THE REACTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL BRUCELLOSIS OF DOGS *

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The experimental studies reported in this paper relate to the general problem of the reaction of mammalian tissues to infection by *Brucella suis*, but with special emphasis upon the response of the cells belonging to the reticulo-endothelial system. This investigation was made as a part of a more comprehensive program, the main purpose of which is to test the possibility of an etiological relation between brucella infection in man and Hodgkin's disease. In previously published papers we have reported studies on experimental brucellosis in a variety of animals, including guinea-pigs,[1] hogs,[2, 3] dogs,[3] and on naturally acquired brucellosis in man,[4, 5] all of which relate to this problem. In all of the experimental studies the strains of brucella employed were derived from cases of typical Hodgkin's disease; a strain of brucella derived from a naturally infected hog was used for the production of comparative infection. In this paper are described in some detail the pathological anatomical findings in a group of dogs in which infection by a strain of *Br. suis* had been maintained for as long as 487 days by repeated inoculations. The bacteriological and immunological observations made in this experiment will be dealt with only in brief; they have been reported in full in a previous communication.[3]

The literature dealing with brucellosis in dogs, both the naturally acquired and the experimental disease, is quite limited; this has been reviewed in one of our earlier communications.[3] At this time only a brief note relating to the previously reported pathological anatomical findings is pertinent. It has been observed that the development of anatomical lesions in both the naturally acquired and the experimental infection is quite unusual; even so, in the hands of practically all experimenters, there appears to have been no great difficulty in establishing infection in the dog as indicated by a significant rise in the agglutination titer for brucella and the recovery of the organisms either from the tissues at autopsy or from the blood during life. The rare instances in which anatomical lesions have been described in brucellosis in dogs and the lesions described were: (a) enlargement and suppuration of the testis (Plantz and Huddleson,[6] Davis [7]), (b) multiple yellowish nodules in the kidney (Thomsen [8]), (c) enlargement of the reticulo-

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endothelial tissues, especially the lymph nodes and the spleen (van der Hoeden [9]), (d) focal, histiocytic, tubercle-like reactions in the liver, spleen, kidneys, and lungs (Feldman, Bollman, and Olson [10]).

Of the descriptions just mentioned, that of Feldman, Bollman, and Olson [10] is most informative. The lesions they describe were found in dogs experimentally infected with organisms obtained from swine and bovines. The dogs were inoculated by mouth and intravenously, only one inoculation being made. The total duration of the experiment was 185 days. The histological descriptions of the lesion by Feldman, Bollman, and Olson, unlike those of the other contributors, are quite comprehensive, and they indicate that the basic reaction in the dog is that which involves a response of the cells of the reticulo-endothelial system. In all of the studies that have been made, the conclusion has been reached that the dog has but little susceptibility to injury by brucella and that whatever lesions may be produced are minimal and transient. In view of this conclusion and in view of our primary objective, that is, to produce in the dog, if possible, a disease resembling Hodgkin's disease in man, it was decided to use, in our experiments, procedures that were significantly different from those utilized by preceding workers. Accordingly, in the experiments, reports of which follow, repeated inoculations were made by different routes and at considerable intervals of time, and the experiments were continued for as long as 300 days in excess of the experimental period in the investigations of Feldman, Bollman, and Olson.

Experiments

Nine healthy dogs were employed in our experiment. Seven of these were used for a study of infection by a strain of brucella obtained from a typical case of Hodgkin's disease in an adolescent boy who had died of the disease after an illness of over 5 years. The strain of brucella recovered from this case (herein referred to as the Brody strain) was of the suis variety. Previously it had been shown to be pathogenic for the guinea-pig, and it had been used in other experiments carried out on the hog; this organism was known to be virulent for the guinea-pig at the time the experiments on the dog were begun. Two of the dogs were used for a comparative study of infection by a strain of *Br. suis* which had been recovered from a natural infection in a hog (herein referred to as strain ABF 36). This organism was known to be pathogenic for the hog and the guinea-pig at the time the dog experiments were begun.

Preliminary studies involving the determination of the agglutination titer for brucella 456, the opsonocytophagic index, and cultures of the blood for brucella were carried out on all of the experimental animals.

Each animal was kept in its own cage from which it was never re-

moved except for inoculation. All animals were kept in a room in which there were also other animals infected by the organisms being employed in the experiment. Special care was exercised in the handling of all animals in this group to prevent cross infection.

Four of the animals inoculated with the Brody strain of brucella received these organisms intravenously. Three of the animals receiving the Brody strain were inoculated intraperitoneally. One of the animals receiving the ABF 36 strain of brucella was inoculated intraperitoneally

TABLE I

Experimental Brucellosis in Dogs: Experimental Data

Dog	Length of experiment in days	Organism and route	Injections	Blood cultures	Positive blood cultures	Days before death of:		Organ cultures at autopsy					
						Last injection	Last positive blood culture	Nodes	Spleen	Liver	Kidney	Testis	Lung
I	198 died	Brody I. V.	21	27	12	14	7	+	+	+	+	−	−
II	487 killed	Brody I. V.	35	42	18	225	233	+	−	−	+	−	o
III	461 killed	Brody I. P.	39	41	4	143	270	−	−	−	−	o	o
IV	261 died	Brody I. V.	28	34	23	7	7	o	+	+	+	+	−
V	38 died	Brody I. V.	4	5	3	8	1	+	+	o	o	o	o
VI	454 killed	Brody I. P.	39	43	o	136		−	−	−	−	−	o
VII	398 killed	Brody I. P.	33	34	8	132	231	−	−	−	−	o	−
IX	186 killed	ABF 36 I. P.	3	5	1	152	152	+	−	−	−	−	o
X	216 killed	ABF 36 I. V.	3	5	3	182	101	+	−	−	−	o	o

and the other intravenously. The inoculations were given repeatedly, usually at intervals of about 1 week. In certain instances the inoculations were given in two series, the second series being started after the animal had been allowed to recover sufficiently from the preceding inoculations to assure prolongation of the experiment.

All of the dogs were bled from the jugular vein at frequent intervals and always preceding inoculation. Blood cultures were made and brucella agglutination titers and opsonocytophagic indices were determined at each bleeding. The essential data relating to the experiment are recorded in Table I. The bacteriological and immunological observations made during the course of this experiment are the subject

of a previous communication in which all of the details may be found.[3] Protocols in summary for each of the experimental animals follow.

Dog I. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog I, a male mongrel, weighed 9.3 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 37; agglutination for brucella 456, negative. The animal died 198 days after the first injection.

Dog I received 21 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 14 injections were given at weekly intervals; from that time injections were given irregularly, at an average of bi-monthly intervals. The last injection was given 14 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.8° and 40° C. Opsonocytophagic index varied widely during the experiment. From an initial 37 it rose gradually to 91.5, then varied between 56 and 80.5. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week and varied from 1:2560 to 1:20480 during the experiment. Brucella was recovered from the blood stream after the first injection, and a total of 12 times in 27 weekly cultures. The positive results were obtained from cultures made 1 week after injections; blood cultures at intervals of 2 or more weeks following inoculations were always negative.

During the first 11 weeks of the experiment the dog appeared well. Then, with continued weekly injections he developed anorexia, rapidly lost weight, and at 14 weeks appeared moribund. At this time multiple shallow ulcers appeared in his mouth. These did not resemble the lesions of black tongue and did not show a characteristic response to adequate parenteral doses of nicotinic acid. Brucella was isolated from the ulcers 15 days after the last previous injection of organisms. When inoculations were discontinued the ulcers slowly healed and the general condition of the animal improved. When injections were started again and given at shorter intervals, the dog seemed to hold his own for a while, but then again developed anorexia, became very weak and cachectic, and at death presented a picture of extreme emaciation. No enlargement of the peripheral lymph nodes was observed during the experiment.

Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog II. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog II, a male mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 50; agglutination for brucella 456, negative. The animal was killed 487 days after the first injection.

Dog II received 35 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The inoculations were given at almost weekly intervals. The animal was then kept for the duration of the experiment without further injections. The last inoculation was given 225 days before death.

Temperature taken every 3 days during the first 2 months ranged between 38.4° and 39.8° C. Opsonocytophagic index varied widely during the experiment, from an initial 50 to a low of 35 and a high of 87. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week, and fluctuated from 1:1280 to 1:20480 during the experiment. Brucella was recovered from the blood stream

after the first inoculation, and a total of 18 times in 42 cultures. All of the positive cultures were obtained 1 week after inoculations; blood cultures at intervals of 2 or more weeks following injections were always negative.

During the first 30 weeks of the experiment the dog appeared well except for periods of anorexia and mild weight loss. He then developed signs of illness, including pronounced lassitude, anorexia, weakness, and conspicuous weight loss. After 37 weeks it appeared that the animal would not survive further injections, and the inoculations were discontinued. The dog gradually recovered and remained well for the rest of the experiment. No peripheral lymph node enlargement was noted during the period of observation.

Dog was killed by intracardiac air, and autopsy was performed immediately after death. Findings are summarized in Table II.

Dog III. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog III, a female mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 32; agglutination for brucella 456, negative. The animal was killed 461 days after the first injection.

Dog III received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These were given at weekly intervals except for the last few injections, which were given at bimonthly intervals. The last inoculation was given 143 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.9° and 39.1° C. Opsonocytophagic index fluctuated widely during the experiment. From an initial level of 32 it varied between 25.5 and 83. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and thereafter fluctuated between 1:1280 and 1:20480. Brucella was isolated from the blood 1 week after the first and the second inoculations, but was recovered only on two subsequent occasions in a total of 41 cultures. No positive cultures were obtained later than 1 week following injection.

During the course of the experiment the dog showed no definite signs of illness. No signs of peritonitis were evident. No peripheral lymph node enlargement could be detected during the experiment.

Dog was killed by intracardiac ether (5 cc.). Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog IV. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog IV, a male mongrel, weighed 8.5 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 28; agglutination for brucella 456, negative. The animal died 261 days after the first injection.

Dog IV received 28 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 19 injections were at weekly intervals; thereafter the inoculations were given irregularly, at an average of bimonthly intervals. The last injection was 7 days before death.

Temperature taken every 3 days for the first 2 months ranged between 38° and 39.5° C. Opsonocytophagic index, initially 28, varied between 44.5 and 90 during the experiment, and did not appear to be correlated with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of 1 week and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was isolated from the blood stream 1 week after the first injection, and a total of 16 times in 19 weekly cultures made during the period of weekly injec-

tions. Of 15 cultures made thereafter, 7 were positive. All positive results were obtained from cultures taken 1 week after injections; blood cultures taken at intervals of 2 or more weeks after inoculations were always negative.

During the first 12 weeks of the experiment the dog appeared well. He then developed anorexia, lassitude, weakness, and lost weight; the weekly injections were discontinued after 18 weeks because it appeared that the animal would not survive much longer. The animal improved, and injections were resumed at bimonthly intervals. It appeared that these were being withstood very well, and weekly injections were resumed, but after the fifth of these the dog showed a rapid decline in weight, appetite, and strength, and died presenting a picture of cachexia. No peripheral lymph node enlargement was noted during the experiment.

Autopsy was performed 3 hours after death. Findings are summarized in Table II.

Dog V. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog V, a male mongrel, weighed 9.0 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 20; agglutination for brucella 456, negative. The animal died 38 days after the first injection.

Dog V received 4 intravenous inoculations of brucella (Brody strain), each injection consisting of 10 billion organisms, at about weekly intervals. The last injection was 8 days before death.

Temperature taken every 3 days ranged between 38° and 40.3° C. Opsonocytophagic index, initially 20, varied between 21.5 and 51.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and ranged between 1:2560 and 1:10240. Brucella was recovered from the blood after the first inoculation, and a total of 3 times in 5 subsequent cultures; all positive cultures were obtained at intervals of 1 week after inoculations.

The animal appeared well until the 38th day when there was sudden onset of a shock-like condition and rapid death. Autopsy performed 1 hour after death revealed the stomach to be filled with impacted shavings. Other findings are summarized in Table II.

Dog VI. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog VI, a male mongrel, weighed 8.4 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative. The animal was killed 454 days after the first injection.

Dog VI received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The injections were given at almost weekly intervals over a period of 47 weeks. The last inoculation was 136 days before death.

Temperature taken every 3 days for 2 months ranged between 37.8° and 39° C. The opsonocytophagic index, initially 7, fluctuated widely during the first 6 weeks, then ranged between 53 and 86.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was never recovered from the blood stream in 43 attempts: 38 of the cultures were made at intervals of 1 week following injection; 3 were made at longer intervals; and 2 were taken 1 day following inoculation.

During the entire experiment the dog remained well. At no time was there evidence of peritonitis, and no enlargement of the peripheral nodes occurred.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog VII. Summary of Protocol

When the experiment was begun, on September 30, 1940, dog VII, a male mongrel, weighed 8.6 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 398 days after the first injection.

Dog VII received 33 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These injections were given at almost weekly intervals over a period of 38 weeks. The last injection was 132 days before death.

The opsonocytophagic index rose rapidly from 7 to a level ranging from 41 to 84 during the experiment. Agglutination of brucella 456, initially negative, rose to 1:5120 and varied between 1:1260 and 1:20480 during the experiment. Brucella was isolated from the blood stream 8 times in 34 cultures. The first positive culture was obtained after the eighth inoculation. All positive cultures were obtained after intervals of 1 week following injection.

Throughout the experiment the dog remained well. At no time was there evidence of peritonitis, nor any enlargement of the peripheral lymph nodes.

Dog was killed with intracardiac ether (1 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog IX. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog IX, a male mongrel, weighed 9 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 60; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 186 days after the first injection.

Dog IX received 3 intraperitoneal inoculations of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The inoculations were given at intervals of 10 days. The last injection was given 152 days before death.

Opsonocytophagic index varied from 58.5 to 75. Agglutination of brucella 456, initially negative, rose to 1:2560 after 1 week and varied between this dilution and 1:10240. Brucella was recovered from the blood stream once in 5 cultures. The cultures were made from 2 to 11 weeks following inoculation. The positive culture occurred 3 weeks following the second injection.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog X. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog X, a female chow, weighed 8 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 13.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 216 days after the first injection.

Dog X received 3 intravenous injections of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The injections were given at intervals of 10 days. The last injection was given 182 days before death.

Opsonocytophagic index rose from 13.5 to 42 at the end of 1 week and varied between that figure and 71. Agglutination of brucella 456, initially negative, rose to 1:10240 after 1 week, and then remained at 1:20480. Brucella was recovered from the blood stream 3 times in 5 cultures made from 2 to 11 weeks following inoculation. The first positive culture was obtained 2 weeks following the first inoculation; other positive cultures were obtained 3 and 7 weeks following the last inoculation.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac air; autopsy was performed immediately afterwards. Findings are summarized in Table II.

Experimental Results

A. Clinical Observations

From the protocols of the respective animals it is clear that a serious infection by brucella occurred in all four of the dogs inoculated intravenously with the Brody strain of organism. The infection was fatal in two of the four and doubtless would have been in a third had the intravenous inoculation been continued following the development of the profound intoxication that occurred in all of the animals in this group. The fourth intravenously inoculated animal in this group died early in the experiment from an acute intoxication produced by the ingestion of a mass of pine shavings that were being used for bedding. All of the animals of the group suffered from anorexia, pronounced loss of weight, great weakness and lassitude, and, finally, in the fatal cases, coma and death. Although the infection established in these dogs provoked the extraordinary clinical signs mentioned, it is evident from the experience with dogs II and IV that the dog has a remarkable resistance to infection by brucella and is capable of a quick and successful recovery from the acute manifestations of the disease when the inoculations are discontinued.

In spite of this great resistance to the infection, however, the organisms tend to persist in the tissues of the animal, particularly in the reticulo-endothelial system, where, as will be described shortly, they continue to provoke a morphological reaction. This is illustrated strikingly in dog II. Although this animal received the extraordinary number of 35 intravenous inoculations, the animal lived for 487 days and at autopsy was found to harbor brucella in the kidney and the lymph nodes. (No organisms had been received by this animal during the last 225 days of its life.) That the dog which has received a series of inoculations intravenously and has developed definite clinical disease is not protected against further inoculations after a rest period of considerable time is shown clearly in the experience with animal IV. This dog died following the resumption of inoculations with signs and symptoms similar to those which it had previously experienced. The disease of which these animals suffered and from which some of them died was a profound bacterial intoxication, as will be confirmed by the pathological anatomical findings to be recorded presently. This intoxication was not always accompanied by bacteremia. In fact, the

bacterial observations on these dogs showed that the dog is able to clear the blood of the inoculated organism within a relatively short time, usually within 3 weeks following the inoculation. After this time the organisms were found only in the tissues.

Judging from our experience with intraperitoneal inoculation of the dog, this route of infection is ineffective in establishing the clinical disease regardless of the number of inoculations administered. Nevertheless, blood cultures following intraperitoneal inoculations were positive on a number of occasions in two of the dogs so inoculated. In these animals the agglutination titers and the opsonocytophagic indices rose significantly, indicating a definite response of the tissues to the organism even though there were no clinical manifestations of disease. Our experience with intraperitoneal inoculation was the same for both strains of organisms employed.

It is worthy of comment that, whereas all the dogs inoculated intravenously with the Brody strain of brucella (an organism recovered from a case of Hodgkin's disease and of relatively low virulence for guinea-pigs) developed profound clinical disease, the one dog inoculated intravenously with the ABF 36 strain of brucella (an organism recovered from a spontaneous infection in a hog and of high virulence for guinea-pigs) developed no clinical evidence of infection. In spite of the absence of clinical disease in this animal, however, the organism was recovered from the lymph nodes at autopsy 182 days after the last inoculation was given. Active, subclinical infection in this animal is definitely indicated by the sustained elevation of the agglutination titer and the opsonocytophagic index, as well as by the demonstration of a positive blood culture 81 days following the last inoculation. The somewhat unexpected reaction of this dog possibly may be explained on the basis of the relatively few inoculations that were given; this animal received only 3 intravenous inoculations. This would seem to indicate that a relatively large number of inoculations, even though made intravenously, is necessary to establish clinical disease in the dog.

All of these clinical observations confirm the impression of previous workers that the dog is highly resistant to infection by brucella. At the same time, however, they indicate clearly that brucella in sufficient quantity is capable of producing even fatal disease in this animal, an observation which does not appear to have been made by previous workers.

B. Anatomical Observations

In the accompanying Table II are summarized all of the gross and histological findings in this group of animals. Of these findings only a few appear to be the result of the action of brucella. Outstanding

TABLE II

Experimental Brucellosis in Dogs: Autopsy Findings

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
I	Endocardial hemorrhage, dilatation	o	o	o	R.E.* hyperplasia pronounced; hemorrhage, iron pigment; plasma cell reaction marked; focal epithelioid reaction	Epithelioid focal granulomata; chronic diffuse glomerulonephritis	o	Severe	198 died	Brody I.V. 21
II	Focal hemorrhage, dilatation	Foreign body; focal bronchial granulomata	Epithelioid granulomata; portal hepatitis	Focal hemorrhage; R. E. hyperplasia; many giant cells of bone marrow type; reticular scarring	Hemorrhage; R.E. hyperplasia	Focal scarring; dilatation of pelvis	o	Severe for 37 wks., clinical recovery after inoculations discontinued	487 killed	Brody I.V. 35
III	Focal hyaline degeneration	o	o	o	R. E. hyperplasia	o	o	o	461 killed	Brody I. P. 39
IV	o	Bronchial pneumonia; parasitic ova	o	o	Hemorrhage; R. E. hyperplasia, moderate	Acute diffuse hemorrhagic glomerulonephritis	Severe gastritis and enteritis	Severe	261 died	Brody I. V. 28

TABLE II (*Continued*)

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
V	o	Confluent lobular pneumonia	o	Epithelioid focal granulomata	R. E. hyperplasia; granulomatous reaction	o	o	None; died from eating bedding	38 died	Brody I. V. 4
VI	Focal hyaline degeneration	o	o	Chronic capsular inflammation with scarring	R. E. hyperplasia, moderate	Focal granulomata	o	o	454 killed	Brody I. P. 39
VII	o	o	o	Capsular scarring; reticular scarring	R. E. hyperplasia pronounced	o	o	o	398 killed	Brody I. P. 33
IX	o	o	o	Reticular scarring; pulp sparse; hyperplasia of malpighian cells	R. E. hyperplasia pronounced; masses of mononuclear cells packing sinuses and replacing lymphatic cords	o	o	o	186 killed	ABF 36 I. P .3
X	o	o	o	o	R. E. hyperplasia with focal epithelioid reaction; iron pigmentation	Focal granulomata	o	o	216 killed	ABF 36 I. V. 3

* R.E. = Reticulo-endothelial.

among the various lesions are the following: (a) dilatation of the heart, (b) focal granulomata in the lymph nodes, liver, and kidney, (c) chronic inflammation and fibrous thickening of the splenic capsule, (d) hemorrhages or hemorrhagic pigmentation of the lymph nodes, (e) acute diffuse glomerulonephritis, (f) pronounced reticulo-endothelial hyperplasia in the lymph nodes, (g) intracellular brucella in the cells forming the sinusoidal reaction of the lymph nodes. Certain of these lesions are discussed briefly.

The most important of these lesions, as they relate to the primary objective of our experiment, is the rather remarkable sinusoidal reaction within the lymph nodes (Figs. 1 to 5). This lesion was found quite uniformly throughout the group of animals even though there was no gross enlargement of the lymphoid tissues. This sinusoidal reaction consisted of the proliferation of a mass of large mononuclear cells accompanied by an accumulation of a great number of polymorphonuclear leukocytes (Fig. 2). The lesion can be described best as a profound reticulo-endothelial reaction in which, as a rule, only the sinusoidal cells participate. However, in some of the nodes so affected a comparable reaction on the part of the reticulum of the lymph nodes occurred. The latter reaction resulted in the development of little foci of large, pale cells of distinctly epithelioid type (Figs. 3 and 4). Although these lesions were the exceptional finding, their presence seemed to leave no doubt that brucella is capable of exciting the growth of both the reticular and the sinusoidal elements of the reticulo-endothelial system. This reticulo-endothelial reaction, although prominent in virtually all of the nodes studied from all of the animals inoculated, is not quantitatively comparable to the reaction of the reticulo-endothelium that takes place in the lymph nodes of the guinea-pig when that animal is inoculated with brucella (Fig. 6); nor is the reaction comparable to that in the hog which we have described in a previous communication.[2] It is important to note that in the cytoplasm of these proliferating sinusoidal cells and the accompanying polymorphonuclear leukocytes, brucella in considerable quantity was demonstrated. This observation was made on the sections from an animal which had been inoculated 8 days before death (Fig. 2). In some of the lymph nodes showing the reticulo-endothelial reaction an extraordinary accumulation of plasma cells was found. These appeared in foci, usually situated outside of the sinuses and in the lymph cords. In lymph nodes showing this reaction the lesion as a whole resembled a genuine granulomatous reaction in its earliest phase of development (Fig. 5). Accompanying the reaction, multinucleated giant cells occasionally were found. These usually were not of the Langhans type. In some instances they resembled

somewhat the multinucleated giant cells seen in Hodgkin's disease. In all of the lymph nodes showing the sinusoidal reaction, variable quantities of fresh blood and iron pigment were found. Many of the sinusoidal cells contained phagocytosed materials of this sort. Accompanying these macrophages, there was always a great accumulation of the large reticulo-endothelial cells showing no evidence of phagocytosis. Although the changes described were pronounced in the lymph nodes in general, in none of these structures was there complete disturbance in the normal architectural relationships.

The little granulomatous foci in the kidney (Fig. 9), the liver (Fig. 10), and the spleen (Figs. 7 and 8) were found in only one or two of the animals. This lesion was especially prominent in the kidneys of one of the animals. Microscopically, the granulomatous focus was a collection of large mononuclear, sometimes epithelioid, cells accompanied by a few polymorphonuclear leukocytes and a few lymphoid cells. In some instances necrosis had occurred in the center of the lesion. No organisms could be demonstrated in any of these lesions. In general, the histological appearance of these little granulomata was identical with that of the foci of reticulo-endothelial hyperplasia found in the lymph cords of the lymph nodes. In view of the similarity of these lesions to those that one finds in brucellosis of the guinea-pig, and in view of the recovery of brucella by culture from nodes showing these lesions, it seems permissible to attribute the renal lesions to the brucella infection even though organisms could not be demonstrated in histological sections.

At this point it is necessary to call attention to the occurrence of focal granulomata of another type in the lungs of one of the dogs. These were quite clearly unrelated to the brucella infection; they were situated about the bronchi and always were associated with foreign bodies.

In two of the dogs the reticular structure of the spleen was particularly dense. This was unassociated with an increase in the number of pulp cells. The interpretation of this condition as a form of reticular scarring seems justifiable, but its relation to the brucella infection is difficult to establish.

The fibrous thickening of the splenic capsule occurred only in the animals inoculated intraperitoneally. Within the capsule at its thickest portion was found a mild chronic inflammatory reaction suggesting that the lesion was originally an inflammatory one (Fig. 8). Thus, it would appear that this lesion represents a brucella effect. No other alterations of the peritoneum that could be related to the inoculations were found.

All of the lesions other than those just discussed we have regarded as incidental findings or as the effects of the general intoxication produced by the long-continued brucella infection. One of these lesions, the diffuse glomerular injury to the kidney, is worthy of brief comment. This occurred in two of the animals and was unrelated to the development of the granulomatous foci. The histological changes found are like those customarily associated with acute and chronic diffuse glomerulonephritis in man. The route of inoculation in both of the animals showing this lesion was the same, that is, intravenous. These lesions are of particular interest in view of the fact that the experimental production of a typical acute diffuse glomerulonephritis by whatever method employed is a difficult accomplishment. The conditions of our experiment were such that one would have expected the development of a focal type of glomerulonephritis, a common form of injury to the kidney in all forms of bacteremia. It should be emphasized that the renal injury found in these two animals is unlike the usual spontaneous nephritis in the dog. All things being considered, it seems justifiable to attribute the nephritis in these animals to the experimental procedures. A discussion of the mechanisms involved in the production of injury to the kidney of the sort seen in these animals is not pertinent to our primary objective in this paper, and so we have dealt with the problem elsewhere.[5]

Comment

In these experiments it seems clear that we have succeeded in producing in the dog an infection by brucella that is recognizable both clinically and anatomically and which, in some instances, has been of sufficient severity to result in the death of the animal. That this has been accomplished only through the utilization of repeated inoculations of the organisms and that the anatomical alterations accompanying the infection are nondestructive and of an extremely mild character seem to indicate clearly that the dog is a highly resistant animal with tissues that are slow to react to infection by brucella. This observation is in harmony with the conclusions of previous workers. In planning our experiment, it had been hoped that the utilization of an animal that is so refractive to infection by brucella might make possible the production of long-standing chronic anatomical alterations in the tissues of the reticulo-endothelial system resembling the changes occurring in Hodgkin's disease. It is obvious that this has not been accomplished. At the same time, it is clear that a chronic infection by brucella does give rise to a pronounced alteration in the character of the lymphoid tissues of the dog. This alteration is the expression of a basic reaction on the part of the reticulo-endothelial cells that *in principle* may

be considered comparable to what occurs in Hodgkin's disease. If brucella is related etiologically to Hodgkin's disease, a possibility which certain recent observations seem to suggest, there must be certain peculiar and highly important factors involved in the relationship that the experimental studies of ourselves and others have not yet disclosed. Theoretically, it appears entirely possible that such factors may exist.

Summary and Conclusions

1. By means of repeated intravenous inoculations of a strain of *Brucella suis* obtained from a case of Hodgkin's disease, a severe, sometimes fatal, form of chronic brucellosis has been produced in dogs. Dogs so affected have been observed for as long as 487 days.

2. It has not been possible to produce clinical disease in dogs by repeated intraperitoneal inoculations of a strain of *Br. suis* obtained from a case of Hodgkin's disease. This was true also when the inoculations were made with a strain of *Br. suis* obtained from a naturally infected hog. Both of these strains of brucella were known to be pathogenic for guinea-pigs.

3. Clinical brucellosis in the dog is characterized by anorexia, loss of weight, weakness, lassitude, and coma. The course of the disease is progressive only so long as the inoculations are continued. The dog is highly resistant to the infection and may recover from the most severe infection if the inoculations are discontinued.

4. Dogs repeatedly inoculated either intravenously or intraperitoneally and without clinical disease may harbor virulent brucella in the tissues of the reticulo-endothelial system for as long as 7 months after the inoculations are discontinued.

5. The most constant anatomical alterations resulting from brucella infection in the dog are found in the lymph nodes. These consist of a pronounced reticulo-endothelial reaction involving both the sinus endothelium and the reticulum cells of the lymphatic cords, without significant enlargement of the nodes. The result of this reaction is the development of focal granulomata of epithelioid character in the lymphatic cords and the formation of great masses of large mononuclear wandering cells which fill and eventually replace the lymphatic channels. Similar focal granulomata of epithelioid character occur occasionally in the kidney, liver, and spleen. A variety of nonspecific lesions occur, including petechial hemorrhages, focal hyaline degeneration of the heart muscle, and acute gastro-enteritis. These appear to be the result of bacterial intoxication.

6. Repeated intravenous inoculations of *Br. suis* produced anatomically typical, acute, diffuse glomerulonephritis in two of four dogs.

7. Prolonged brucella infection in dogs gives rise to a marked pro-

liferative reaction on the part of the reticulo-endothelial system of a granulomatous character, but it does not produce an anatomical alteration of these tissues comparable to that which characterizes human Hodgkin's disease.

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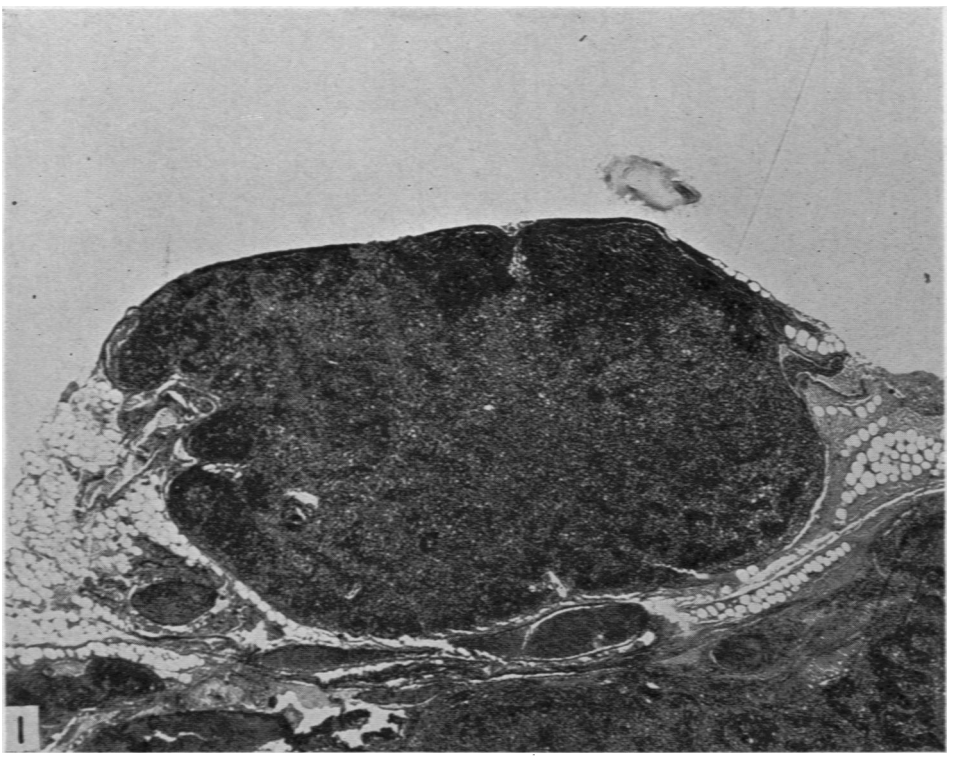
DESCRIPTION OF PLATES

PLATE 127

FIG. 1. Lymph node from dog V. This animal had received 4 intravenous inoculations of *Brucella suis* (Brody strain) which had been obtained originally from a case of Hodgkin's disease of 5 years' duration. This low-power photomicrograph shows a widespread sinusoidal reaction and replacement of the lymphoid cells by new cells. This reaction is typical of all the inoculated animals. Figures 2, 3, and 4 show the cell types involved in the reaction. × 5.

FIG. 2. Early sinusoidal reaction consisting of the accumulation of polymorphonuclear leukocytes and reticulo-endothelial hyperplasia. The lymph node is the same as shown in Figure 1. Only a few of the proliferating reticulo-endothelial cells are active phagocytes. Brucella was demonstrated in the macrophages and the polymorphonuclear leukocytes. × 485.



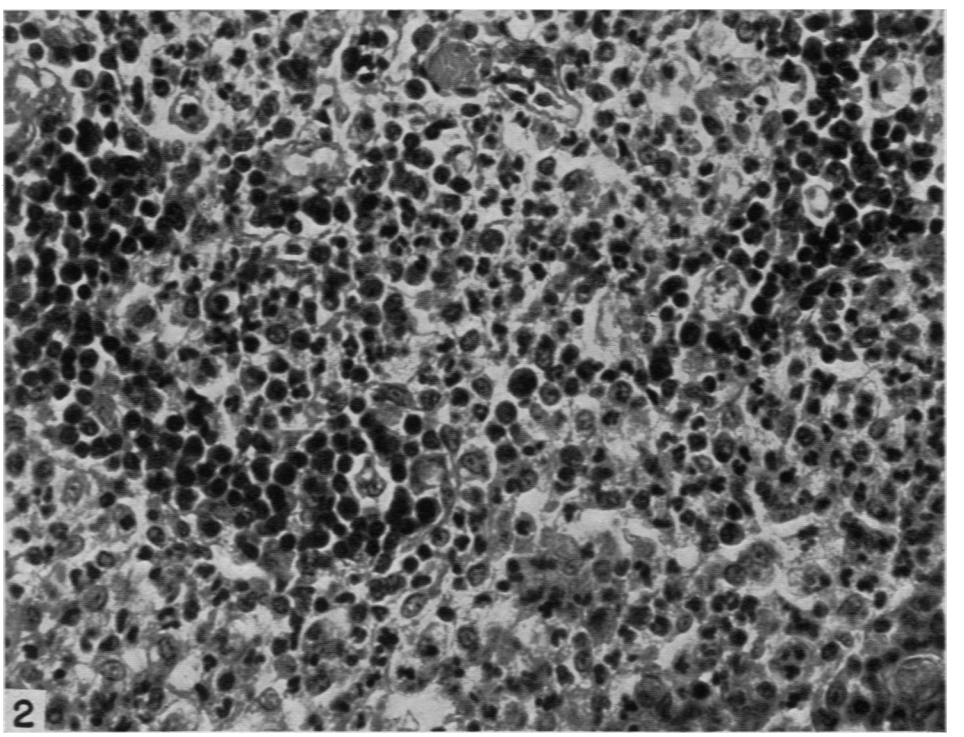


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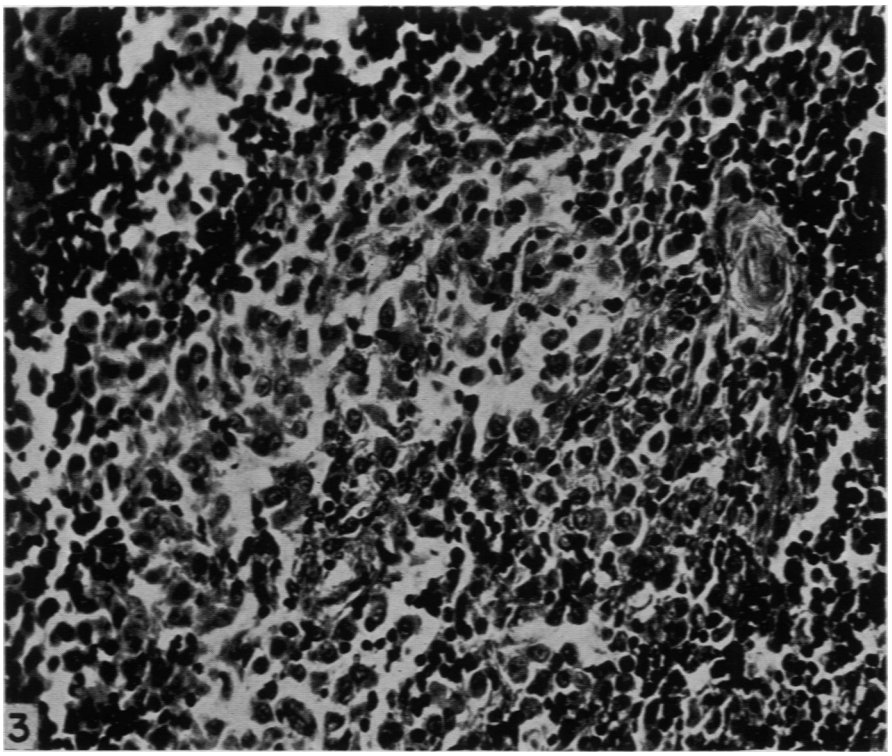
PLATE 128

FIG. 3. Epithelioid transformation of the proliferating reticulo-endothelial cells in a lymph node from dog IX. This animal had received 3 intraperitoneal inoculations of *Br. suis* (ABF 36 strain), originally recovered from a naturally infected hog. The reaction is a genuine granuloma and is identical with that which occurs in the guinea-pig (Fig. 6.) A lower power view of the node from which this photograph was made is shown in Figure 5. × 365.

FIG. 4. Focal proliferation of the reticulum cells of a lymphatic cord accompanied by a marked sinusoidal reaction in a lymph node from dog I. For comparison with the guinea-pig reaction shown in Figure 6.



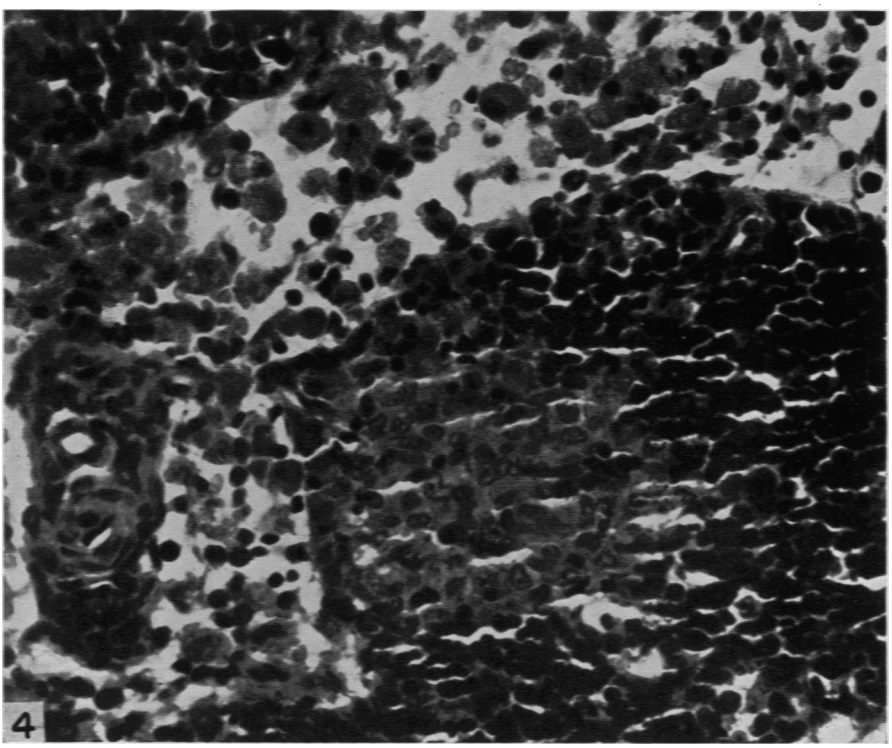


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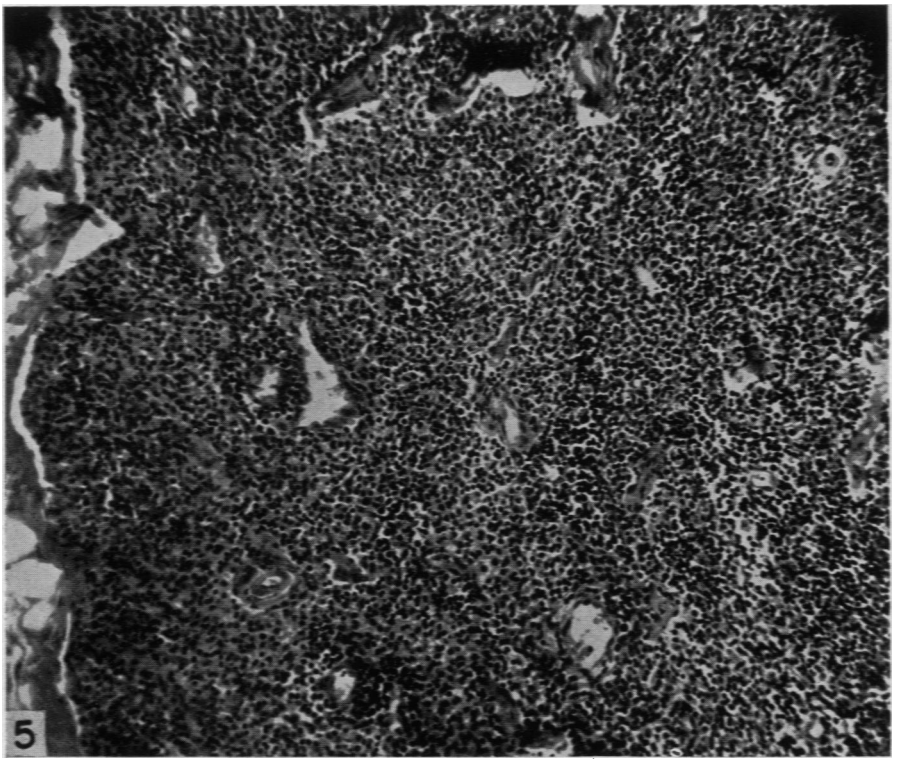
PLATE 129

FIG. 5. Typical early reaction of the lymph nodes accompanying infection by *Br. suis* (ABF strain). This lymph node from dog IX was virtually replaced by the reticulo-endothelial reaction; grossly it resembled the node shown in Figure 1. Although the cells vary greatly in morphology, the reaction at this stage is not epithelioid except in scattered foci. These foci are pictured in Figure 3. In some areas the foci coalesce and produce a picture not unlike that in the guinea-pig reaction (Fig. 6). × 157.

FIG. 6. A typical granulomatous transformation of the lymph node in a guinea-pig infected with *Br. suis* for comparison with the reaction in the dog's node. × 137.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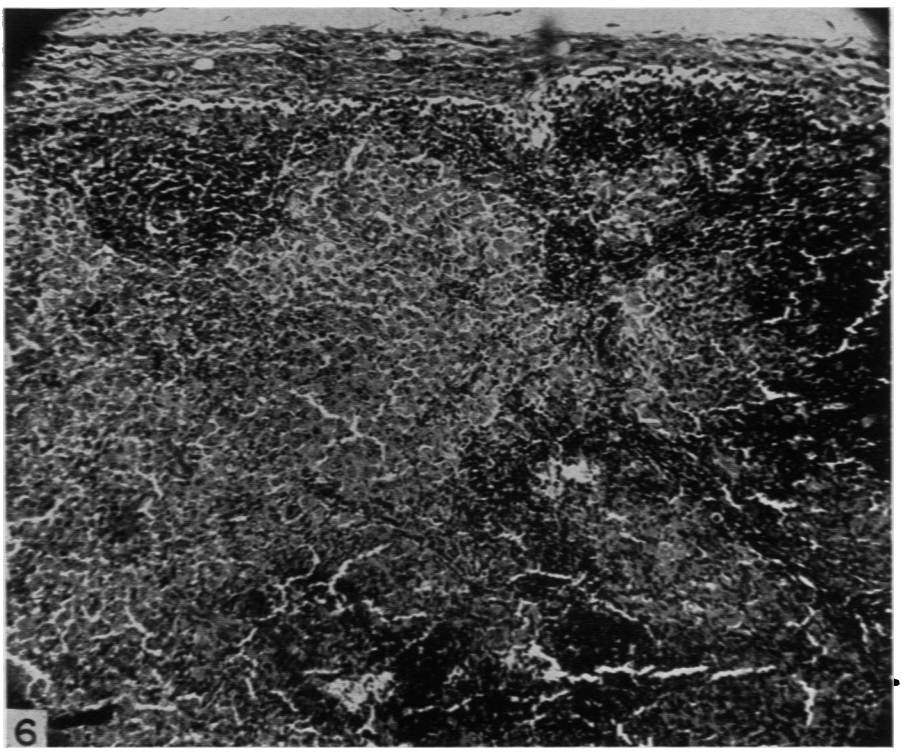
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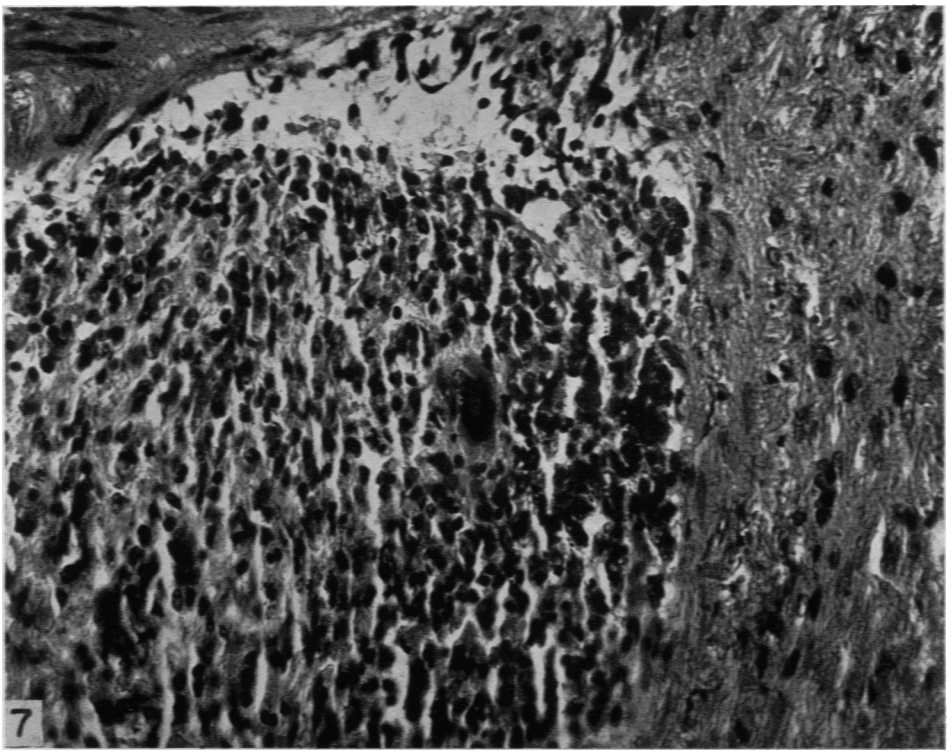
PLATE 130

FIG. 7. A small granulomatous focus in the spleen just beneath the capsule, with a large giant cell of megakaryocytic type. Giant cells of other types, some with one nucleus and others with several nuclei such as those pictured in Figures 2 and 4, are more common in the typical reaction to brucella. Dog VI, from which this section was taken, received 39 intraperitoneal inoculations of *Br. suis* (Brody strain) and lived for 454 days. The lymph nodes showed the reaction pictured in Figure 1, and the kidneys showed focal granulomata like that pictured in Figure 9. × 375.

FIG. 8. A necrotic inflammatory lesion in the capsule of the spleen of dog VI. The section is from the spleen shown in Figure 7. × 182.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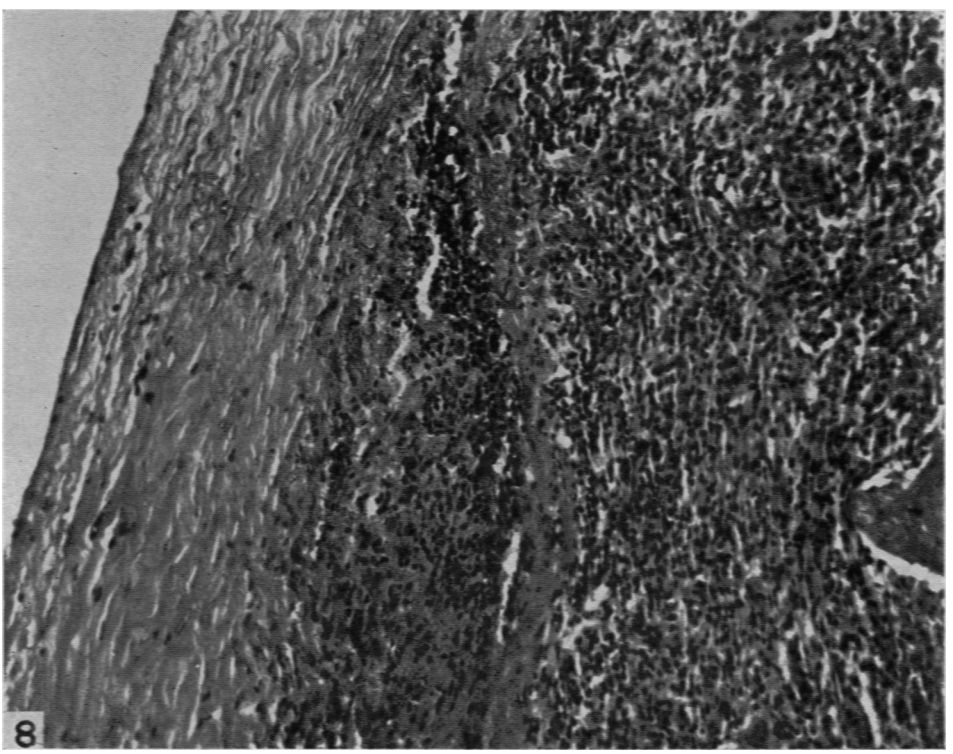
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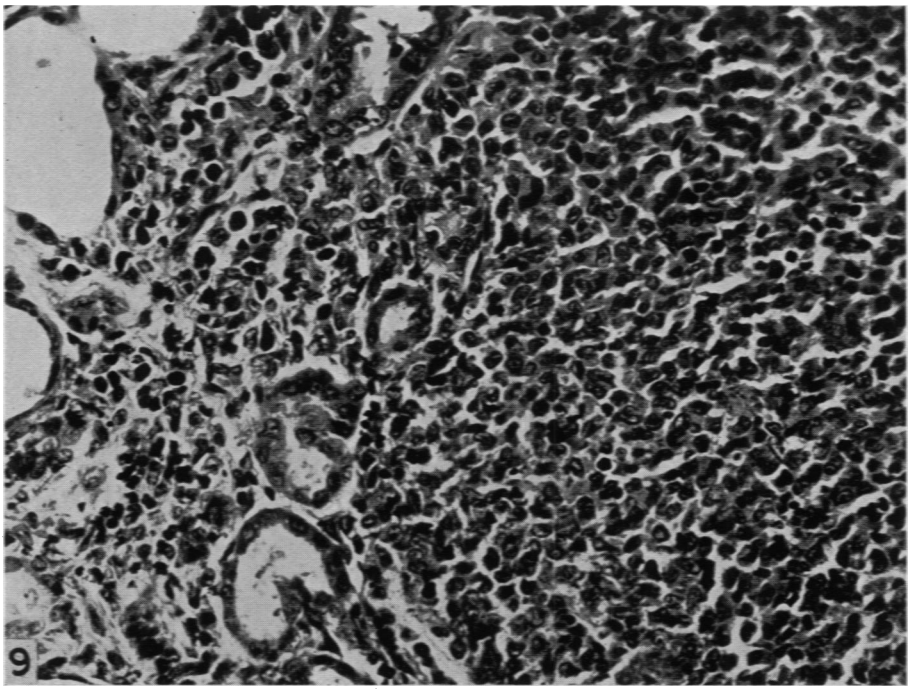
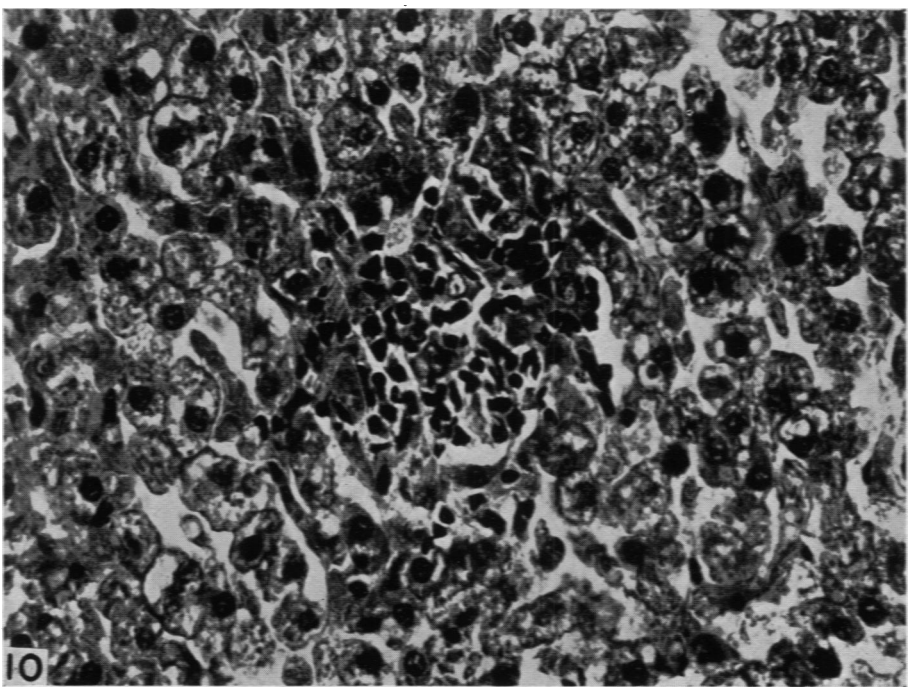
PLATE 131

FIG. 9. Focal granuloma in the kidney of dog I. The typical reacting cells are reticuloendothelial, but there is also a scattering of polymorphonuclear leukocytes; many of these are eosinophils. Lesions of this type were numerous in two of the dogs. × 365.

FIG. 10. Focal granuloma in the liver of dog II. This animal had received 35 intravenous inoculations of *Br. suis* (Brody strain) and was killed 487 days after being infected. Lesions of this sort are like those seen in the guinea-pig liver. They are not numerous. × 485.





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