important. Reactions which started after thirty minutes no doubt had less agglutinins in the milk and many of these were due to Schern-Gorlisch reactions. These late reactions are borderline cases and are only of such value as they can be further substantiated by other known methods, such as the whey agglutination, rapid agglutination, and fresh milk agglutination.

Norell and Olson (192) of Sweden, published results of investigations on the value of serologic milk examinations by means of the ring test. The ring test antigen which they used apparently gave the best results so far.
They incubated herd samples for fifty minutes without obtaining a particularly great number of nonspecific reactions. The ring test proved to be far more sensitive on herd samples than did the slow, whey-agglutination test.

When the results with herd milk ring tests were compared with the blood tests of individual animals, Seitz and Jorgensen (229) of Denmark, found only a 3.8 percent disagreement.

Christiansen (42) reported results on 6,266 herds that had been blood tested at about the time of performing the milk ring test. There was a complete agreement between the two tests in 93.2 percent of the tested herds, partial disagreement in 2.4 percent, and complete disagreement in 4.4 percent, when only one ring test was taken into account. Ring tests carried out simultaneously with blood tests on 3,952 individual animals showed an 84.3 percent agreement, partial disagreement in 4.1 percent, and total disagreement in 11.6 percent of the instances. In 82.3 percent of instances of total disagreement, the ring test was positive and the blood test negative. There was noticeably less agreement between the blood and the ring tests when these tests were applied to samples from cows recently fresh, from strippers, or from cows infected with mastitis.

Bruhn (28) reported that, by means of the ring test,
he was able to detect 97 of 121 (80.2 percent) cows which were blood reactors at 1:20 or higher. There were 24 instances of positive blood and negative ring tests. The ring test failed in three instances, in one with the blood-agglutination titer 1:100, and in two cases with the titer of 1:200. Bruhn concluded that these negative ring test reactions in samples from individual cows which are strong reactors are usually due to the lack of cream-rising capacity of the milk. When these samples are mixed with ring test-negative pooled milk, positive reactions are obtained.

Roepke, Clausen, and Walsh (120), in reporting results of preliminary studies on slightly over 6,000 herds in Minnesota, found a 75 percent efficiency for the milk and cream ring test when compared to the blood serum-agglutination test in locating infected herds.

Roepke, Paterson, Driver, Clausen, Olson, and Wentworth (215), in reporting subsequent studies in Minnesota, found an agreement between the ring test and the blood serum-agglutination test on 96.2 percent of 8,469 herds tested. The ring test was positive for 68 percent of 385 herds considered infected on the basis of blood tests. Studies in 107 infected herds which were negative to the ring test revealed that in 69 herds (65 percent) the reactor animals were not in production at the time of milk collection. The ring test was positive for 88 percent of
the herds in which infected animals were contributing to the can sample.

Two modifications of the *Br. abortus* ring test have been described. The one described by King (142) consisted of drawing ring test antigen and milk into a capillary tube (0.8 by 90 mm.), and observing the tube for the presence of clumping of the antigen. On 428 individual cow samples tested by King, agreement with the blood test was 92.7 percent. On herd milk samples, Morse et al. (123) found the ring test to be slightly more accurate than the capillary tube test. A short time later, Blake et al. (19) described a milk plate test. He reported that this test was positive on milk from 18 blood test positive cows that shed *Br. abortus* in their milk, and negative for 19 cows that were negative to blood tests and cultural tests. However, tests on 22 cows that were positive to the blood tests but negative on cultural examinations varied from negative to positive.

Several factors may affect the results of the ring tests. The inclusion of milk from mastitis udders, colostrum and milk from cows in late lactation may account for some of the positive reactions given by milk from blood test negative herds (Bruhn (28)) and Holm (113). The data obtained by Roepke (215) suggests that calf or adult vaccination with *Br. abortus* strain 19 vaccine may
interfere with the reliability of the ring test, but to what extent has not been determined.

Agglutination Test on Bull Semen. The possibility of testing the plasma of bull semen for Brucella agglutinins was suggested by the work of Bendixen and Blom (6). Christensen (41) found that in 24 of 28 bulls with Brucella infection of the genital organs the agglutinin titer of the semen plasma was higher than that of the blood serum.

Agglutination Test on Uterine Fluid. Jepson and Vindekilde (134) reported the local formation of agglutinins in the genital organs of Brucella infected cows. Several cows were observed in which the fluid from the uterine mucosa gave a higher agglutinins titer than their blood serum. A tampon method for the collection and examination of uterine fluid is described. Later Plastridge et al. (199) used this technique to collect and examine cervico-vaginal mucus for Vibrio fetus agglutinins.

Other Tests Used to Diagnose Brucellosis in Animals. Attempts have been made to diagnose brucellosis in cows by injecting Brucella cells, or products of Brucella cells, and observing the animals for evidence of hypersensitivity. Most of these tests have been generally regarded as inferior to the blood serum agglutination test.

McFadyean and Stockman (179) injected cattle intravenously with "abortin", a filtrate of Br. abortus cultures,
and observed the animals for a rise in temperature. Other workers including Mohler and Traum (184), and Meyer and Hardenbergh (183) found the test to be unreliable.

Many workers have used intradermal injections of killed suspensions of Br. abortus cells and extracts of these cells in attempts to diagnose brucellosis. Edgington and Broerman (60) reported that more animals reacted positively to the intradermal test than to the blood serum agglutination test. Some animals from which Brucella had been recovered were found to be negative to the intradermal test. Live et al. (155) and Live and Stubbs (153) reported that intracutaneous injections of filtrates of Br. abortus cells gave unreliable results. Intradermal tests were carried out by Ottosen and Plum (194) using an extract of Br. abortus cells. It was thought that the test was useful when used in recently infected herds. Huddleson (117) used the protein nucleate fraction of Brucella cells as an allergic agent for detecting Brucella skin allergy in human beings. Its usefulness in detecting Brucella allergy was reported to be highly satisfactory.

An ophthalmic test was studied by Fitch and Donham (77). The test was not sufficiently reliable to justify its general use.

Huddleson (117) found that the neutrophilic leucocytes in whole citrated blood of human beings and animals that had recovered from brucellosis, phagocytized Brucella cells in
large numbers in a phagocytic system. He also observed that leucocytes in the blood of those who had no past or present history of infection showed little if any phagocytosis. This diagnostic test was called the opsonocytophagic test.

**Bacteriologic Methods.** In routine work, use of bacteriologic techniques is limited largely to the examination of aborted fetuses for the purpose of determining the presence or absence of pathogenic micro-organisms, especially *Br. abortus*, *Vibrio fetus*, *Trichomonas fetus*, and pyogenic bacteria. Quite often the uterine exudate and placental tissue are grossly contaminated with saprophitic bacteria that prevent satisfactory cultural tests. However, the author found the vaginal swab to be one of the best sources of Brucella, especially if it is taken within the first 12 hours following the abortion or calving.

A staining technique developed by Koster and described by Plastridge (198) may be quite helpful in detecting *Br. abortus* in placental tissue.

Cultural techniques on milk have been described by Edgington and King (62, 63, 65), and King et al. (140), and many other investigators (9, 85, 29, 61). The addition of antibiotics to the culture media has been a great help in the isolation of Brucella from milk and vaginal exudate.

Huddleson (117) was one of the first to use guinea
pig inoculation as a means of isolating \textit{Br. abortus} from the various tissues and body fluids of animals.

Many methods have been devised for differentiating the different species of Brucella. Probably the most commonly used method is the dye plate method described by Huddleson (118).

**Attempts to Differentiate Vaccinal from Infection Titers.** A method for differentiating between vaccinal and infection titers of \textit{Br. abortus} in cattle was suggested by Dick, Venzke, and York (55). These workers reported that any animal not stimulated to the production of Brucella agglutinins by the intramuscular injection of 5 ml. of strain 19 vaccine within a maximum of 15 to 17 days, was an infected animal. Animals exhibiting any blood titer increase following the injection of the vaccine was classified as noninfected. It was suggested that these animals possessed titers due to previous vaccination. Venzke (250, 251) has made additional reports on the use of this diagnostic test. Barner \textit{et al.} (3) reported that there was no rise in titer in 10 of 12 injected animals when the test was applied. King \textit{et al.} (141) and Manthei (163) identified only 40.0 and 31.4 percent, respectively, of infected cattle by this method.

Barner, Oberst and Atkeson (4) recently reported on the anamnestic reactions on noninfected vaccinated and infected cattle following the intramuscular injection of
5 ml. of dead strain 19 vaccine. All of the noninfected cattle showed a rise in titer; whereas, at the same time none of the infected cattle showed a rise in titer. Manthei (163) reported similar results when using dead strain 19 vaccine.

Traum and Maderious (248) first introduced the whey agglutination test as a possible method of differentiating between infected and noninfected cattle with comparable blood serum agglutinin titers for brucellosis. They reported 79.4 percent of the cattle with whey titers of 1:25 or higher had udder infection; whereas, only 1.7 percent of the cattle with whey titers less than 1:25 had udder infection. Blake and Manthei (18) got comparable results using a similar technique.

Venzke (250) reported the whey agglutination test was less specific than the anamnestic reaction in differentiating between the vaccinal and infection titers for brucellosis in cattle. Barner et al. (3) stated that studies using whey agglutination test were discontinued because of so many variations and lack of correlation in results.

In 1950, van Drimmelan (57) reported that cattle with vaccinal titers could be differentiated from those with infection titers by use of the milk ring test. He considered cattle whose milk was not positive above the 1:4 dilution as free from brucellosis. Holm, Eveleth, and
Rheault (113) found 19.6 percent of the vaccinated and 69.2 percent of the nonvaccinated animals were classified as infected by virtue of a positive ring reaction in the 1:5 dilution of milk. By employing the same criterion of infection, Blake and Manthei (18) classified 35.5 percent of the noninfected vaccinated animals and 97.4 percent of the infected animals correctly.

The use of electrophoretic techniques is fairly new in veterinary literature but has been reported to a limited extent in the study of some of the animal diseases. Bradish et al. (25) and Bradish and Brooksby (23, 24) were the first to suggest that electrophoretic changes in ox serum associated with the development of vesicular stomatitis were less pronounced and less regular than those associated with foot-and-mouth disease. Within the last year or so several investigators (219, 254, 197, 59) have reported differences in the electrophoretic patterns of the serums of disease-free animals and animals infected with various agents.

Methods of Prevention

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.

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.

Control Based on Periodic Blood Tests and Segregation of Reactors

The discovery that the blood serum agglutination test could be used to diagnose brucellosis in cattle suggested the possibility of a control program based on use of the test. Results obtained with the test, and the finding that calves born to positive dams became nonreactors and could be used as additions to a negative unit, led Rettger et al. (211) to suggest the following control measures: (1) periodic blood tests on adult cattle, (2) segregation and gradual disposal of positive reactors, (3) use of negative bulls, (4) caution in the purchase of new animals, and (5) burning or burial of aborted fetuses and afterbirths.

The work of Barnes (5), and Fitch et al. (75) suggested that the vaccination of adult cattle failed to control abortion in herds. However, it was indicated that it was possible for a breeder to maintain a clean and an infected herd in separate barns on the same premises. Rettger et al. (209) found in testing 75 herds that a testing and segregation program was effective in eradicating infec-
tion from herds in which the infection tended to be sta-
tionary, but not so effective in herds with rapidly
spreading infection.

In 1933, Birch et al. (17) recommended three plans
for controlling Bang's disease: (1) sale of reactors,
(2) complete segregation, and (3) partial segregation.
In 33 badly infected herds, infection was eliminated
from 12 and satisfactorily reduced in 18. Seven herds
with few reactors were freed from the infection.

Following these early reports control programs based
on the blood test were set up in most states. Cooperative
state-federal programs were started about 1934 for the
dual purpose of reducing both the incidence of infected
animals and the total populations of cattle. Under these
programs herds were tested at state and federal expense,
positive reactors were slaughtered, and an indemnity paid
for condemned animals.

In order to standardize and coordinate these pre-
liminary procedures, the U. S. Livestock Sanitary Associa-
tion in 1947 recommended four plans of control in infected
herds. These plans were approved by the Bureau of Animal
Industry and were as follows:

Plan "A" - test and slaughter with or without calf
vaccination.

Plan "B" - test, calf vaccination and temporary re-
tention of reactors.
Plan "C" - calf vaccination without test of any part of the herd.

Plan "D" - adult vaccination. Plans B, C, and D were to be temporary measures to be used by herd owners who were not in position to immediately undertake control Plan A.

The eradication of brucellosis, however, demands more than voluntary adoption of one of the proposed plans. To be most successful an orderly systematic program must be put into effect, requiring participation of all cattle owners under a plan which would result in eliminating the disease in the most prompt and practical manner.

Impetus has recently been added to the eradication program by the adoption of milk ordinances by several of the large cities in the United States. These ordinances require that only milk from brucellosis negative herds will be accepted for distribution within the cities.

Control by Vaccination

Br. abortus vaccines, both killed and living, have been studied for many years by many investigators in an attempt to control brucellosis. As early as 1906 Bang employed killed bacteria for the prevention of the disease. He, as well as Buck and Creech (30), failed to obtain good protection in animals that were repeatedly treated with killed vaccine. One of the chief fallacies in the use of vaccines was that many investigators were thinking of the clinical signs of the disease, namely, 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 under­
stood. It is now known that the prevention of infection
is just as important, if not more so, than the prevention
of the clinical signs of the disease.

Following the earlier disappointing experience with
killed vaccine, Bang (1) reported results which demonstrated
some protective effects in sheep, goats, and cattle, when
live virulent Br. abortus organisms were injected sub­
cutaneously into animals before conception had taken place.

After investigators in other European countries, in
America, and elsewhere determined that the same species of
bacterial agent was responsible for the disease in cattle
in their respective countries, as was found by Bang in
Denmark, they also began to study the immunizing effects
of living and killed cultures of Br. abortus.

Following favorable reports by Stockman (240) and
McFadyean and Stockman (179) on the field observations of
cattle injected with live and killed organisms, many labora­
tories all over the world produced and distributed live cul­
ture vaccine for use in preventing bovine brucellosis.

The method in most countries called for the vaccination
with virulent cultures of Br. abortus of all non-reacting,
non-pregnant heifers and cows at about two months before breeding. The main purpose of this recommendation was to avoid the danger of causing abortion by the use of live active cultures in pregnant animals.

In 1919, the Bureau of Animal Industry (B.A.I.), U.S.D.A., permitted commercial biological concerns to produce and distribute vaccine. Vaccination was carried out on a large scale in this and other countries using virulent cultures of Br. abortus. Many investigators in various parts of the world conducted projects on field and experimental herd vaccination. With few exceptions, results indicated that the use of the virulent cultures of Br. abortus in non-pregnant cows protected the majority of the animals against abortion.

Strain 19 Vaccine

Investigations by Hart and Traum (106) and others indicated that in vaccinating lactating cows, Br. abortus was frequently becoming established in the udder and thus the cows became carriers, of virulent Br. abortus. During the period when live virulent cultures were being used for vaccinating cattle, several reports appeared which showed that Br. suis had been isolated from cow's milk. Vaccines were found on the market which contained Br. suis (Mohler et al. (186)). It was mainly for this reason that the use of virulent Br. abortus cultures were
discouraged, and in 1932 the B.A.I. no longer permitted the indiscriminate use of live virulent Br. abortus cultures in the production of the vaccine.

Since 1919, many investigators from institutions in the United States and elsewhere have worked with cultures of Br. abortus and other species having reduced virulence. The primary objective was to find a vaccine which would produce good protection against Brucella infection and at the same time not produce the carrier state in the animals.

Giltner, Huddleson, Clark and Schlingman (84), using an avirulent strain vaccine on a large number of animals in the field, reported an abortion rate of 3.6 percent for the treated group and 18.4 percent in the untreated group. These experiments were conducted on animals without regard to age.

In 1938, Meyer and Huddleson (182) again reported on the use of an avirulent vaccine that had been changed so that after large and repeated doses of the living vaccine the animals remained negative to the agglutination test. They reported that while there was a significant difference between the incidence of infection in the vaccinated animals and those in the control group, nevertheless, the vaccinated animals did not develop sufficient immunity to last one year.

In 1929, Cotton, Buck and Smith (51,52) began a long series of experiments with several avirulent strains of
Br. abortus from which strain 19 was selected as the most promising.

Sixteen heifers (age not given) were vaccinated, bred from two to 11 months later, and exposed to infection (conjunctival method) when pregnant. Of the five heifers given vaccine of low virulence (strain 11), four calved normally, one died during calving and two became infected. All of the five given vaccine of medium virulence (strain 19) and the six which were injected with the strain of high virulence (strain 484) produced vigorous calves. One strain 19 vaccinate showed uterine infection. Seven of the eight controls aborted, and all eight showed uterine and colostral infection after calving. The use of strains of high virulence, such as strain 484, was considered highly objectionable because of the possibility of causing udder infection.

The combined results of four experiments conducted by the B.A.I., U.S.D.A. were given by Plastridge (198). Of 53 calf-vaccinated animals exposed to infection when pregnant, 96 percent calved normally, and 13.2 percent showed uterine or udder infection. In comparison, 26 percent of the controls calved normally and 83 percent became infected following exposure. In these experiments, calf vaccination, using strain 19 vaccine, was about 84 percent effective in protecting cattle against infection with Br. abortus.
Birch (13) and Birch et al. (15) conducted experiments in which vaccinated and control heifers were exposed to infection during the sixth to seventh month of pregnancy by keeping them in a pen in which virulent infectious material was maintained. During their first pregnancy, 2.8 percent of the vaccinates aborted and 8.5 percent became infected. In comparison, 26 percent of the controls aborted and 60.8 percent became infected. During the second pregnancy, 3.5 percent of the vaccinates and 25 percent of the controls aborted.

Meanwhile, field experimentation on a large scale, beginning in 1934, was started and has continued up to the present time.

Butler, Warren, and Marsh (35) were some of the first investigators to use the strain of low virulence prepared by the B.A.I. under field conditions. No conclusions were drawn concerning the resistance developed in their vaccinated animals. Their work did indicate that heifers vaccinated at four to twelve months of age returned to a negative status much more quickly than those vaccinated over twelve months of age.

Stevens (239) used strain 19 vaccine in 130 heavily infected herds. He found that of the 1,027 cattle vaccinated, the abortion rate in over 200 calvings was only 1.5 percent.

About the same time, Hardenbergh (102), Haring and
Traum (104), Rabstein and Welsh (207), Thompkins (243), and Lothe (159) reported very favorable results from the use of strain 19 vaccine in controlling brucellosis in infected herds. Rabstein and Welsh suggested that the length of time agglutinins persist following vaccination depended on at least three factors: (1) the age at vaccination, (2) whether or not the animal was subsequently exposed to virulent Brucella organisms, and (3) the relative proportion of rough (R) and smooth (S) types of organisms in the vaccine used.

During the period from 1936 to 1941 the Bureau of Animal Industry vaccinated 17,000 calves ranging from five to seven months of age. This work was carried on in 260 infected herds in 24 states. At the start of the experiment approximately 29.2 percent of the adult cows were positive to the blood test. In 1940, Mohler et al. (185) reported that 96.2 percent of the calvings were normal and only 5.1 percent of the normal calving animals were positive to the blood agglutination test. No data was given as to the number of reactors removed during this period.

In general, the results of experiments and field trials indicate that calf vaccination protects about 97 percent of the animals against abortion from brucellosis and about 80 percent against infection. However, exposure
to large numbers of virulent *Br. abortus* may overcome the resistance of calf-vaccinated animals. It has been the author's experience that the age of the animals challenged may also be a factor in measuring the resistance of animals. This is shown by experiments conducted at the Ohio Agricultural Experiment Station (62,63,65). The results showed that the incidence of infection following conjunctival exposure of adult vaccinated cows (5 to 12 years of age) to 1,500,000 virulent organisms (strain 2308) was zero percent. In two other experiments, calfhood vaccinated heifers were exposed to 750,000 strain 2308 organisms. The incidence of infection was 16.7 and 20.0 percent. In the 1948 Annual Report of the Bureau of Animal Industry results showed that the incidence of infection following conjunctival exposure of calf vaccinated animals to 15,000,000; 74,000; and 370,000 virulent organisms was 72.7, 22.2, and zero percent respectively.

Vaccination is usually done on calves between six and eight months of age. While it is generally thought that calves under six months of age develop less resistance from the vaccine than older ones, there is no well controlled research which would prove this to be true. Well controlled work is needed to clarify this point. In general, calves over nine months of age tend to retain vaccinal blood titers.

**Duration of Resistance.** Another controversial point is the question of duration of resistance in cattle vac-
cinated with strain 19.

Birch et al. (16) challenged calf vaccinated animals in different pregnancies by placing them in a barn in which known infected cows were kept. The results indicated that the resistance did not decrease with age.

Manthei et al. (168) challenged five experimental groups of calf-vaccinated animals during their first, second, third, fourth, and fifth pregnancies. The results indicated that the resistance did not decrease with time. In fact, the older animals appeared to be more resistant to artificial conjunctival exposure than the younger animals.

In general, the results of investigations in both the laboratory and field trials, indicate that the resistance induced by calfhood vaccination with strain 19 does not decrease with time. However, it has been suspected by some investigators that the resistance decreased after the first pregnancy.

Revaccination. Berman and Beach (8) and Berman et al. (9) have recently reported their findings on this problem. The revaccination of blood negative heifers caused positive blood titers to develop for a period of about four months, and persistent suspicious titers thereafter. When these animals were challenged, 94 percent of the controls became infected, 21 percent infection occurred in the group vaccinated once at eight months of age and 28 percent
Infection occurred in the group vaccinated at eight months and again at 14 months of age. Twenty-five percent of the animals vaccinated at eight months and again at 20 months became infected. These results are in agreement with those of Plastridge et al. (201). They indicated that there was little need for revaccination when good quality vaccine was used.

**Persistence of Agglutination Reactions Resulting from Calfhood Vaccination.** The number of calfhood vaccinated heifers which react positively or suspiciously after two years of age depends upon at least two factors: (1) the exposure to Brucella or other antigenically related organisms following vaccination, and (2) age at the time of vaccination. Haring and Traum (104) found the percentages of animals with negative agglutinin titers 24 months following vaccination to be as follows: vaccinated at four to eight months - 99 percent; vaccinated at eight to 12 months - 91 percent; vaccinated at 12 to 16 months - 83 percent, and unbred heifers vaccinated when over 16 months of age - 50 percent. In another study, Haring and Traum (103) divided heifers into five age groups at the time of vaccination; four to six months, six to eight months, eight to 10 months, 10 to 16 months, and heifers over 16 months. The percentage of animals in each group that were negative to the blood test 24 months after vaccination was 100, 90, 75, 60, and 15 percent, respectively.
Apparently there are a few animals that do not exhibit agglutination reactions following vaccination. Birch et al. (16) observed one animal that showed no agglutinin response to the original vaccination nor to a second. They indicated that this animal probably possessed a high degree of natural resistance. The author has observed two animals that exhibited no agglutinin response to conjunctival exposure of virulent Br. abortus.

**Intracutaneous Injection of Strain 19 Vaccine.** The intracutaneous method of injecting strain 19 vaccine was suggested by Rabstein and Cotton (206). Twenty-nine calves were injected with 0.2 ml. of strain 19 vaccine into the caudal fold, and 12 calves by the usual subcutaneous injection of 5 ml. of the same vaccine. Blood samples were collected periodically and examined for agglutinins, and by the opsonocytophagic test described by Huddleson (117). Three months following vaccination, the agglutinin reactions of all the calves vaccinated intracutaneously were negative while 50 percent of the calves vaccinated subcutaneously reacted suspiciously or positively. More recently, Cotton (46) repeated this work and obtained similar results. None of the animals in these experiments were challenged to compare the resistance of the groups.

McDiarmid (173) vaccinated heifers when 15 to 18 months of age. They were then bred and challenged at about the fifth month of pregnancy. The incidence of in-
Infection was as follows: one of eight vaccinated subcutaneously, one of 10 vaccinated with 0.2 ml. intradermally, and one of 10 vaccinated with 1.0 ml. intracaudally. All of the 12 controls became infected. McDiarmid concluded that intradermal and intracaudal vaccination conferred a resistance comparable to that produced by the subcutaneous method.

Gregory (90) vaccinated 129 calves when seven to 10 months of age; 67 by the intracaudal method and 62 subcutaneously. The subcutaneously vaccinated group exhibited a mean maximum titer of 1:423 compared to 1:793 for those injected intracaudally. There was little or no difference in the number of animals exhibiting negative agglutinin titers 12 months post-vaccination. Later Gregory (91) challenged about 40 animals in each group during their first pregnancy. There was no significant difference in the abortion rate between the two groups.

Manthei et al. (167) vaccinated 41 heifers between 12 to 13 months of age; 21 intradermally with 0.2 ml., 14 with 5 ml. subcutaneously, and six with 0.2 ml. subcutaneously. Seventy-eight weeks after vaccination the agglutinin titers were below 1:100 in 52 percent of the intradermal group, in 36 percent of those injected with 5 ml. of vaccine subcutaneously, and in 50 percent of those injected with 0.2 ml. vaccine subcutaneously. The degree of resistance to subsequent challenge was similar in all three groups and not
related to the post-vaccinal agglutinin titer.

At about the same time, Manthei (164) vaccinated three groups of calves ranging from four to eight months of age. The percentage that exhibited a positive agglutinin reaction 12 months after vaccination was 9.4 percent for those injected in the caudal fold, 3.8 percent for those injected subcutaneously, and 1 percent for those injected intradermally. There was no significant difference in the degree of immunity produced by the three methods. Similar results were reported by Buddle (32).

Apparently there is little or no distinct advantage in the intradermal and intracaudal methods of vaccination over the usual subcutaneous method except possibly in the saving in the amount and cost of the vaccine used.

Huddleson's Mucoid Vaccine

Huddleson (123, 122) found that a mucoid strain of \textit{Br. suis} when injected into guinea pigs gave rise to a specific growth inhibiting antibodies and a high degree of active immunity, without the development of positive reactions to the blood serum agglutination test. Huddleson and Bennett (126) used the vaccine in 22 infected herds and three brucellosis-free herds. Of the 772 adult animals in the infected herds that were negative at the time of vaccination, 23 exhibited positive blood titers during the subsequent 14-month period. Thirty-three animals aborted, but Brucella bacteria were recovered from only nine. In the three blood
negative herds all the 73 adult vaccinated animals reacted negatively at the end of the one year period of observation.

Eight hundred ninety-nine blood test negative cattle and 179 suspicious or positive reactors were vaccinated with M vaccine in Michigan by Clark and Phelps (43). They concluded that the Brucella M vaccine did not produce persistent blood titers even in mature cattle, and that the vaccine had little or no therapeutic value.

Killham et al. (138) reported a decrease in the incidence of positive reactors in Brucella M vaccinated herds from 28 to 8 percent during an eleven month period of observation. In comparison, the incidence decreased from 26 to 17 percent in revaccinated herds during a seven month period. Some of the reactors were slaughtered during the period of observation.

Huddleson (119), in an effort to answer the criticism of omission of controls in previous work, vaccinated 17 head with M vaccine and allowed 15 head to serve as controls. These animals were all located in three infected herds. Following a period of 12 months of observation, he reported that none of the vaccinates became positive to the blood test, while eight of the controls exhibited reactor titers.

Edgington and King (62) were the first to report on the use of M vaccine in cattle under well controlled laboratory conditions. In the first experiment adult animals
beef cows 7 to 12 years old) were selected from a herd which had been maintained as a non-reactor group over a period of five years prior to the test. Pregnancies were established in 25 cows, 11 of which had received strain 19 vaccine and 8 Brucella M vaccine, while six served as nonvaccinated controls. The cows were challenged with 1,500,000 Br. abortus (strain 2308) viable organisms, by way of the conjunctival route, approximately nine months after vaccination and six to seven months in gestation. The percentage of the animals infected by the experimental challenge was zero percent of the strain 19 vaccinates, 12.5 percent of the M vaccinates and 66.7 percent of the nonvaccinated controls.

In a similar experiment, Edgington and King (65) divided heifers from 8 to 15 months of age into three groups, five received strain 19 vaccine, six were given M vaccine and six served as controls. Approximately ten months after vaccination and during the fourth to the sixth months of pregnancy, the animals were given a conjunctival challenge of 750,000 viable organisms (strain 2308). The incidence of infection in the three groups was 20, 50, and 83.3 percent, respectively.

In a third experiment, Edgington and King (63) divided 26 heifer calves, averaging nine months of age, into three groups. Six received strain 19 vaccine, nine were given M vaccine, and 11 were retained as nonvaccinates. Approxi-
mately 15 months following vaccine administration and during the mid-gestation period, they were given an exposure challenge. The percentages of infection in this test were 16.7, 77.8, and 90.1, respectively.

Edginton and King concluded that while neither vaccine gave complete protection, under the conditions of these tests, strain 19 afforded greater protective value than did the M vaccine. Neither vaccine produced a recognizable transmission of infection to other nonvaccinated animals in direct association with the vaccinates. Mucoid vaccine was used with no apparent ill effect, irrespective of the stage of gestation at the time of vaccination, and did not produce prolonged reactor titers in non-infected cattle, regardless of the animal’s age.

These results were later confirmed by Green (89) and other investigators.

Berman et al. (10) vaccinated groups of calves and sexually mature heifers with strain 19 and M vaccines. During the first pregnancy, the vaccinates and the non-vaccinated controls were exposed to Br. abortus strain 2308 by the conjunctival route. One-half of the animals in each group received a challenge of $6 \times 10^5$ organisms, and the other half were given $6 \times 10^6$ virulent organisms. They concluded that the strain 19 vaccinates were significantly more resistant to the challenge dosage than the controls. The M vaccinates were not significantly more
resistant than the controls.

Bryan et al. (29) compared the resistance developed in heifers given mucoid vaccine and strain 19 vaccine. The heifers were vaccinated when four to eight months of age and challenged by the conjunctival instillation of from 4 to 14 million virulent organisms. Positive agglutinin titers were exhibited by 45 percent of the strain 19 vaccinates, 82 percent of the M vaccinates, and 88 percent of the controls. It was concluded that under the conditions of the experiment, M vaccine failed to provide a significant degree of resistance in calfhood vaccinated cattle. The infective dose of the virulent organisms was almost 10 times as large as that used by Edgington and King.

Woods (258) studied the value of Huddleson's mucoid vaccine when used under field conditions in 14 Illinois herds. He concluded that the vaccination of animals of all ages and in all stages of gestation was not harmful. The use of the vaccine did not cause persistent agglutination titers, and only rarely did the titer last 90 days. Most of the herds included in these trials resumed calfhood vaccination with a strain 19 since Brucella infection occurred in a disturbing number of first calf, M vaccinated cattle in infected herds.
Other Immunizing Agents Used to Control Bovine Brucellosis

Bacterins (killed cells), cell fractions and extracts, nonvirulent cultures, nonagglutinogenic cultures, weakly virulent cultures, moderately virulent cultures, and highly virulent cultures have all been used in attempts to develop resistance to brucellosis.

Killed cells of *Br. abortus* were first tried by Bang (2); however, the results were not encouraging. In 1931, James and Graham (133) refuted the claim that repeated intravenous injections of *Br. abortus* bacterin would cause blood test positive cattle to return to negative status.

*Br. abortus* cells killed with formaldehyde, mercuriochrome, thionin, pyronin, chinosol, methylene blue, iodine and heat were found to be useless as immunizing agents by Zeller and Stockmeyer (261) and Gwatkin and Panisset (95).

McDiarmid (174) tested the intramuscular injections of formalin killed *Br. abortus* cells suspended in the lanolin and liquid paraffin. He concluded that while the lanolin vaccine did increase resistance, strain 19 vaccine was more effective in preventing infection.

In 1952, Schlingman and Manning (222) reported that the subcutaneous injection of alum precipitated, ultraviolet inactivated *Br. abortus* cells seemed to increase resistance to natural exposure to virulent Brucella.

Priestly (202) found that the injections of trichlor-
acetic acid extracts of *Br. abortus* failed to protect guinea pigs against Brucella challenge.

Huddleson (121) reported good protection in guinea pigs against Brucella infection by injecting them with a specially prepared water soluble agent. The agent failed to protect susceptible cattle. An agent prepared by Live et al. (152) produced good resistance in guinea pigs. Some increase in resistance was induced in cattle by the injection of a trypsin digested suspension of cells prepared by Paterson and Pirie (195).

Huddleson (125) and Cotton et al. (51, 52) evaluated the resistance induced by the use of nonvirulent strains of *Br. abortus*. The results indicated that the vaccines gave some protection but not of sufficient value to warrant further trials.

McEwen and Roberts (178) reported the isolation and use of a Brucella strain (strain 1+5) which seemed to produce some resistance in cattle. Later, McEwen (175, 176) substituted a rough substrain (strain 1+5 (20)) for the original strain 1+5 and found that the newly prepared vaccine offered promise as an immunizing agent to prevent bovine brucellosis.

Edwards et al. (66, 67) reported that strain 1+5 (20) was not only inferior to strain 19 as an immunizing agent, but presented evidence that the strain mutated and became viru-
lent when injected into nonpregnant lactating cattle.

The stability of the two vaccines was studied by Taylor and McDiarmid (242). After seven passages through pregnant cattle, strain 19 appeared to be unchanged in respect to its virulence for guinea pigs and in its ability to grow in air. Under the same handling, strain 45 (20) became CO₂ sensitive and highly virulent.

Chemotherapy

Prior to the advent of the sulfonamides, numerous chemical agents were used in an attempt to alter the Brucella-agglutinin titer of reacting cattle. Graham et al. (88) observed that acriflavine, trypan blue, trypacin A, colloidal carbon, thionine, alkali, hypiodite, and pyronine failed to affect the blood-serum agglutinin titer. Cotton and Swope (147) have recently shown that sodium para-amino-benzoic acid, administered subcutaneously every four hours for 21 days at a dosage level of 3 Gm. per kilogram of body weight, would produce complete tissue sterilization provided the treatment was initiated three days after the guinea pigs were infected with Brucella. These workers found that when therapy was started fourteen days after the guinea pigs were infected, only 80 percent of the infected individuals showed tissue sterilization.

Sulfonamides have been employed during the past decade in an attempt to treat Brucella infection. Huddleson (120)
was the first to report that the sulfonamides were value-
less in brucellosis therapy in cattle and guinea pigs.
Chinn (40) treated infected guinea pigs at the time of
infection with sulfanilamide, and observed sterile spleens
and livers from the autopsied pigs. Hamman and Huddleson
(100) showed that prontosil and sulfanilamide had little
effect on the blood-serum agglutination titer or the shed-
ding of Brucella organisms in the milk from Brucella in-
fected cows treated over a period of five to seven weeks.
Rajcevic and Okljesa (208) administered streptozol (sul-
fanilamide) for ten-day intervals over a period of six
months to cows that were naturally infected with Br.
abortus and observed that little effect was obtained on
the blood-serum agglutinin titer. It was also observed
that streptozol did not prevent nonreacting cows from be-
coming reactors following contact exposure to infected
animals. Dumaresq (58) employed massive doses of sulfanila-
mide in the treatment of guinea pigs and showed that the
drug retarded the Brucella infection. This worker also
treated two naturally occurring cases of bovine brucellosis
with 30 to 60 Gm. of sulfanilamide daily for eleven to
thirteen days, and observed that the dosage level was toxic
for the host, but did not cause the blood-serum agglutinin
titer to recede nor did it free the mammary gland of the
organisms.

Hamman and Huddleson (101) observed that sulfapyridine
had some bacteriostatic effect on Brucella in vitro, but
did not alter the agglutinin titers nor the organisms in
infected guinea pigs. Wilson and Maier (256) treated
Brucella-infected guinea pigs at the time of infection
with sulfapyridine, and observed that the livers and
spleens were sterile at the time of autopsy. Live, Stubbs,
and Gardiner (156) obtained only moderate results in treat­
ing Brucella-infected cows known to shed organisms in the
milk with sulfapyridine. These investigators (154) also
employed sulfathiazole in the treatment of cows known to
eliminate Br. abortus in the milk, obtaining only mediocre
results.

Schuhardt, Rich, and Beal (227) treated cattle with
sulfadiazine for Brucella infection, but reported no cures.
Huddleson (124) fed sulfadiazine at the rate of 0.2 Gm.
daily per guinea pig for thirty days. The guinea pigs were
experimentally infected with either Br. suis or Br. meli­
tensis. At the close of the first 24-hour feeding period,
each pig was injected intraperitoneally with 2 ml. of nor­
mal rabbit serum. The results were encouraging and indi­
cated that experimentally induced Brucella infection in
guinea pigs might be cured rapidly.

With the discovery of penicillin, there developed a
few experiments designed to treat brucellosis. T'ung (249)
found that eight of fifteen strains of Brucella tested
showed some susceptibility to penicillin in vitro. Berman
Irwin, and Beach (11) found that penicillin effected no cures in Brucella-infected cattle. Bunnell, Hutchings, and Donham (34) treated guinea pigs experimentally infected with \textit{Br. suis} and found that penicillin in varying dosage levels, at various periods of time following exposure, failed to alter significantly the course of the disease.

Following the development of streptomycin, it was found that Brucella organisms were susceptible to its action \textit{in vitro} (220,98,44). Live, Sperling, and Stubbs (151) concluded that streptomycin-treated guinea pigs infected with \textit{Br. abortus} were protected by the antibiotic. Gilman and LeGrow (82) treated guinea pigs infected with \textit{Br. abortus} with streptomycin administered intraperitoneally for a short period of time and observed that, under the conditions of the experiment, the disease was not overcome nor altered in its course. Kelly and Henley (137) have shown that streptomycin is of little value in the treatment of guinea pigs infected with \textit{Br. suis}. Similar results were obtained in the treatment of human patients with streptomycin alone (71,205).

Recently Kolmer (146) has reviewed the subject of the synergistic or additive activity of chemotherapeutic compounds against various micro-organisms. This report has stimulated the combination of two or more drugs, antibiotics, or biological products in the treatment of infectious dis-
Watts, Boley, and Greig (253) repeatedly treated four Brucella-infected cattle with sodium sulfamethiazine and transfusions of whole blood or normal serum. The treatment did not reduce the blood-serum agglutinin titer nor did it prevent three of the cows from shedding Brucella in their milk.

In human medicine, Pulaski and Amspacher (204) were the first to report that a combination of streptomycin and sulfadiazine would serve as successful therapy for human brucellosis. Other workers have confirmed this work and have shown that sulfadiazine synergizes with the activity of streptomycin in its action on Brucella elimination from the infected individual. As a result, a high proportion of acute human cases of brucellosis have been reportedly cured (135,237).

Holm and McNutt (174) produced bacteriological cures in experimental brucellosis in guinea pigs with streptomycin and sulfadiazine administered subcutaneously and orally. The drug-antibiotic combination was as effective when administered ten days after the animals were infected as when treatment was initiated on the first day following infection. It was also found that the combination was as effective when administered once daily as when given five times daily. Holm and Moore (115) observed that subcutaneous administration of streptomycin, plus oral administra-
tion of sulfadiazine on alternate days, was as effective as daily treatments to guinea pigs experimentally infected with *Br. abortus*. These workers also found that treatment every other day was as effective when initiated at the time of guinea pig infection as when it was started ten days following the experimental infection. Under the conditions of the experiment, *Br. abortus* was not isolated from the spleens of 70 to 100 percent of the treated guinea pigs.

Since the development of aureomycin, several reports have been published on its activity against *Brucella* infection. Spink et al. (235) administered aureomycin orally to human patients infected with *Br. melitensis*. The most recent information published on this experiment indicated that only three of the twenty-four treated patients developed a recurrence of fever and positive blood cultures.

Heilman (108) treated experimental *Brucella* infections in mice with aureomycin, chloromycetin, streptomycin, sulfonamid, and dihydrostreptomycin. Aureomycin combined with streptomycin or with dihydrostreptomycin was found to be the most effective method of treating *Brucella* infections in mice. Under the conditions of the experiment, it was found that none of the sulfa-antibiotic combinations completely sterilized the mice spleens.

Herrell (109) treated three human cases of *Br. abortus* infection and one case of *Br. suis* infection with oral
aureomycin and dihydrostreptomycin. No recurrence of fever or positive blood cultures were reported.

Holm and Moore (116) recently reported on the use of aureomycin and sulfadiazine administered subcutaneously and orally to guinea pigs experimentally infected with Br. abortus. The combination reduced the spleen size in the guinea pigs, but was not as effective as the streptomycin and sulfadiazine in eliminating the infection. Best results were obtained when the treatment was initiated ten days following infection. The aureomycin alone and aureomycin plus sulfadiazine produced toxic effects in the guinea pigs.

Larsen and Gilman (149) treated 4 acutely affected cows with aureomycin. The aureomycin failed to arrest the Brucella infection, or to modify its usual course as observed in the treated cases.
EXPERIMENTAL PROCEDURE

The Use of Huddleston's Brucella M Vaccine
Under Field Conditions

The prevention of brucellosis in cattle has challenged the minds of investigators for a half century or more. Its eventual eradication depends upon the effectiveness of preventive methods as well as the removal of the infected animals.

A suitable immunizing agent for brucellosis should meet the following requisites: a) it should not be harmful, that is, it should not produce a progressive type of disease or establish a carrier state in the species of animal in which it is injected; b) the agent should not cause the production of specific serum agglutinins that persist for more than a few months and thus increase the difficulty of distinguishing infected animals from those not infected by means of the serological test; c) it must engender sufficient resistance in susceptible animals to prevent the spread of the disease in herds while the infected animals are being eliminated, and to prevent the introduction of the disease in disease-free herds.

Despite the fact that Br. abortus strain 19 has been shown by controlled laboratory and field experiments to confer a useful degree of resistance to brucellosis in cattle, its use has certain disadvantages. Probably the most important disadvantage of the vaccine is that its
use stimulates the production of agglutinins which may persist at a diagnostic level for years. This persistence of titer interferes with the disease control program because there is no good practical method for differentiating the vaccinal from infection titers. Because of this and other disadvantages, a continuing search has been made for improved Brucella vaccines. One of those recently advanced as an improved vaccine has been Huddle-son's mucoid vaccine.

Edgington and King (62,63,64,65) were the first to study Brucella M vaccine under well controlled conditions. They reported that while Brucella M vaccine did not produce persistent blood agglutinin titers, its use did not seem to produce as good a resistance as did strain 19 vaccine. These results were later confirmed by Bryan et al. (29) and Berman et al. (10).

The above results were obtained when the experimental cattle were exposed by an artificial challenge dosage of from $7.5 \times 10^5$ to $14 \times 10^6$ virulent organisms. Since the true dosage and virulence of field exposure was not known, it was thought that the resistance engendered by Brucella M vaccine might make it a satisfactory preventive treatment under field conditions. It was with this objective in mind that the following work was initiated.
Materials and Methods

The vaccine used in these field studies was supplied through the courtesy of Dr. I. Forest Huddleson from current production lots of the Brucella Laboratory, East Lansing, Michigan. The vaccine was injected subcutaneously at the upper portion of the area immediately posterior to the scapula. The individual dose of vaccine injected was 1 ml.

The blood-serum agglutination test was conducted by the usual tube method. Serum in twofold dilutions ranging from 1:25 to 1:12,800 and a standardized B.A.I. antigen were used. The tests were read following incubation at 37° C. for 48 hours.

Whenever possible, aborted fetuses were obtained from the vaccinated and control animals that aborted and were examined for the causative agent. Milk samples from animals in certain herds were examined at different periods for the presence of the vaccine strain and the causative micro-organism. Attempted recovery of Brucella organisms was limited to the inoculation of Tryptose agar plates with milk, stomach contents of the aborted fetus, and when possible, vaginal swabs taken from the recently aborting dam. Duplicate plates were inoculated with each material. One of each set of plates was incubated under increased CO₂ tension. Following a minimum of four days of incubation at 37° C. colonies showing Brucella characteristics
were selected for further identification. Saline suspensions of the selected cultures were used as the antigen in agglutination tests with known positive and negative Br. abortus serums. Huddleson's dye plate method was used for species determinations.

The Brucella M vaccine was used in 12 Ohio dairy herds. At the beginning of the experiment, Brucella infected animals were present in all 12 herds. The infection had been diagnosed from three to 18 months prior to the use of the vaccine.

Four hundred and eighty-seven cows and heifers were vaccinated with Huddleson's Mucoid vaccine at the beginning of the test. All of the animals were negative to the blood agglutination test at the time of vaccination (table 1). Sixty cows, negative to the agglutination test, were not vaccinated and served as controls.

The chief criterion used to determine the absence or presence of brucellosis in the animals before and after the injection of vaccine was the blood-serum agglutination titer of each animal. All the animals were bled and tested on the day of, or a short time before vaccination, and at intervals of three to four months during the two-year period of observation. In certain herds the animals were bled and tested at weekly or monthly intervals after injection in order to observe the rise and fall in the blood agglutination titer.
Table 1. Blood Test Negative Animals Injected with "M" Vaccine at the Beginning of the Test

<table>
<thead>
<tr>
<th>Herd</th>
<th>Number of Adults Vaccinated</th>
<th>Number of Heifer Calves Vaccinated</th>
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</tr>
<tr>
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<td>18</td>
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<td>52</td>
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</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>Grand Total</strong></td>
</tr>
<tr>
<td></td>
<td>429</td>
<td>58</td>
</tr>
</tbody>
</table>

Results

Some of the adult Brucella M vaccinated animals exhibited a one to two dilution rise in blood titer approximately 10 to 15 days following the injection of the vaccine. In all instances the blood titers declined to a negative level within 90 days after vaccination except those animals that became permanently infected with the virulent organisms.

The vaccination of some lactating animals resulted in some reductions of milk flow, but no serious postvaccinal reactions were seen.

No trend regarding the effect of the use of Brucella
M vaccine on reproduction was observed. No therapeutic value from the use of the vaccine on infected animals was demonstrated.

The classification, as determined by the blood agglutination test, of all Brucella M vaccinated animals at the termination of the tests is shown in table 2.

Table 2. The Classification of Brucella M Vaccinated Animals at the Termination of the Test

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<td>4</td>
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<tr>
<td>Removed during Exp.</td>
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</table>

One hundred and two of the 429 Brucella M vaccinated adults exhibited a positive agglutination titer at the end of the two-year period of observation. Forty cows showed
a suspicious reaction and 287 remained negative. Twelve of the calfhood-vaccinated animals became positive to the blood test and 42 remained negative.

During this same period there were 45 abortions, eight premature and 490 normal calvings in the adult vaccinated animals. Seven heifers aborted and 24 calved normally. This data is summarized in table 3.

Table 3. Calvings During the Test Period

<table>
<thead>
<tr>
<th>Herd No.</th>
<th>Adults</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Premature</td>
<td>Aborted</td>
<td>Normal</td>
<td>Premature</td>
<td>Aborted</td>
</tr>
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<td>6</td>
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<td>5</td>
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<td>8</td>
<td>45</td>
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</table>

In all herds except one, approximately 10 percent of the negative adult animals were left as nonvaccinated controls. These animals as well as the permanently infected cattle were kept together with the Brucella M vaccinated animals. No attempt was made to segregate any of the groups of animals.
In all, there were 60 cattle which served as non-vaccinated controls at the beginning of the experiment. All were negative to the blood agglutination test when the test began. The titers of the controls at the termination of the experiment and the calvings which took place during the two-year period of observation are summarized in table 4.

Twenty-one cows and heifers became positive, nine became suspicious and 30 remained negative to the blood agglutination test during the two-year period. During this same period there were 11 abortions, three calved prematurely and 37 calved normally.

A comparison of the Brucella M vaccinates and the nonvaccinated controls made at the termination of the experiment is summarized in table 5.

Approximately 36.9 percent of the controls became infected, according to the blood agglutination titer while over the same period of time, and under the same exposure 23.4 percent of the Brucella M vaccinates exhibited reactor titers. These differences were more marked when the calvings of these two groups were considered. Approximately 27.4 percent of the controls aborted or had premature calves while only 8.8 percent of the Brucella M vaccinates aborted or had premature calves.

Discussion

This experiment was designed primarily to evaluate the
<table>
<thead>
<tr>
<th>Herd Total No.</th>
<th>Adults</th>
<th>Heifers</th>
<th>Calvings</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5 (9)</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6 (4)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 (8)</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8 (3)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>9 (3)</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>10 (3)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11 (6)</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12 (7)</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>60</td>
<td>29</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 5. A Comparison of the Brucella M Vaccinates and the Controls at the Termination of the Experiment

<table>
<thead>
<tr>
<th></th>
<th>Total Number in Experiment</th>
<th>Titer at Termination of Experiment</th>
<th>Total Calvings</th>
<th>Calvings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Remained Negative</td>
<td>Suspicious</td>
<td>Positive</td>
</tr>
<tr>
<td>Brucella M Vaccinates</td>
<td>487</td>
<td>67.5%</td>
<td>9.1%</td>
<td>23.4%</td>
</tr>
<tr>
<td>Controls</td>
<td>60</td>
<td>52.6%</td>
<td>10.5%</td>
<td>36.9%</td>
</tr>
</tbody>
</table>
resistance engendered in cattle by Brucella M vaccine when challenged by maintaining them in herds where brucellosis was known to exist.

Under the conditions of this experiment the data show that the use of Brucella M vaccine did produce a certain degree of resistance to a field exposure of virulent Brucella organisms. Statistical analysis, by the chi-square test, of the incidence of reactor titers in the M vaccinated animals showed them to be significantly different from the incidence of reactors in the non-vaccinated controls. While it is true that there was a significant difference at the 1 percent level, in the incidence of abortion between the two groups, nevertheless, since the causative agent associated with the abortions was determined in only a few instances, these observations were not used as a criterion for evaluating the vaccine.

Several important factors should be considered in evaluating the Brucella M vaccine under the conditions of this experiment. First of all, the number of non-vaccinated controls was not as large as was desired. These animals were selected largely by the owners and in a good many cases these were animals that were not too valuable, often because of poor breeding performances. There would, therefore, be some question as to the random sampling of this group.

Another factor which should be considered in the evaluation of the Brucella M vaccine when used under the
conditions of this test is that of the status of animals at the time of vaccination. It has been demonstrated by McEwen et al. (177) and Bendixen and Blom (6) that more than 200 days may elapse before cattle show a significant agglutination titer after a known exposure to Br. abortus. This is especially true in herds where infection is known to exist. Therefore, one cannot be sure, on the basis of one blood test, that all test negative animals were free from the infection at the time of vaccination. Ordinarily, a sizeable number of controls would tend to minimize the importance of this source of error.

The results seem to confirm those obtained in our controlled laboratory studies of Brucella M vaccine (62, 63, 64, 65). In these trials it was concluded that although Brucella M vaccine did not offer the protection afforded by the use of strain 19 vaccine, nevertheless, Brucella M vaccine was not entirely devoid of value as an immunizing agent against bovine brucellosis.

The vaccine did not cause blood serum agglutinins to develop for an S-phase antigen in a titer higher than 1:100 dilution. As a rule, specific agglutinins in the titer of 1:25 could not be demonstrated in the serum of the animals 90 days after injection of the vaccine.

Summary

Brucella M vaccine was injected subcutaneously into
serologically negative cows and heifers located in 12 privately owned dairy herds to determine its capability of engendering an active immunity against brucellosis.

Each of the 12 herds contained animals showing serological evidence of brucellosis, and in most of them, infected animals had been present only a short time before vaccine administration. In 11 of the 12 herds blood negative non-vaccinated animals were left to serve as controls.

Of the 487 animals that were negative to the agglutination test at the time of the injection of the Brucella M vaccine, 114 or 23.4 percent became reactors during the 24-month observation period. During the same period, 21 or 36.9 percent of the non-vaccinated controls became reactors. Approximately 27.4 percent of the control animals aborted or had premature calves while only 8.8 percent of the Brucella M vaccinates aborted or had premature calves.

Some of the Brucella M-vaccinated animals exhibited a one or two-dilution rise in blood titer approximately 10 to 15 days following the injection of the vaccine. In all instances the blood titers declined to a negative level within 90 days after vaccination except those animals that became permanently infected with the virulent organisms.

The vaccination of some lactating animals resulted in some reductions of milk flow, but no serious post-vaccinal reactions were observed.
The Application of a Suggested Method for Differentiating Vaccinal and Infection Titers in Cattle Known to Be Infected with Brucella Abortus

A method for differentiating between vaccinal and infection titers of Br. abortus in cattle was suggested by Dick, Venzke, and York (55). These workers reported that any animal not stimulated to the production of Brucella agglutinins by the intramuscular injection of 5 ml. of strain 19 vaccine within a maximum of fifteen to seventeen days was an infected animal. Animals exhibiting any blood serum titer increase following injection of strain 19 vaccine was classified as non-infected and it was suggested that these animals possessed titers due to previous vaccination. Venzke (250, 251) has made additional reports on the use of this diagnostic test. Barner, Oberst, and Atkeson (3) administered Br. abortus strain 19 vaccine to vaccinated cattle exhibiting varying serological reactions, known infected cattle, and known infected cattle previously vaccinated as adults. These workers concluded that the anamnestic reaction was of value (as a diagnostic measure) in differentiating between vaccinal and infection titers.

It was the purpose of the present study to expand the observations under experimental conditions and to determine the efficacy and the practicability of this diagnostic test.

Materials and Methods

In general, the methods outlined by Dick et al. (55)
were followed. The serum titer was determined by the serial dilution tube test method rather than the rapid plate agglutination test since it was believed that the latter did not show as reliable maximal titers, especially in the high dilutions. Titers were determined on all animals at least twice, approximately two weeks apart. If titers were stationary or declining, the animals were injected with 5 ml. of Br. abortus vaccine (B.A.I. strain 19) subcutaneously. The animals were bled approximately two weeks following the test injection of the vaccine, and the titers compared with the prevaccination determinations.

Those animals which showed a rising titer when the herd testing was completed were dropped from the experiment, since it was one of the premises that the test would be of significance only on animals exhibiting stationary or receding titers. Obviously, animals of negative status (showing no agglutinin titer at 1:50) could not be included.

The maximal titer was assigned to the highest dilution giving a complete reaction or to the next higher dilution provided it showed an incomplete agglutination.

For the purpose of this investigation and in accordance with data presented by Dick et al. (55) and Barner et al. (3), a significant rise in titer was defined as an increase of at least one complete dilution. Less than one complete dilution increase was considered as not significant.
At all prevaccination bleedings, quarter milk samples were cultured for Br. abortus. A tryptose (difco), penicillin, sodium azide agar, enriched with thiamine hydrochloride was used.

Ten herds, totaling 162 cattle, were studied. Of this number, 45 were found to be shedding Br. abortus in their milk and were designated as infected animals.

Results

When the differential test was applied to the 45 known infected animals, it was found that the agglutinin titers increased at least one complete dilution in 27 (60 percent) of the animals injected with the test injection of strain 19 vaccine. Therefore, if the correct interpretation of this test has been applied, 60 percent of these known infected animals would be considered carrying titers due to vaccination rather than to infection. A summary of dilution changes for the group of infected cattle is shown in table 6.

The mean number of dilutions changed was 0.73 and the standard deviation around this mean was 1.1. Statistical analysis revealed that there was a significant difference, at the 1 percent level, between the blood serum agglutinin titer dilution change of these infected cattle before and after injection with strain 19 vaccine.
Table 6. Changes in Agglutination Titer Following the Injection of Infected Cattle with Brucella Abortus Strain 19 Vaccine

<table>
<thead>
<tr>
<th>No. of dilutions changed</th>
<th>No. cattle in group</th>
<th>Total No. of dilutions changed</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>-1</td>
<td>6</td>
<td>-6</td>
</tr>
<tr>
<td>Totals</td>
<td>45</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 7 shows the results when this differential test was used on a group of experimentally infected cattle, a part of the 45 cattle previously mentioned. This infected herd contained eight animals vaccinated with Huddleston's mucoid vaccine (M), one strain 19 vaccine, and seven non-vaccinated controls. The vaccinates were injected with the two types of vaccine approximately twelve months prior to the experimental exposure. The test vaccine (strain 19) was used seven months following the experimental exposure (BAI, strain 2308). Nine of the 16 infected animals exhibited a rise in serum agglutinin titer of at least one complete dilution following the injection of the test vaccine. Of the nine animals exhibiting an increase in titer, five were Brucella (M) vaccinates, and four were controls. Titers of two of the infected Brucella (M) vaccinates (6 and 17) rose one dilution, two (23 and 36) rose two dilutions,
Table 7. The Application of the Differential Test on Cattle Experimentally Infected with *Brucella abortus*

<table>
<thead>
<tr>
<th>Cow (No.)</th>
<th>Titer Injected str. 19 vac.</th>
<th>Titer 6-14-52</th>
<th>Previous history</th>
<th>Tissue from which Br. abortus isolated</th>
<th>Classification according to differential test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,600</td>
<td>1,600</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>2</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>3</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>4</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>5</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>6</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>7</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>8</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>9</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>10</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>11</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>12</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>13</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>14</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>15</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
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<tr>
<td>16</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
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<tr>
<td>17</td>
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<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>18</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>19</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>20</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>21</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>22</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>23</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>24</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>25</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>26</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>27</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>28</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>29</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>30</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>31</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>32</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>33</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>34</td>
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<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>35</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>36</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>37</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>38</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
<tr>
<td>39</td>
<td>6,400</td>
<td>6,400</td>
<td>M (s.)</td>
<td>MK, Vs.</td>
<td>Infected</td>
</tr>
</tbody>
</table>

All animals were exposed with BAI strain 2308 on Nov. 1, 1951. C = complete reaction; I = incomplete reaction; M = Huddleston's mucoid vaccine; B = blood; F = fetus; MK = milk s = subcaneous i.v. = intravenous; Vs. = vaginal swab Control = nonvaccinated.
and one (24) rose three dilutions. There was a titer rise of one dilution in each of three controls (21, 22, and 25), and a rise of four dilutions in one control (39). All of the animals injected had stationary or declining titers for two pretest bleedings twenty days apart. If the procedure for conducting this test is applied as specified by the original authors, then 9 of 16 (56.25 percent) of these infected animals would be classified incorrectly as vaccinates; whereas seven (43.75 percent) would be diagnosed correctly as possessing agglutinins due to infection.

Table 8 shows the fluctuations of agglutinin titers of nonvaccinated cattle known to be infected with Br. abortus. These animals were artificially exposed to virulent Br. abortus at approximately 5 to 6 months in gestation. Maximal titers were reached approximately two or three months following exposure. A slow receding, fluctuating, agglutinin titer was observed for many months following abortion or calving.

The titer response of calves vaccinated between seven and twelve months of age with strain 19 is shown in table 9. When these unexposed calves were vaccinated subcutaneously with strain 19 vaccine, the maximal agglutinin production occurred approximately seven to fourteen days following injection of the vaccine. Without active virulent infection, their titers receded rather rapidly and fluctuated around 1:50 or 1:100 for several months. All of these calves
Table 8. Fluctuations of Agglutinin Titers of Nonvaccinated Cattle Experimentally Infected with *Brucella abortus*

<table>
<thead>
<tr>
<th>Cow (No.)</th>
<th>Exposed with BA I strain 2388</th>
<th>Postexposure titers</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-27-49</td>
<td>11-19-49</td>
<td>12-20-49</td>
<td>1-19-50</td>
<td>3-7-50</td>
</tr>
<tr>
<td>1</td>
<td>10-6-49</td>
<td>I 100</td>
<td>I 3,200</td>
<td>C 12,800</td>
<td>I 12,800</td>
</tr>
<tr>
<td>2</td>
<td>10-6-49</td>
<td>I 25</td>
<td>I 200</td>
<td>I 3,200</td>
<td>I 3,200</td>
</tr>
<tr>
<td>3</td>
<td>10-6-49</td>
<td>I 200</td>
<td>I 800</td>
<td>I 12,800</td>
<td>I 12,800</td>
</tr>
<tr>
<td>4</td>
<td>10-6-49</td>
<td>I 25</td>
<td>I 50</td>
<td>C 1,600</td>
<td>C 1,600</td>
</tr>
<tr>
<td>5</td>
<td>10-6-49</td>
<td>I 100</td>
<td>I 200</td>
<td>C 12,800</td>
<td>C 12,800</td>
</tr>
<tr>
<td>6</td>
<td>10-6-49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>C 800</td>
<td>I 3,200</td>
</tr>
<tr>
<td>8</td>
<td>11-6-51</td>
<td>I 25</td>
<td>I 400</td>
<td>I 800</td>
<td>I 1,600</td>
</tr>
<tr>
<td>9</td>
<td>11-6-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>11</td>
<td>11-6-51</td>
<td>I 200</td>
<td>I 12,800</td>
<td>I 6,400</td>
<td>I 6,400</td>
</tr>
<tr>
<td>12</td>
<td>11-6-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>14</td>
<td>11-6-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>16</td>
<td>11-6-51</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>17</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>18</td>
<td>11-6-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>20</td>
<td>11-6-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>11-6-51</td>
<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
<tr>
<td>22</td>
<td>11-6-51</td>
<td></td>
<td></td>
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<td>I 50</td>
<td>I 100</td>
<td>I 200</td>
<td>I 400</td>
</tr>
</tbody>
</table>

I = incomplete agglutination; C = complete agglutination; — = no sample.
Table 9. Fluctuations of Agglutinin Titers of Unexposed Cattle Vaccinated Between Seven and Twelve Months of Age with Strain 19 Vaccine

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Time of vaccination</th>
<th>Postvaccination Titers</th>
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<tbody>
<tr>
<td></td>
<td>1950</td>
<td>1951</td>
</tr>
<tr>
<td></td>
<td>9-20 10-18 11-17 12-18 1-18 2-19</td>
<td>3-10 4-18 9-18 11-1</td>
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<tr>
<td>27</td>
<td>8-21-50</td>
<td>I 3,200 I 200 I 50 I 25 I 50 I 50</td>
</tr>
<tr>
<td>28</td>
<td>8-21-50</td>
<td>I 800 I 200 I 50 I 50 I 50 I 25</td>
</tr>
<tr>
<td>29</td>
<td>8-21-50</td>
<td>I 800 I 100 I 25 I 50 I 100 I 25</td>
</tr>
<tr>
<td>30</td>
<td>8-21-50</td>
<td>I 800 I 100 I 100 I 50 C 50 I 50</td>
</tr>
<tr>
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<td>8-21-50</td>
<td>I 800 I 100 I 25 I 100 I 50 I 50</td>
</tr>
<tr>
<td>33</td>
<td>8-21-50</td>
<td>I 3,200 I 200 I 100 I 200 C 100 I 50</td>
</tr>
<tr>
<td>35</td>
<td>8-21-50</td>
<td>I 3,200 I 200 I 100 I 50 I 100 I 50</td>
</tr>
</tbody>
</table>

I = incomplete agglutination  C = complete agglutination  --- = no sample
exhibited titers fluctuating one dilution or more around
1:50 or 1:100 as late as twelve months following vaccina-
tion.

Discussion

Titers of both infected animals and those vaccinated
with strain 19 tend to fluctuate considerably, especially
after peak titers have been reached. Temporary increases
of one to two complete dilutions in unexposed strain 19
vaccinates and in infected animals without intentional
re-exposure have been observed when tested at semi-monthly
and monthly intervals.

Similar observations have been reported by Metzger
and Shuart (181). These workers reported that when adult
cattle previously unexposed to Br. abortus are vaccinated
with strain 19 vaccine, the peak of agglutinin production
occurred at about ten days following vaccination. The
concentration of agglutinins declined rather rapidly to a
titer of 1:50 or 1:100. Without superimposed infection,
the titers fluctuated about this level for periods varying
from several months to three or four years. When the vac-
cinated adults became infected with a virulent strain of
organisms, either soon after or a considerable period of
time following vaccination, the concentration of aggluti-
nins increased at a rapid rate.

Edgington and Donham (61) reported that nonvaccinated
heifers exposed to a virulent strain of *Br. abortus* prior to breeding and re-exposed during pregnancy showed a rise and considerable fluctuation in agglutinin titers.

Winter (256) observed that some strain 19 vaccinates which showed negative readings following vaccination would, at some later date following calving, show marked increases in titer. These fluctuations persisted for variable periods, in some cases for as long as a year. There seemed to be no direct relationship to the period of gestation or any other known factor. Such fluctuations occurred and recurred at various periods and seemed to be of little importance rather than to seriously mar the blood record.

The results of the present study indicate that a titer increase of one complete dilution is well within the limits of normal fluctuation. Data from other brucellosis studies (61,181,257) indicate that it is not uncommon to observe such a fluctuation of titer in vaccinated and infected individual animals. In view of these observations, it would appear that an increase in titer of but one complete dilution should not be considered as a significant increase in conducting a biological test of this kind.

The results in table 7 show that 60 percent of known infected cattle exhibited a rise in titer following injection with strain 19 vaccine. These results are not in accord with the premise that infected cattle should show no rise in titer following the injection of the test vaccine.
While this and other studies (12,139,140,253) indicated that the use of the test vaccine elicits a greater average titer increase in strain 19 vaccinates than in Brucella infected animals, nevertheless, blood serum titers of infected animals may increase following injection of strain 19 vaccine and in response to re-infection. Therefore, if it is assumed that titers of Brucella infected animals do rise, either in response to Brucella antigen stimulation or to normal titer fluctuation, the question immediately arises, what should be considered a diagnostic titer rise? Should less than one complete dilution be considered a rise? Should the critical point be one dilution, two dilutions, or more?

If the diagnostic rise in titer following the test vaccine injection is considered to be an increase of one complete dilution, then of the 45 known infected animals used in this experiment, 27 (60 percent) would be improperly classified by the differential test. If it is assumed that the diagnostic level should be two complete dilutions, then 9 (20 percent) of the infected animals would be improperly classified. Such results would be of questionable value as an aid in the control of brucellosis.

A rise in titer following the injection of the test vaccine, as defined in this study and as used in the two previous reports, must be redefined if the test is to be of value in differentiating between vaccinal and infection titers.
Summary

1) A suggested method for differentiating between vaccinal and infection titers was applied to cattle known to be infected with Br. abortus.

2) Forty-five known infected cattle were injected with strain 19 as a test vaccine. Agglutinin titers increased one complete dilution or more in 27 (60 percent) of the animals injected.

3) Agglutinin titers increased two or more complete dilutions in 9 (20 percent) of the animals injected.

4) If the data were to be interpreted on the basis of one dilution titer rise, as indicated by previous reports, it would show that 60 percent of known infected animals used in this experiment would be considered as exhibiting titers due to vaccination when the differential test was applied.

5) What should be considered a significant rise in titer following the injection of strain 19 as a test vaccine into cattle carrying titers due to infection and vaccination is discussed.

6) The results obtained in this experiment using strain 19 vaccine as a means of differentiating between titers due to vaccination and those resulting from infection indicate that the test under the present method of use is of questionable value.
The Use of Paper-Strip Electrophoresis as a Means for Differentiating Vaccinal and Br. abortus Infection Titers

The problem of cattle with low blood agglutinin titers of 1:50 to 1:200 has always caused confusion and indecision in the control and eradication of bovine brucellosis. According to reports from some states, the number of suspects has apparently increased following an accelerated calf vaccination program. Indiscriminate vaccination of over-aged heifers and adult cattle has added to the confusion by increasing the number of animals with suspicious and low reacting vaccinal titers which can not now be differentiated from infection titers.

Considerable research has been conducted to determine the significance of suspicious blood agglutinin reactions for brucellosis of cattle as well as differentiation between vaccinal and infection agglutinin titers. The different procedures investigated or under investigation are: Comparison of prevaccinal and postvaccinal titers of cattle following the injection of viable or dead strain 19 vaccine, the whey agglutination test, the milk ring test employing the dilutions technique, filter paper chromatographic techniques, the treatment of blood sera with heat, the use of acidified antigen, and the agglutination absorption with non-specific antigens.

In summarizing the data presented on the various tests employed for differentiation of vaccinal and infection
titers for brucellosis in cattle, several conclusions are apparent.

The anamnestic reactions produced by the injection of viable strain 19 vaccine failed to identify a significant number of bacteriologically proved infected animals and the persistence of secondary blood agglutinin titers make the test incompatible with a sound brucellosis control program.

Although the whey agglutination test has certain limitations, it has generally given more consistent and reliable results than either the anamnestic reaction produced with strain 19 vaccine or the milk ring test. However, the test cannot be applied to males, non-lactating cows and heifers; and it is not reliable in cattle during the early and late stages of lactation because of numerous false positive reactions.

The lack of uniformity and consistency of results with the milk ring test make its use as a differential test highly questionable.

The finding of the inadequacy of the above mentioned differential tests stimulated the present preliminary investigations on the use of paper-strip electrophoresis as a possible means of differentiating Brucella vaccinal and infection titers.

Paper-strip electrophoresis is one method for the characterization of proteins. Proteins, having a specific
electrical charge, will migrate in an electrical field. When filter paper is wet with a conducting solution and a bridge is formed between two reservoirs of the solution, each containing an electrode, a source of direct current passed between the electrodes will pass through the filter paper. When a protein solution, e.g., serum is applied to the filter paper, the protein molecules will migrate on the paper strip at a velocity dependent upon the electrical charge of the molecule. As different protein molecules have different charges, they will migrate at different rates and so separate one from the other.

Wall (252) found that the proteins of human serum separated on paper-strip electrophoresis into five main components designated as albumin, alpha$^1$, alpha$^2$, beta and gamma globulins. Properly performed paper-strip electrophoresis can not only differentiate these five components, but roughly quantitate the relative amounts of these components.

The present electrophoretic studies were undertaken (a) to ascertain the value of an experimental paper-strip electrophoretic apparatus in the characterization of bovine serum proteins; (b) to learn what fundamental differences, if any, existed between the serum globulins of animals vaccinated with Brucella strain 19 vaccine and the globulins of animals infected with virulent Br. abortus; and with this information (c) develop, if possible, a
means for differentiating vaccinal from infection titers.

Materials and Methods

This study was initiated approximately two years ago. Twenty-three cattle, divided into calfhood vaccinated, adult vaccinated, and adult infected groups were studied in this experiment.

The eight heifer calves and the eight adult cows were located in the Ohio Agricultural Experiment Station's main dairy herd. This herd had been free of brucellosis for the past six years. The heifer calves were housed with other vaccinated and nonvaccinated animals of approximately the same age. With the exception of two, all of the adult vaccinated cows had been raised at the Station. Two animals were purchased from a brucellosis-certified herd in Northwestern Ohio. All except one of the seven nonvaccinated infected animals studied were located in a private herd near Columbus, Ohio, and were maintained there during the period of observation. Infection had been present in the herd for approximately 1 year. The remaining infected animal was housed in the cattle isolation unit at the Station. This animal was infected as a result of artificial exposure with strain 2308.

The group of heifer calves were injected with strain 19 vaccine when six to eight months of age. All were negative to the sero agglutination test at the time of
vaccination. The adult vaccinated group averaged approximately three years of age at the time of vaccine administration and were negative to the sero agglutination test.

Blood Serum Agglutination Test: The usual tube method of testing was used throughout the experiment for titer determinations. Serums in dilution ranging from 1:25 to 1:12,800 and a standardized ARS antigen were used. The tests were read following incubation at 37° C. for 48 hours.

Blood samples were collected from the vaccinated animals at the time of vaccination and at two and four week intervals following vaccination. Blood samples were collected from the infected animals twice, approximately one month apart.

Immunization of the Animals: The animals were immunized by the subcutaneous injection of 6 ml. of strain 19 vaccine in the area immediately posterior to the scapula. The vaccine was obtained from a commercial veterinary supply house.

Electrophoresis: The percentage distribution of the serum proteins was determined from the analysis of the electrophoretic patterns using the apparatus in Figure 1. The horizontal cell was designed and made by Dr. Walter Frajola, Hoster Memorial Laboratory, Ohio State University, Columbus 10, Ohio. This type of equipment had been used experimentally in Frajola's laboratory to separate the various fractions of human serum and to study the relation-
ship of these fractions to a human disease. The variable voltage regulated power supply shown in Figure 1 was obtained from the Heath Company, Benton Harbor, Michigan.

The electrophoretic technique used in these experiments was similar to that used in Wall's (252) laboratory. The following steps were followed in the electrophoresis of the bovine serum proteins:

1. Both ends of the cell were filled with fresh barbitone buffer (ionic strength 0.05, pH 8.6) until the electrodes were well covered. The buffer in the cell was equalized and the cell leveled.

2. Whatman 3 mm. filter paper (18¾" by 22½") was wet with buffer solution and partially dried by blotting with a towel.

3. Approximately 0.01 ml. of serum was applied by the use of a micro-pipette approximately midpoint on the paper where a pencil line had been previously drawn for a guide. Recently, a special applicator, designed by Scientific Products Corporation, American Hospital Supply Corporation, Evanston, Illinois, had been used. The use of the special applicator made it possible to get a better distribution of the sample on the filter paper.

4. The paper was then placed in the cell and the system allowed to equilibrate for approximately 15 minutes.
Figure 1. A Photograph of the Variable Voltage Regulated Power Supply and the Experimental Horizontal Cell Used in Electrophoretic Studies
5. The current was then set at approximately 15 MA -
(260 - 280 volts) and allowed to run for five to
six hours. The amount of time necessary to get
good separation of the proteins was determined
by a stained control sample applied with the
other samples at each run. The temperature out-
side the cell was maintained as uniformly as
possible during the period of separation.

6. The paper was then removed from the cell and
dried in a horizontal position in a drying oven
for 20 minutes at 120° C. This fixed the protein
on the paper and the paper was then stained by
submerging in a bromphenol-blue bath (0.01 per-
cent bromphenol blue with 5 percent zinc sulfate
and 5 percent acetic acid) for 16 hours.

7. The paper was then washed three times, 10 minutes
each time, in a 2 percent acetic acid solution.
The paper was then transferred to a bath contain-
ing 0.75 gram of sodium acetate in 100 ml. of a 2
percent acetic acid and allowed to remain for 10
minutes.

8. The paper was then dried in a drying oven for 10
to 15 minutes at 120° C.

9. The paper was then cut into strips and the protein
components were quantitated by scanning the strips
in the calibrated recording photometer shown in
Figure 2. This particular piece of equipment was obtained from the Scientific Products Division, American Hospital Supply Corporation, Evanston, Illinois.

Results

Paper-strip electrophoresis, using the above described apparatus and technique, was found to be one method which can be used to characterize the proteins of bovine serum. Usually bovine serum separated on the paper strips into four main components designated as albumin, alpha, beta and gamma globulins (Figures 3 and 4).

During the early part of this work much time was devoted to the development and improvement of the technique for use with the experimental horizontal type cell, since this was the first time this particular type cell had been used to separate the proteins of bovine serum.

In this early work the serum from two calves was collected and electrophorograms made at weekly intervals beginning at the time of birth and continuing for a period of five months. One of the calves was colostrum fed and the other was colostrum deprived.

The electrophorograms prepared from the serums collected from the two calves 24 hours after birth, at one month of age, and at four months of age are shown in Figures 5, 6, 7. The outstanding characteristic of the serum from the colostrum deprived calf (no. 1185) was the high
Figure 2. A Photograph of the Recording Photometer Used in the Electrophoretic Studies
Figure 3. A Photograph of an Electrophorogram Showing the Four Main Components of Bovine Serum
Figure 4. A Photograph of a Stained Filter Paper Showing the Four Main Components of Bovine Serum
Albumin 24 hours after nursing
Globulins Calf 1187

Figure 5. A Photograph of Two Electrophorograms
Showing the Distribution of Serum Proteins of a
Calf (1187) 24 Hours after Ingestion of Colostrum
and at One Month of Age
Figure 6. A Photograph of Two Electrophorograms Showing the Distribution of Serum Proteins of Colostrum-Deprived Calf (1185) 24 Hours after Birth and One Month of Age
No Colostrum
4 months of age
Calf 1185

Albumin
4 months of age
Calf 1187

Globulins

Figure 7. A Photograph of the Electrophorograms Showing the Distribution of the Serum Proteins of the Colostrum-Fed (1187) and Colostrum-Deprived Calves (1185) at Four Months of Age.
concentration of albumin. On the other hand, the gamma globulin fraction was distinguished by its extremely small size or complete absence (Figure 5).

Twenty-four hours after calf 1187 had ingested colostrum the gamma globulin had risen to approximately 70 percent of the total globulins. In a month the gamma globulin fraction of the colostrum-fed calf had decreased from a value of 70 percent to approximately 15 percent. During the same period, the alpha globulin fraction decreased and the beta globulin increased. The electrophorogram of the colostrum-deprived calf showed a decrease in the alpha fraction and an increase in the beta globulins, and no change in the gamma fraction during the first month of age. At four months of age the electrophorograms of the two calves looked very similar in appearance (Figure 7).

These results are similar to those reported by San Clemente and Huddleson (219) and Pierce (196) using the modified Tiselius apparatus.

The electrophoretic distribution of the serum globulin fractions of the vaccinated and infected cattle are shown in tables 10, 11, 12.

There was no difference in the alpha globulin values between the calfhood vaccinates and the adult vaccinates. However, in the infected animals the alpha globulin fraction made up only 21.014 percent of the total globulins.
### Table 10. The Percentage Distribution of the Globulin Fractions of the Serums of the Calves Two Weeks Following Vaccination with Strain 19 Vaccine. (Determined by Paper-Strip Electrophoresis)

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Alpha Globulin</th>
<th>Beta Globulin</th>
<th>Gamma Globulin</th>
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<tbody>
<tr>
<td>1274</td>
<td>26.9</td>
<td>24.3</td>
<td>4.8</td>
</tr>
<tr>
<td>1273</td>
<td>35.5</td>
<td>21.3</td>
<td>4.3</td>
</tr>
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<td>1278</td>
<td>34.0</td>
<td>21.8</td>
<td>4.1</td>
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<td>1271</td>
<td>34.9</td>
<td>25.4</td>
<td>3.7</td>
</tr>
<tr>
<td>1275</td>
<td>34.9</td>
<td>28.1</td>
<td>3.6</td>
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<tr>
<td>1195</td>
<td>23.8</td>
<td>27.1</td>
<td>4.0</td>
</tr>
<tr>
<td>1179</td>
<td>35.0</td>
<td>25.6</td>
<td>3.9</td>
</tr>
<tr>
<td>1107</td>
<td>31.6</td>
<td>30.7</td>
<td>3.9</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>32.075</strong></td>
<td><strong>25.575</strong></td>
<td><strong>42.60</strong></td>
</tr>
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</table>

### Table 11. The Percentage Distribution of the Globulin Fractions of the Serums of Adult Cattle Two weeks Following Vaccination with Strain 19 Vaccine. (Determined by Paper-Strip Electrophoresis)

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Alpha Globulin</th>
<th>Beta Globulin</th>
<th>Gamma Globulin</th>
</tr>
</thead>
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<td>408</td>
<td>38.3</td>
<td>20.0</td>
<td>41.7</td>
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<td>431</td>
<td>26.0</td>
<td>21.9</td>
<td>52.1</td>
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<td>426</td>
<td>28.2</td>
<td>21.8</td>
<td>47.0</td>
</tr>
<tr>
<td>418</td>
<td>37.2</td>
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<td>537</td>
<td>31.6</td>
<td>20.5</td>
<td>47.9</td>
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<tr>
<td>419</td>
<td>34.9</td>
<td>23.4</td>
<td>41.7</td>
</tr>
<tr>
<td>424</td>
<td>32.6</td>
<td>18.7</td>
<td>48.7</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>32.47</strong></td>
<td><strong>21.24</strong></td>
<td><strong>46.27</strong></td>
</tr>
<tr>
<td>Animal No.</td>
<td>Alpha Globulin</td>
<td>Beta Globulin</td>
<td>Gamma Globulin</td>
</tr>
<tr>
<td>------------</td>
<td>----------------</td>
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<td>18.2</td>
<td>19.2</td>
<td>62.6</td>
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<td>1076</td>
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<td>19.6</td>
<td>51.0</td>
</tr>
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<td>21.7</td>
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</tr>
<tr>
<td>32</td>
<td>20.9</td>
<td>19.4</td>
<td>59.7</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>21.043</strong></td>
<td><strong>19.2</strong></td>
<td><strong>58.76</strong></td>
</tr>
</tbody>
</table>
compared to a value of approximately 32 percent for the vaccinates.

The beta globulin fraction made up approximately 25.6 percent of the total globulin in the calfhood vaccinates, 21.24 percent in the adult group.

It appeared that Br. abortus infection produced a tremendous increase in the gamma globulin peak in spite of the fact that the average titer of the vaccinated group was one dilution higher than the infected group. The gamma globulin fraction made up approximately 58.76 percent of the total globulin in the Brucella infected group, 46.27 percent in the adult vaccinates, and 42.6 percent in the calfhood vaccinates. The average seroagglutinin titers for these same groups was found to be 1:900, 1:1600 and 1:800, respectively.

**Discussion**

This experiment was designed primarily (a) to ascertain the value of an experimental paper-strip electrophoretic apparatus in the characterization of bovine serum proteins, more especially the serum globulins; (b) to make a preliminary study of the fundamental differences between the serum globulins of Brucella strain 19 vaccinated and Brucella infected cattle, and with this information (c) evaluate the possibility of using this technique as a means for differentiating vaccinal from infection titers.
Under the conditions of this experiment the data show that this experimental apparatus can be used as a reliable means for the characterization of serum protein of the bovine. The results compare favorably with those obtained by the use of the conventional Tiselius method as reported by San Clemente and Huddleson (219), and Pierce (196).

The data show that certain fundamental differences did occur in the serum globulins between the strain 19 vaccinates and the Brucella infected animals. Statistical analysis, by the analysis of variance test, indicated that there was a significant difference, at the one percent level, between the gamma globulin values of the infected group and the strain 19 vaccinated groups.

The gamma globulin values of the infected group ranged from 51 to 65.3 percent, while the vaccinated groups exhibited values of 36.7 to 52.1 percent. There was an overlapping of only one animal number 431, an adult vaccinate.

The fact that the differences between the two groups was rather clear cut demonstrates the feasibility for using this technique as a possible differential test. The data further suggest the need for additional work involving greater numbers of animals.

Several important factors should be considered in evaluating this technique as possible differential test. First of all, *Br. abortus* organisms were isolated from only
one of the so-called infected group. The diagnosis of
the remaining six animals was based on the sero agglutinin
titers and on the history of infection in the herd. While
none of these animals had been vaccinated with strain 19,
nevertheless, strain 19 vaccinates were present in the
same herd. The spread of strain 19 organisms from vac­
cinates to nonvaccinated animals has never been reported
in this country. It has been reported in Germany by
Lembke et al (150).

Another factor which should be considered in the eval­
uation of this test is that of the time of sampling, blood
was collected from the vaccinates prior to or at the time
of vaccination and again at two and four week intervals
following vaccination. The sampling of the infected ani­
mals was done without regard as to time and stage of in­
fecion inasmuch as this information was not known. While
there was little difference in the patterns of the serums
collected from the vaccinates at the two and four week
postvaccination periods, it would seem that this phase of
the work needs more critical study.

San Clemente and Huddleson (219) conducted extensive
studies on the absorption of antiserum of high titer. It
was found that Brucella agglutinin antibody was associated
with the gamma globulin fraction. However, the increased
production of gamma globulin was not the function of any
particular antigen alone. It would, therefore, seem that
disease free, and bacteriologically known infected animals should be used in a critical evaluation of a test of this kind.

**Summary**

Paper-strip electrophoresis, using an experimental type horizontal cell, was found to be one method which can be used to characterize the proteins of bovine serum. Using this technique, bovine serum separated into four main components designated as albumin, alpha globulin, beta globulin and gamma globulin.

The electrophoretic patterns of a series of colostrum deprived, colostrum fed, strain 19 calfhood vaccinate, strain 19 adult vaccinate and Brucella infected bovine serums have been obtained using the paper-strip technique. The concentrations of the electrophoretic protein components have been computed from the patterns. The outstanding characteristic of the serum of a newborn colostrum deprived calf was the extremely high concentration of alpha globulin in contrast to a negligible, or almost complete absence of gamma globulin. Within 24 hours after the ingestion of colostrum by a normal calf the gamma peak accounted for almost 70 percent of the total globulins. Within four months all protein components of the serums of the two calves were in the relative concentration usually found in young normal heifers.
Vaccination of calves and adult cattle with Brucella strain 19 vaccine caused some change in the normal serum distribution, especially an increase in the gamma globulin fraction (for example see Figures 7 and 8). _Br. abortus_ infection produced an even greater increase in the gamma globulin peak (Figure 9). The gamma globulin fraction made up approximately 58.76 percent of the total globulins in the Brucella infected group, 46.27 percent in the adult vaccinates, and 42.6 percent in the calfhood vaccinates.

The data show that there was a highly significant difference between the gamma globulin values of the infected group and the Brucella strain 19 vaccinated groups.

The possibility of using this technique as a means for differentiating vaccinal and infection titers is discussed.

The Influence of Some Shipping Fever Bacterins on the Brucella Sero Agglutinin Titer of Cattle

In recent years there have been several reports of Brucella calfhood vaccinated cattle in brucellosis free herds showing increasing titers to the Brucella sero agglutination test a short time after being injected with some of the commercially prepared bovine bacterins. Wart vaccine, mastitis bacterins, and hemorrhagic septicemia bacterins have also been reported as being capable of producing this type of reaction.
Figure 8. A Photograph of the Electrophorograms Showing the Distribution of the Serum Proteins Prior to and Two Weeks after Vaccination with Strain 19 Vaccine. (Cow No. 408)
Figure 9. A Photograph of the Electrophorograms Showing the Distribution of the Serum Proteins Prior to, and Two Weeks after Vaccination with Strain 19 Vaccine (Cow No. 426)
Figure 10. A Photograph of the Electrophorograms Showing the Distribution of the Serum Proteins Prior to, and 2½ Months after Brucella Infection
Within the last year, Berman (7) stated that he was able to produce significant titers in strain 19 vaccinated cows by injecting them with commercial bacterins containing *Pasteurella multocida*. Working with bacterins prepared in his own laboratory, Berman demonstrated that a cross reaction occurred between Brucella and the encapsulated (fluorescent) *Pasteurella* serotypes C and D.

In 1956, Hollister (112) reported that he observed a similar phenomenon in a certified Brucella free herd in Pennsylvania. Twenty-two days after this herd of 283 dairy cattle had been injected with Bacterin Formula #1 (composed of *Pasteurella*, *Corynebacterium*, *Streptococcus* and *Staphlococcus*), 21 percent were found to be positive and 14 percent were suspicious to the sero agglutination test. Within 90 days all significant blood titers had disappeared.

These and other reports serve to point up the importance of a study of this problem inasmuch as the use of such bacterins, if found to be the cause of these reactions, could interfere, seriously with the present brucellosis control program.

This experiment was designed primarily to make a preliminary study of the effects of some hemorrhagic septicemia biologicals on the sero agglutination titers of Brucella vaccinated and nonvaccinated cattle.
Materials and Methods

The bacterins and anti-hemorrhagic septicemia serums used in this study were obtained from the current production lots of four different commercial biological manufacturers. The bacterins were designated A, B, C, D, and E and were composed primarily of Pasteurella species and Corynebacterium species of organisms. The bacterins were injected subcutaneously at the lower portion of the area immediately posterior to the scapula. The individual dose of bacterin varied from five to ten milliliters.

The blood serum agglutination test was conducted by the usual tube method. Serum in twofold dilutions ranging from 1:25 to 1:200 and a standardized Agriculture Research Service, U.S.D.A., antigen were used. The tests were read following incubation at 37° C. for 48 hours.

Twenty-three cows ranging from two to eight years of age were injected with hemorrhagic septicemia bacterins and antiserums. Of this number, five had been calfhood vaccinated with Brucella strain 19 vaccine, eight had been injected with Brucella M vaccine, and the remaining ten were nonvaccinated. All of these animals were located in the Ohio Agricultural Experiment Station Dairy Herd. These herds have been free of brucellosis for several years.

Sero agglutination titers were determined prior to, and at the time of the injection of the biological, and
at three to seven-day intervals following the injections for a period of approximately six weeks. The sero agglutination titers at the time of injection of the biologicals ranged from negative to suspicious.

Results

Data showing the vaccination history of each animal, the preinjection titers, the material injected and the postinjection titers are summarized in tables 13 and 14.

It was found that some titer fluctuation occurred in most of the animals during the six week observation period. However, the fluctuation was not sufficient to change the classification of most of the animals. Two Brucella M vaccinates, 1142 and 1127, and one strain 19 vaccinate 1089, changed from a negative to suspicious status but returned to negative within a period of 30 days following the injection of the biologicals. A nonvaccinated animal 1083, showed a suspicious reaction before injection of the bacterin, exhibited a positive reaction six days after injection and remained positive for the remainder of the period of study.

Discussion

Various explanations have been suggested as possible causes of the fluctuation of Brucella titers such as described above.

Many prefer to refer to this phenomenon as an example
<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Vaccination History</th>
<th>Titer 1955</th>
<th>Titer 1956</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12-19 12-28</td>
<td>1-3 1-6 1-9</td>
<td>1-16 1-20 1-24 1-30 2-1 2-6</td>
</tr>
<tr>
<td>990</td>
<td>Nonvac. I--- C Bact.</td>
<td>---- I--- ----</td>
<td>D Bact. ---- ---- ----</td>
</tr>
<tr>
<td>1016</td>
<td>Bruc. M 10-4-49</td>
<td>I--- I--- II-- II-- II-- II-- II-- II--</td>
<td></td>
</tr>
<tr>
<td>1077</td>
<td>Nonvac. I--- C Bact.</td>
<td>I--- I--- II-- II-- II-- II-- II--</td>
<td></td>
</tr>
<tr>
<td>1083</td>
<td>Nonvac. CII- A Bact.</td>
<td>CII- CII- CCI- CCI- CCI- CCI- CCI- CCI-</td>
<td></td>
</tr>
<tr>
<td>1089</td>
<td>St. 19 7-26-51 I---</td>
<td>II-- CI-- CI-- CI-- II-- II-- CI-- II-- II--</td>
<td></td>
</tr>
<tr>
<td>1167</td>
<td>Nonvac. I--- D Bact.</td>
<td>---- ---- ---- ---- ---- I--- ----</td>
<td></td>
</tr>
<tr>
<td>1177</td>
<td>Nonvac. ---- A Bact.</td>
<td>---- ---- ---- ---- ---- ---- ----</td>
<td></td>
</tr>
<tr>
<td>1404</td>
<td>Nonvac. I--- ----</td>
<td>---- I--- ---- ---- ---- ---- I---</td>
<td></td>
</tr>
<tr>
<td>1138</td>
<td>St. 19 10-21-52 I---</td>
<td>---- I--- ---- ---- I--- I--- I--- ----</td>
<td></td>
</tr>
<tr>
<td>1142</td>
<td>Br. M 7-2-52 ----</td>
<td>---- I--- I--- I--- I--- CI-- C--- I---</td>
<td></td>
</tr>
</tbody>
</table>

I = Incomplete agglutination; C = Complete agglutination; - = No agglutination
Titer represents dilutions ranging from 1:25 to 1:200
Table 14. The Effect of Some Hemorrhagic Septicemia Biologicals on Brucella Sero Agglutination Titers

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Vaccination History</th>
<th>Titer 1-30-56</th>
<th>Titer 2-1-56</th>
<th>Titer 2-6-56</th>
<th>Titer 2-13-56</th>
<th>Titer 2-21-56</th>
<th>Titer 2-27-56</th>
<th>Titer 3-5-56</th>
<th>Titer 3-12-56</th>
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</thead>
<tbody>
<tr>
<td>926</td>
<td>Nonvac.</td>
<td>D Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>985</td>
<td>9-5-51</td>
<td>A Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1007</td>
<td>8-29-49</td>
<td>D Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1008</td>
<td>8-29-49</td>
<td>A Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1054</td>
<td>9-22-50</td>
<td>C Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1107</td>
<td>2-8-52</td>
<td>A Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1127</td>
<td>9-9-52</td>
<td>C Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1165</td>
<td>Nonvac.</td>
<td>C Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1232</td>
<td>8-6-54</td>
<td>D Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>1233</td>
<td>Nonvac.</td>
<td>A Bact.</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

Anti-Hem. Sept. serum ----

I = Incomplete agglutination; C = Complete agglutination; - = No agglutination

Titer represents dilutions ranging from 1:25 to 1:200
of an anamnestic reaction. While it is true that detectable antibody may disappear from an animal after stimulations of antigen ceases, however, many doubt that production ever completely ceases during the lifetime of an animal. After antibody has disappeared, an injection of antigen will usually cause it to reappear much sooner than it did originally. Furthermore, the injection of, and possibly exposure to, unrelated antigens may cause antibody to form which may be specific for those antigens to which the animal had been previously exposed.

There is good evidence that there may be cross reaction between Brucella and certain types of Pasteurella and other organisms which may be responsible for this change in titer.

Winter (257) observed that some strain 19 vaccinates which showed negative readings following vaccination would at some later date following calving show marked increase in titer. These fluctuations persisted for variable periods, in some cases for as long as a year. There seemed to be no direct relationship to the period of gestation or any other known factor. Such fluctuations occurred and recurred at various periods and seemed to be of little importance rather than to seriously mar the blood record.

King et al. (141) reported that a Brucella titer change of one complete dilution was within the limits of normal fluctuations found in Brucella vaccinated and Brucella
infected animals. This and other brucellosis studies (61,181,257) indicated that an increase in titer of but one dilution should not be considered as a significant increase when conducting a biological test such as the Brucella sero agglutination test.

Since none of the animals in this test showed more than one dilution rise in the sero agglutination titer following the injection of certain hemorrhagic septicemia bacterins it would appear that the change in titers noted could have been caused by normal fluctuations rather than the stimulation by the bacterins.

Summary

Twenty-three Brucella vaccinated and nonvaccinated animals were injected with seven different hemorrhagic septicemia bacterins and antiserums.

Brucella sero agglutination titers were determined prior to, at the time of, and at three to six day intervals following the injection of the biologicals.

Considerable fluctuations of Brucella sero agglutination titer was noted in most animals.

In only four animals did the titer rise sufficiently to cause their reclassification. Three of the four reclassified animals returned to the former classification status within 30 days following injection of the bacterins.

Results obtained in this experiment indicate that
certain bacterins may stimulate the production of Brucella sero agglutinins in animals previously sensitized with Brucella organisms. However, it has been proven that sero agglutination titers of Brucella vaccinated and Brucella infected animals appear to fluctuate from time to time. Additional work is needed to determine whether or not this change in titer was caused by the influence of the antigen or no more than usually found in the normal fluctuating titers of Brucella vaccinated and infected individual animals.

The Survival of Br. abortus, U.S.D.A. Strain 2308, in Manure Pit Studies

Bang (2) in 1897, proved that Br. abortus was incriminated in causing contagious abortion of cattle. Since then considerable investigational work has been done on the disease. We now know, among other things, that the organism is eliminated from the vagina and thus may contaminate fecal material and bedding. Methods have been developed by which the disease may be controlled.

Those working with the disease are frequently concerned with the problem of the disposal of manure and other wastes from Brucella infected animals. This is particularly important where disease free animals, used in production studies, are maintained on the same farm. Investigators are frequently asked how long the manure from infected animals must be held in manure pits before it is safe to be spread
on pastures to be used by disease free animals.

This work was undertaken to determine (a) the temperatures within a manure pit where wastes from Brucella infected animals was stored, and (b) the effect of these temperatures on the survival time of Br. abortus strain 2308 under these conditions.

Materials and Methods

The manure pit used in these studies is 26 x 19 x 6 feet. Three sides, part of the roof and the floor are of concrete construction. One side and part of the roof were constructed of removable doors to facilitate the emptying of the pit by machinery (Figure 11). A concrete 10-inch wall divides the pit in half. Small wooden lids in the roof of the pit were removed at the time of adding wastes to the pit (Figure 12). The pit was entirely closed except during the filling and emptying operations.

Wastes including feces, urine and bedding (usually straw) from 15 adult cattle, was added to this pit during the periods of observations. The cattle were housed in individual stalls in an adjacent cattle isolation building. The quarters were cleaned and new bedding added daily except on Sundays.

The temperatures within the manure pit were determined by securing a laboratory grade, armored thermometer (graduated -15° to 105° C. in 1° divisions) to a six foot pole and then the pole was lowered to the desired depth in
Figure 11. A Photograph Showing the Construction of the Manure Pit
Figure 12. A Photograph Showing the Doors on the Top of the Manure Pit
the pit. Readings were made at least once and sometimes twice daily. Readings were usually taken approximately one foot from the bottom of the pit where the manure was the deepest. Readings were also made at the same depth inside the pit but against the outside wall. A few readings were taken on the surface of the manure.

The mean weather temperatures were taken from the monthly weather reports prepared by Mr. J. H. Wilson, Ohio Agricultural Experiment Station's weather observer.

*Br. abortus*, strain 2308, was used throughout the following survival investigations. This strain does not require increased carbon dioxide tension for growth and is widely used in the United States as a challenge culture to evaluate the efficacy of various Brucella vaccines.

The organism was grown on tryptose agar slants and then transferred to tryptose broth, and incubated at 37° C. for 48 hours. The number of cells was not determined. Viability was determined by transferring portions of these inoculated materials to tryptose agar plates. One ml. of inoculum was dispersed over the surface of the medium with a sterile glass spreader. All plates were incubated aerobically at 37° C. for 14 days before final determinations were made. The colonies were identified as Brucella on the basis of colony morphology and saline suspensions of the selected cultures were used as the antigen in agglutination tests with known positive and
negative *Br. abortus* serums.

**Broth Cultures**

Ten cc. of a 48-hour broth culture of *Br. abortus*, strain 2308, was added to each of two sterile tubes. The tubes were wrapped in foil and one placed in the manure pit by securing it to a wooden pole and driving it into the deepest part of the manure pile. The remaining tube was incubated at 37° C. for the period of observation.

**Manure and Broth Cultures**

Approximately 15 grams of feces of normal consistency and 10 cc. of saline were mixed, divided equally into two test tubes. The tubes were plugged with cotton and then thoroughly sterilized by autoclaving. Ten cc. of a 48-hour broth culture of *Br. abortus*, strain 2308, was added to each tube and thoroughly mixed with the contents. The tubes were wrapped in foil and one placed in the manure pit and the other incubated at 37° C. for the period of observation.

**Results**

The following tables illustrate the results from each group:
Kept in Manure Pit
(near bottom) Temperature 158° F.

<table>
<thead>
<tr>
<th>Number of hours</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth culture</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Duplicate Culture in Incubator

<table>
<thead>
<tr>
<th>Number of hours</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth culture</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Kept in Manure Pit
(near bottom) Temperature 158° F.

<table>
<thead>
<tr>
<th>Number of hours</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure and Broth culture</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>C</td>
</tr>
</tbody>
</table>

Duplicate Culture in Incubator

| Manure and Broth culture | + | + | + | + |

C = contaminated  + = Brucella growth  - = No Brucella growth

Broth cultures of *Br. abortus*, strain 2308, were killed in less than four hours when placed near the bottom of the manure pit. When a similar broth culture was mixed with sterilized manure and placed in the same lo-
cation of the pit, Brucella could not be cultured from
the mixture after a four-hour period. Duplicate cul-
tures placed in the incubator at 37° C. were viable for
at least an 18-hour period. Cultures placed near the
top of the manure mass survived for at least 48 hours.

The temperatures within the manure pit and the mean
weather temperatures for a comparable period are shown
in Figures 13 and 14. Figure 13 shows the temperatures
recorded for an observation period during the summer
months of August-September, while Figure 14 shows the
readings for a similar period during the winter months
of December-January. Observations were started at the
time of, or soon after, the pit had been emptied and
refilling began. Usually the manure and wastes from the
15 cattle filled one side of the pit in approximately
four weeks. Observations were made during this filling
period and continued for a two-week period after the pit
had been filled.

The maximum pit temperature recorded during the sum-
mer period was approximately 170° F. The maximum for the
winter period was found to be approximately 158° F. These
maximum temperatures were reached usually seven to ten days
after the filling process began. The temperatures remained
near the maximum level for about a week and then slowly de-
clined until at the time of emptying, the recordings were
found to be approximately 20 to 25 degrees lower than the
Figure 13. Manure Pit and Mean Weather Temperatures for a Summer Observation Period
Figure 14. Manure Pit and Mean Weather Temperatures for a Winter Observation Period
maxima.

As was expected, temperatures near the outside walls and in corners were somewhat lower than in the center of the mass. The maximum readings in the corners and near outside walls ranged from 132° to 144° F. The surface temperatures ranged from 113° to 120° F.

Discussion

It is recognized that in this work natural conditions have not been duplicated. A factor which one should consider in the duration of viability of Br. abortus is the presence of other organisms and their products. Common saprophytic and pathogenic bacteria, molds, and yeasts probably have a deleterious effect on this organism. It would seem reasonable to assume that, since these factors did not enter into the work, the organism probably would not have lived any longer when subjected to the corresponding influences under more natural conditions. Although the organism was killed within four hours when placed near the bottom of the pit it did survive for at least 48 hours when placed on top of the manure mass.

While Cameron (36) found that Br. abortus remained viable in feces under experimental conditions for over 100 days, it is extremely doubtful if it would survive that long under natural conditions, whether on top of a manure pit or spread on pasture. In the pit there would
be rotting and fermentation while in the pasture the sunlight would exercise rapid germicidal action.

It is an important fact that the organism remains viable only for a relatively short time in urine. If we assume that feces and urine are excreted in the proportion of about four to one in weight, then there would probably be a reduction in the duration of viability of the organism in mixed feces and urine. Neither the chemical analysis nor the pH of the manure was determined in this experiment.

Additional and more detailed studies of this nature are needed because of the increased expansion of facilities for carrying on animal disease research of this nature.

Summary

The manure and other wastes from 15 adult cattle housed in individual box stalls filled a manure pit (13' x 9' x 6') in approximately four weeks.

Temperatures within the pit were recorded during the filling period and for at least two weeks thereafter. The maximum pit temperature recorded during a summer period was approximately 170° F. while the maximum observed during a comparable period during the winter months was 158° F. These maximum readings were usually reached seven to ten days after the filling process began. The temperatures
remained near the maximum level for about a week then slowly declined until at the time of emptying the recordings were found to be approximately 20 to 25 degrees lower than the maxima.

Broth cultures of Br. abortus, strain 2308, and a similar culture mixed with sterilized manure, were found to be non-viable when examined four hours after being placed in the pit near the recording thermometer.

The Treatment of Brucella abortus Infection in the Bovine

With the discovery of each disease, a satisfactory method of therapy is always sought. A satisfactory and practical treatment for brucellosis in cattle remains to be developed. It is realized that even though an effective therapy is developed it probably would not be the answer to the control and eradication of bovine Br. abortus infection. Effective bovine brucellosis therapy can only be an aid in the overall control and eradication program.

Recent advances in the treatment of human brucellosis have stimulated new interest in the bovine infection in the field of veterinary medicine. In addition, the development of the sulfonamides and the various antibiotics has generated new impetus to the difficult problem of brucellosis therapy in cattle.

Brucellosis therapy in cattle is beset with numerous problems. Initially a chemical, drug, biological agent,
or antibiotic, either alone or in combination, should be nontoxic to the host at the effective therapeutic dosage. It is essential that a satisfactory margin of safety exist between the therapeutic and toxic dosage level. Secondly, after an effective therapeutic agent is found, it is necessary that some practical method be developed for determining when a treated animal is completely free of the infective agent. Thirdly, Br. abortus infection in the bovine animal exists as a more or less chronic infection with localized tissue areas harboring the organisms, and not as an acute bacteriemia as is thought to be the case in the infected human patient. Usually, acute infections respond more readily to therapy than do chronic infections.

Materials and Methods

The present study was undertaken in an attempt to determine the value of (a) sodium sulfadiazine administered intravenously and orally, accompanied by bovine blood plasma transfusions, accompanied by colloidal manganese intravenously; (b) sodium sulfadiazine administered intravenously and orally, accompanied by calcium chloride complex streptomycin intramuscularly; and (c) aureomycin administered intravenously, accompanied by calcium chloride complex streptomycin intravenously. The treatments were administered to mature cattle experimentally infected with Br. abortus B.A.I. strain No. 2308, and to animals naturally
infected. The presence of the disease in the artificially and naturally infected cows both before and following treatments was based upon actual isolation of the organism from the milk, vaginal secretions, or placenta, and positive blood serum agglutination.

The infected cows described in group 1, 2, and 3 were selected from two naturally infected private herds. Brucella infection had been present in these herds for slightly more than one year. Animals described in group 4 were selected from an artificially infected group with Br. abortus BAI strain No. 2308. Milk from these 20 agglutinin positive animals was cultured, and 16 were selected on the basis of their consistent shedding of Brucella organisms.

The various groups were treated as follows:

Group 1 (table 15)—Cows 1, 5, 7, and 9 received 1,000 ml. of bovine plasma intravenously to which had been added 10 Gm. of sodium sulfadiazine. All animals in this group were given sulfadiazine orally every twelve hours for four days at the rate of approximately 1 gr. per pound of body weight per day. Bovine plasma was prepared from blood of animals negative to the Brucella agglutination test.

Group 2 (table 16)—Colloidal manganese (Colmetanese\*) was administered intravenously to each of 4 animals at the rate of 125 ml. per day for ten days. Animals 4 and 8

\*Colmetanese, Farnsworth Laboratories, Chicago 13, Illinois
Table 15. Effect of Sulfadiazine and Blood Plasma Transfusions on *Brucella abortus* Infection in Dairy Cattle

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Post-treatment</th>
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<th>16 Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave. blood titer</td>
<td>Milk Culture</td>
<td>Ave. blood titer</td>
<td>Milk culture</td>
</tr>
<tr>
<td>Cow No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25,600</td>
<td>+</td>
<td>12,800</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>a. Plasma</td>
<td></td>
<td>6,400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Sulfadiazine</td>
<td></td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25,600</td>
<td>+</td>
<td>12,800</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>a. Plasma</td>
<td></td>
<td>12,800</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>b. Sulfadiazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>25,600</td>
<td>+</td>
<td>12,800</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>a. Plasma</td>
<td></td>
<td>6,400</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>b. Sulfadiazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12,800</td>
<td>+</td>
<td>12,800</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>a. Plasma</td>
<td></td>
<td>3,200</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>b. Sulfadiazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>+</td>
<td>200</td>
<td>I</td>
</tr>
</tbody>
</table>

I = incomplete agglutination; + = *Br. abortus* isolated from milk; - = unable to isolate *Br. abortus* from milk.
Table 16. Effect of Sulfadiazine and Colmetanese on *Brucella abortus* Infection

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Ave. blood titer</th>
<th>Milk culture</th>
<th>Pretreatment</th>
<th>Ave. blood titer</th>
<th>Milk culture</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I 400</td>
<td>+</td>
<td>a. Colmetanese</td>
<td>I 800</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12,800</td>
<td>+</td>
<td>a. Colmetanese</td>
<td>I 12,800</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I 12,800</td>
<td>+</td>
<td>b. Sulfadiazine</td>
<td>I 12,800</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I 12,800</td>
<td>+</td>
<td>a. Colmetanese</td>
<td>I 12,800</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I 6,400</td>
<td>+</td>
<td>b. Sulfadiazine</td>
<td>I 25,600</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* = incomplete agglutination;  + = *Br. abortus* isolated from milk
received in addition to the Colmetanese, sulfadiazine orally by balling gun, at the rate of approximately 0.5 gr. per pound of body weight every twelve hours for eight days.

Group 3 (table 17)—Streptomycin was administered intramuscularly to each of the 3 animals in this group. The dosage rate was 5 mg. per pound of body weight every six hours for 72 hours. Animal 361 also received intramammary infusions of 0.5 Gm. of streptomycin per quarter per day for three days. Animals 49 and 361 received, in addition to streptomycin, sulfadiazine orally at the rate of 0.5 gr. per pound of body weight every twelve hours for four days.

Group 4 (table 18)—Aureomycin was administered intravenously to each animal of this group. The dosage was 5 mg. per pound of body weight per day, and administered every twelve hours. The treatment was continued for five days in all animals except cow 1, which developed a very severe phlebitis, and treatment was discontinued the third day. In addition to the above treatment, cow 20 received intramammary infusions of 200 mg. of aureomycin per quarter per day for three days. Cows 11 and 537 received, in addition to aureomycin, streptomycin intramuscularly at the rate of 5 mg. per pound of body weight per day for six days. Streptomycin was given every twelve hours.
Table 17. Effect of Sulfadiazine and Streptomycin on Brucella abortus Infection in Dairy Cattle

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 Days after treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ave. blood culture</td>
</tr>
<tr>
<td>Cow No.</td>
<td>Ave. blood titer</td>
<td>Milk titer</td>
</tr>
<tr>
<td>49</td>
<td>I 51,200 +</td>
<td>a. Streptomycin \n b. Sulfadiazine</td>
</tr>
<tr>
<td>361</td>
<td>I 51,200 +</td>
<td>a. Streptomycin intramuscularly \n b. Streptomycin intramammary \n c. Sulfadiazine</td>
</tr>
<tr>
<td>4</td>
<td>I 25,600 +</td>
<td>a. Streptomycin</td>
</tr>
</tbody>
</table>

I = incomplete agglutination; + = Br. abortus isolated from milk; - = unable to isolate Br. abortus from milk
Table 18. Effect of Aureomycin and Streptomycin on 
Brucella abortus Infection in Dairy Cattle

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Ave. blood titer</th>
<th>Milk culture</th>
<th>Treatment</th>
<th>Ave. blood titer</th>
<th>Milk culture</th>
</tr>
</thead>
</table>
| 1       | 3,200           | +            | a. Aureomycin*  
b. Streptomycin   | I 3,200      | +            |
| 11      | 12,800          | +            | a. Aureomycin  
b. Streptomycin   | I 12,800      | +            |
| 20      | 6,400           | +            | a. Aureomycin intramammary infusion | I 12,800 | + |
| 537     | 6,400           | +            | a. Aureomycin  
b. Streptomycin   | I 6,400      | +            |

I = incomplete agglutination; + = Br. abortus from milk

*Aureomycin discontinued at end of third day
Blood serum agglutination tests and milk cultures were made before, during and following treatment at frequent intervals throughout the experiments. In addition to the above observations, free sulfonamide, streptomycin, and aureomycin blood levels were determined at frequent intervals.

Results

The results of the experiments are shown in tables 15, 16, 17, and 18.

In all cases, except for two animals which received Colmetanese alone, there was an apparent cessation of the shedding of Brucella organisms from approximately the third day of treatment to three to ten days following treatment.

In all treated animals, except cow 1 and cow 10 in group 1, recurrent infection was noted. The necessity of disposal of all animals in group 1, sixteen days after treatment, did not permit further examination of the two nonshedders. However, it would appear probable that, had examinations been made at later periods, organisms would have been recovered from these 2 animals also.

Only slight variation in the blood serum agglutinin titers was noted when pretreatment and post-treatment titers were compared. The titers were about the same as in nontreated infected animals.
Free sulfadiazine in cattle to which the drug had been administered by balling gun varied from 1.0 to 3.0 mg. per 100 ml. in milk and ranged from 2.0 to 9.3 mg. per 100 ml. of blood serum. Blood levels of streptomycin in cattle treated intramuscularly varied from 12 to 66 units per milliliter, and aureomycin levels ranged from 0.2 microgram to 1.6 microgram per milliliter of blood serum.

Discussion

Sulfadiazine, streptomycin, aureomycin, and various combinations of these therapeutic agents caused a temporary cessation of shedding of Brucella organisms in the milk of treated animals. However, recurrent shedding occurred in 14 or 16 animals treated. From the two animals in which infection had no recurrence, milk and postmortem specimens were only available for sixteen days of observation.

The data seem to pose the question of just where the bacteriostatic effect of the drugs used takes place. It still remains to be established whether the bacteriostatic effect of the drug takes place in vivo or in vitro.

Summary

1) Blood plasma, sulfadiazine, streptomycin, Colmetanese, and aureomycin, singly or in combination, were administered to 16 cattle shedding Brucella organisms in
their milk.

2) The use of the therapeutic agents, singly or in combination, apparently caused a temporary cessation in the shedding of Brucella organisms in most of the animals treated. Recurrent infection was noted in all animals in which studies were completed; however, the two animals in which infection did not recur were under observation for only a sixteen-day period.

3) The use of therapeutic agents, singly or in combination, had no apparent effect on the blood agglutination titers of the animals treated.
GENERAL SUMMARY AND CONCLUSIONS

Brucella M vaccine was injected subcutaneously into blood agglutination test negative cows and heifers located in 12 privately owned dairy herds to determine its value as an immunizing agent against brucellosis.

Of the 487 animals that were negative to the seroagglutination test at the time of the injection of the vaccine, 23.4 percent became reactors and 8.8 percent aborted or calved prematurely during the subsequent 24-month observation period. During the same period, 36.9 percent of the 60 nonvaccinated controls became reactors and 27.4 percent aborted or calved prematurely. Under the conditions of this experiment, the data show that the use of Brucella M vaccine produced a certain degree of resistance to a field exposure of virulent Brucella organisms.

The results obtained in the experiment in which live Brucella strain 19 vaccine was used as a means for differentiating vaccinal from infection seroagglutination titers indicated that the test was neither practical nor accurate enough to assist materially in the present brucellosis control program.

Paper-strip electrophoresis, using an experimental type horizontal cell, was found to be a reliable method for the characterization of the proteins of bovine serum.
The data show that certain fundamental differences occurred in the serum globulins between the Brucella strain 19 vaccinates and the Brucella infected animals. The gamma globulin values of the infected group ranged from 51 to 65.3 percent, while the vaccinated groups exhibited values of 36.7 to 52.1 percent. The fact that the differences between the two groups was so clear cut indicated the feasibility of using this method as a possible differential test. The data further suggests the need for additional work involving greater numbers of animals.

Considerable fluctuation of Brucella sero agglutination titer was noted when 23 Brucella vaccinated and nonvaccinated cattle were injected with several types of hemorrhagic septicemia biologicals. The titer-rise was sufficient to cause the reclassification of four animals. Three of the four reclassified animals returned to the former negative status within 30 days following the injection of the bacterins. Under the conditions of this experiment, it appeared that the injection of the antigens may have influenced the concentration of sero agglutinins. However, these data seem to pose the question of whether the change in titer was due, in some way, to the injection of the antigens or whether the change was no more than usually found in the fluctuation of titers of Brucella vaccinated and infected individual animals.
The manure and other wastes from 15 adult cattle housed in individual box stalls filled a manure pit (13' x 9' x 6') in approximately four weeks. Temperatures were recorded during the filling period and for at least two weeks thereafter. The maximum pit temperature recorded during a summer period of observation was approximately 170° F. while the maximum observed during a comparable period during the winter months was 158° F. These maximum readings were usually reached seven to ten days after the filling process began. The temperatures remained near the maximum levels for about a week, then slowly declined until at the time of emptying, the recordings were found to be approximately 20 to 25 degrees lower than the maxima.

Broth cultures of *B. abortus*, strain 2308, were found to be nonviable when examined four hours after being placed in the pit near the recording thermometer.

The use of various therapeutic agents, singly or in combination, apparently caused a temporary cessation in the shedding of *Brucella* organisms in most animals treated. However, recurrent infection was noted in all animals in which studies were completed.

The use of the therapeutic agents, singly or in combination, had no apparent effect on the blood agglutination titers of the animals treated.
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I, Nelson Byron King, was born near Williamsburg, Ohio, August 14, 1914. I received my secondary education in the public schools of Williamsburg, Ohio, and my undergraduate training at Ohio State University, which granted me the Bachelor of Science in Agriculture degree in 1941. I received the Degree Doctor of Veterinary Medicine from Ohio State University in 1948. While in residence at the University from 1944 to 1948, I was employed as a Research Engineer at Battelle Memorial Institute, Columbus, Ohio. Following graduation in March, 1948, I was appointed an Instructor in the Department of Veterinary Science of the Ohio Agricultural Experiment Station, a position I now hold. While employed by the Station I was granted the Degree Master of Science from Ohio State University in 1950. Following graduation in 1950, I have continued my work in animal disease research while completing the requirements for the Degree Doctor of Philosophy.