A STUDY OF BRUCELLA ABORTUS INFECTED TISSUES AS AN IMMUNIZING AGENT IN BRUCELLA INFECTIONS

THESIS FOR THE DEGREE OF M. S.

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THESIS

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CONTENTS

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INTRODUCTION

Because of the economic losses which the Brucella disease causes annually and because the causative organism is a source of danger to humans, a multitude of workers have been diligently seeking to find some agent whereby Brucella infections can be suppressed, but to date the literature reveals no known, safe, reliable, agent giving highly successful results unless it is Huddleson's (1) vaccine which has given promising results. This agent however, is still considered to be in the experimental stage.

Successful immunizing agents appear to have been developed against rabies, rinderpest, canine distemper, Rocky Mountain spotted fever, yellow fever, and other diseases by using specific infected tissues, after rendering them non-infective. In view of the results obtained in the diseases just mentioned, a study of Brucella abortus infected tissue was undertaken with the idea of determining its value as an immunizing agent in the control of Brucella infections.

The spleens of Br.abortus infected guinea pigs were selected as the main tissue to be studied, due to the fact that Br.abortus causes marked pathological changes in this organ.

The exudate from a Br.abortus infected bovine fetus and the udder of a cow harboring Br.abortus were also studied, but not in detail.

HISTORICAL REVIEW

The use of infected tissues as an immunizing agent in disease of unknown etiology and in virus disease has been a development of the last five or six years. Perhaps the first use of infected tissue as an immunizing agent was practiced by the Ancient Jews of Bagdad against the Oriental sore in which case a portion of the sore was smeared on the back of a susceptible person and after the disease had manifested itself, the person was rendered immune against future infection.

Of recent years Spenser and Parker (2) 1925 have succeeded in immunizing guinea pigs against Rocky Mountain spotted fever by the injection of a phenolized emulsion of tick virus. Ticks were allowed to feed for three days on guinea pigs. They were then eviscerated and the viscera ground ten or fifteen minutes with a little sand and sterile salt solution. The emulsion was diluted with enough salt solution so that each cubic centimeter contained the equivalent of two ticks. Phenol was added to 0.5 per cent. This method is under observation at the present time. The investigators have found virus neutralizing substances in the blood of vaccinated guinea pigs, rabbits and man.

Duncan and Laidlow (3) (1926) in thier work on canine distemper showed that the spleen of the ferret and dog is one of the main reservoirs of the virus of that disease, and that a potent vaccine could be made therefrom by the mere

process of chemical (formalin) attenuation. Likewise Curasson and Deply (4) have shown that the vaccine of choice against renderpest is a formalized spleen emulsion derived from "virus calves" employed in the hyperimmunization of steers for anti-renderpest serum. Staub (5) produced an immunizing agent against fowl pest by the same method. In Spain spleen emulsions from infected hogs are employed in an immunization against hog cholera.

Duran-Reynal and J. R. Murphy (6) showed in their work on chicken tumors that ground muscle from susceptible chickens fix in vitro in a number of instances, the agent of the filterable chicken tumor and in a lesser degree inactivates it. The power of fixation by the chicken muscle is far greater than its inactivating power.

Duran-Reynal (7) in a study of organs from normal and immunized animals found that the extracts of brain and testicle tissues from immune rabbits, brought in contact with Levarditi or Noganchi strain of vaccine virus will fix or inactivate the virus. The kidney, probably skin, brain and liver extracts possess enhancing properties, but to a lesser degree than the testicle extract. On the other hand, spleen, blood lymph nodes, and bone marrow not only fail to enhance but actually suppressed or restrained entirely the vaccinal skin infection.

Pijoan (8) showed that the addition of testicular extract to cultures of twenty different bacteria just prior to inoculation enhances their infectious activity to a high

degree. Spleen extracts on the other hand never give rise to enhancement, and often caused the lesion to be less than would ordinarily be the case.

Kelser (9) believes that the active principle in rabies vaccine is something other than the dead virus. He thinks that it is possibly a cellular principle resulting from the reaction between the virus and tissue cells, or possibly a particular stage of the virus which occurs only in the tissue cells. He argues that it is firmly linked with the tissue cells and that it is not capable of being readily separated from them by ordinary methods.

In an address by E.Hindle (10) on yellow fever, he reports the use of a vaccine made from the liver of a Rhesus monkey in a late stage of the disease. This vaccine, states this speaker, conferred a high degree of protection on monkeys. This work has been confirmed in Paris and Rio-de-Janerio. This vaccine has proven successful on man and is now in use in Brazil. Plans are being made for its use in West Africa.

H.Zinsser and Castaneda (11) have experimental evidence that guinea pigs can be completely or partially protected by three injections of typhus tunica material in which there are moderate numbers or Rickettsiae. The tunica material was treated twenty four to forty eight hours with two per cent formalin before inoculation.

EXPERIMENTAL METHODS

Infected Spleens: The infected spleens for this study were obtained from guinea pigs that had been injected intraperitoneally or received an interpalpebral instillation of a culture to Br. abortus recently isolated from milk or an infected bovine fetus.

Six weeks from the time of inoculation the guinea pigs were killed and blood was collected for the agglutination test. The spleens showing enlargement and gross pathological changes were removed under aseptic conditions, ground in a sterile meat chopper and further ground in a mortar with a pestle until a homogenous emulsion was obtained. Sterile 0.85 per cent NaCl solution was added until the emulsion contained twenty per cent of spleen by weight. This spleenic emulsion was smeared out on liver agar plates (12) and liver agar plates containing gentian violet (1-200,000). Half of the plates were incubated in the presence of five to ten per cent carbon dioxide gas and the other half aerobically at 37°C. for three days. If the plates upon examination showed Br. abortus colonies, chloroform of formalin was added until two per cent of the chemical was present in the emulsion. The material was then placed in the ice box for five to ten days after which time the material was tested for sterility as described above. If, upon examination, there was no visible growth, the emulsion was considered sterile and ready for use.

Some idea of the changes produced in the size of the spleen by Br.abortus may be had from the accompanying photograph.

1. normal spleen.
2. spleen wt. 1.9 grams
3. spleen wt. 3.95 grams
4. spleen wt. 4.3 grams

Untreated spleenic emulsion was prepared as above except the emulsion was not treated by any agent, but used soon after being prepared. The spleen tissue was cultured, upon removal from the guinea pigs.

INFECTED FETAL EXUDATE: The exudate from a Br.abortus infected fetus was obtained from the gravid uterus of a slaughtered cow. The gravid uterus was brought into the

laboratory, seared with a flame, opened up with sterile instruments, and the exudate scraped from the fetus. This exudate was emulsified in sterile 0.85 per cent NaCl solution, smeared on liver agar plates containing gentian violet. Half of the plates were incubated in the presence of five to ten per cent carbon dioxide gas, and the other half aerobically at 37°C. for three days. If Br.abortus was found in pure culture, the exudate emulsion was chemically (chloroform) treated and again tested for sterility as above. If no visible growth was observed, the material was considered sterile and ready for use.

IMMUNIZATION TREATMENT

Guinea pigs were injected subcutaneously or intraperitoneally with various amounts of the twenty per cent spleenic emulsion as prepared above. Four weeks after injection all of the treated guinea pigs along with untreated ones (controls) were fed cultures of Br.abortus that had been recently isolated from milk and a fetus. Six weeks after the feeding of the cultures, all of the guinea pigs were killed. Blood was collected for the agglutination test. Parts of the spleen, kidney, liver and lungs were smeared on liver agar plates and liver agar plates containing gentian violet (1-200,000). These plates were then placed in jars, from which the atmospheric oxygen had been replaced by five or ten per cent carbon dioxide and incubated at 37°C. for three days, after which time the plates were examined and the findings recorded.

EXPERIMENTAL WORK

Experiment No. 1

The results of experiment number one are shown in table number I.

Four groups of five guinea pigs were inoculated with twenty per cent spleenic emulsion (chloroform treated) subcutaneously and intraperitoneally respectively, with 2.5 cc. and 5 cc. amounts.

Six weeks after the inoculation of the spleenic material all of the guinea pigs were killed. Blood was collected for the agglutination test. Parts of the spleen, kidney, liver and lungs were smeared on liver agar plates and liver agar plates containing Gentian violet (1-200,000). These plates were placed in jars from which the atmospheric oxygen was replaced by five to ten per cent carbon dioxide and incubated at 37°C. for three days after which time the plates were examined and the cultural findings recorded.

The results of this experiment coincide with the results obtained from the plating of the chloroform treated twenty per cent spleenic emulsion in that Br. abortus was not obtained in either instance, nor was there any detectable agglutinins produced in the guinea pigs as a results of the injection of the material, hence chloroform treated twenty per cent spleenic emulsion does not produce any detectable changes in the guinea pigs.

Experiment No. 2

The results of this experiment may be found in table number II.

In this experiment twenty guinea pigs were injected with the chloroform treated (twenty per cent) spleenic emulsion subcutaneously and intraperitoneally respectively in 2.5 cc. and 5 cc. amount. Four weeks after the inoculation these pigs were fed newly isolated strains of Br.abortus on the feed. Six weeks after feeding of the cultures all of the guinea pigs were killed. Blood was collected for the agglutination test. Parts of the spleen, kidney, liver and lungs were smeared on liver agar plates and liver agar plates containing Genitan violet (1-200,000). These plates were then placed in jars. The atmospheric oxygen was replaced by five to ten per cent carbon dioxide and incubated at 37°C. for three days, after which time the plates were examined and the findings recorded. It is evident from the results that no protection was had from the injection of chloroform treated (twenty per cent) spleenic emulsion, before being exposed to cultures of Br.abortus by feeding. All of the treated pigs showed infection equally as great as did the untreated pigs. The organism was recovered from the tissues and complete agglutination was had in the 1:500 dilution of the blood serum of all of the pigs.

Experiment No. 3

The results of experiment number three are shown in table number III.

In this experiment ten guinea pigs were given repeatedly subcutaneously injection of Br.abortus infected chloroform treated (twenty per cent) spleenic emulsion at intervals of three days. Five pigs received three injections and five injections respectively at three days intervals in 2.5 cc. amounts. Four weeks after the last injection the pigs were fed newly isolated strains of Br.abortus on the feed. Six weeks after being fed cultures all of the pigs were killed. Blood was collected for the agglutination test. Parts of the spleen, liver, kidney and lungs were smeared on liver agar plates containing gentian violet (1-200,000). These plates were placed in closed jars from which five to ten percent of the atmospheric oxygen was replaced by carbon dioxide, and incubated at 37°C. for three days. The plates were then examined and the cultural findings recorded.

It is evident from the results that no protection was had from repeated injection of chloroform treated (twenty per cent) spleenic emulsion after artificial exposure to Br.abortus. All pigs treated and untreated showed a high degree of infection. Br.abortus was recovered from the tissues and a complete agglutination was had in the 1-500 dilutions of the blood serum.

Experiment No. 4

The results of this experiment are shown in table number IV.

In this experiment five guinea pigs were injected subcutaneously with 2 cc. of formalin treated (twenty per cent

spleenic emulsion). Four weeks after injection the pigs were fed newly isolated strains of Br.abortus on the feed. Six weeks after being fed the cultures all of the pigs were killed. Blood collected for the agglutination test. Parts of the spleen, liver, kidney and lungs were smeared on liver agar plates containing gentian violet (1-200,000). These plates were placed in closed jars from which the atmospheric oxygen was replaced by five to ten per cent carbon dioxide and incubated at 37°C. for three days. The plates were examined and the cultural findings recorded.

As in the preceding experiments the formalin treated spleenic emulsion did not afford any protection from subsequent artificial exposure to Br.abortus as infection was obtained in all pigs as determined by cultural and serological tests.

Experiment No. 5

The results of experiment number five are shown in table number V.

Five guinea pigs received three subcutaneously injection of abortus exudate (chloroform treated) from a bovine fetus at intervals of three days. Four weeks after the last injection blood was collected for the agglutination test. A few days later the pigs were fed newly isolated strains of Br.abortus in feed. Six weeks after being artificially exposed all of the pigs were killed. Blood was collected for the agglutination test. Parts of the spleen, liver, kidney and lungs were smeared on liver agar plates containing

gentian violet (1-200,000). These plates were incubated for three days at 37°C. in jars from which the atmospheric oxygen had been displaced by five to ten per cent carbon dioxide. These plates were examined and the cultural findings recorded.

Injection of fetal exudate did not stimulate the productions of agglutinins upon being injected into the body of guinea pigs. All pigs so injected were negative to the agglutination test four weeks after injection of fetal exudate.

It is evident from the results that no protection was had from the injection of bovine fetal exudate against artificial exposure to Br.abortus. Organism was recovered from the tissues of all pigs autopsied, hence there was no detectable immunity.

Experiment No. 6

The results of this experiment are shown in table number VI.

Five guinea pigs were injected subcutaneously with fresh untreated (chemically) 10 per cent spleenic emulsion. Three of the pigs received 1.5 cc. and two received 3.0 cc. Four weeks after inoculation the pigs were bled from the heart, and the blood serum tested for Br.abortus agglutinins. Two pigs showed a titer. These two pigs were killed. The remaining three pigs were artificially exposed to cultures of Br.abortus. Parts of the spleen, liver, kidney, and lungs were smeared on liver agar plates containing gentian violet (1-200,000). These plates were incubated at 37°C. in an

atmosphere of five to ten per cent excess carbon dioxide. After three days the plates were examined and the cultural findings recorded.

The findings in the two pigs killed are in accordance with that of other workers. The pigs became infected as the result of injecting untreated infected spleens. The results of the remaining pigs can not be included in this thesis due to lack of sufficient time to lapse before the pigs can be autopsied.

Experiment No. 7

A goat was given five subcutaneous injections of formalized spleen emulsion and one injection of a fresh untreated infected spleen emulsion at three days intervals. The goat received 5 cc. of the spleen emulsion in each of the first two injections of 10 cc. in each at the third and fourth injections and 20 cc. in the fifth injection. The sixth injection constituted one ground up spleen that was enlarged about four times. Six weeks after this last injection this goat was fed ten agar slants of Br.abortus. The goat was bled once a week and the blood serum tested for Br.abortus agglutinins.

Milk samples were taken and the cream was smeared on liver agar plates with and without gentian violet (1-200,000). Rennin was added to the milk to coagulate it. The milk serum was tested for Br.abortus agglutinins. It appears that the goat was immunized against infection as the animal did not show an increase in agglutination titer after being fed cultures

of Br.abortus. The milk was negative to Br.abortus both culturally and seriologically. The goat gave birth to two normal kids in April, 1931.

Experiment No. 8

A heifer was given two subcutaneous injections of formalin treated spleenic emulsion, and one injection of an untreated infected spleen at intervals of one week. Two months after the last injection the heifer was exposed to infection by placing several drops of a culture of Br.abortus under the lower eye lid and by feeding cultures.

This heifer developed a titer after the subcutaneous injection of an untreated infected spleen, but in a short time the titer had begun to decrease (table #8). Upon exposure to cultures of Br.abortus the titer again increased, but not beyond that of the first instance and has remained there. Sufficient time has not elapsed to determine the absence of infection in this animal, hence no conclusion can be drawn at this time as to the degree of protection obtained.

Table No. 1

Guinea pig	Method of inoculation	Amount of Spleenic emulsion injected	Organs from which Br. abortus was recovered at autopsy	Agglutination reaction at autopsy
1	Subcutaneously	2.5 cc.	None	-
2	"	"	"	-
3	"	"	"	-
4	"	"	"	-
5	"	"	"	-
6	"	5.0 cc.	"	-
7	"	"	"	-
8	"	"	"	-
9	"	"	"	-
10	"	"	"	-
11	Intraperiton-eally	2.5 cc.	"	-
12	"	"	"	-
13	"	"	"	-
14	"	"	"	-
15	"	"	"	-
16	"	"	"	-
17	"	"	"	-
18	"	"	"	-
19	"	"	"	-
20	"	"	"	-

- = No agglutination

Table No. 2

Guinea pig No.	Method of inoculation	Chloroform treated spleen emulsion injected 4/10/30	Fed cultures of Br.abortus	Autopsy material collected	Tissues from which Br. abortus was recovered at autopsy	Agglutination reaction at autopsy
1	Subc.	2.5 cc.	5/13/30	6/28/30	spl.li.kid.lu.	+++++
2	"	"	"	"	spl.kid.	+++++
3	"	"	"	"	spl.li.kid.lu.	+++++
4	"	"	"	"	" " " "	+++++
5	"	"	"	"	" " " "	+++++
6	"	5.0 cc.	"	"	spl.	+++++
*7	"	"	"		no tissues cult.	+++++
8	"	"	"	"	spl.	+++++
9	"	"	"	"	spl.li.kid.lu.	+++++
10	"	"	"	"	" " " "	+++++
11	Intraperit.	2.5 cc.	"	"	spl.li.kid.lu.	+++++
12	"	"	"	"	" " " "	+++++
13	"	"	"	"	spl.	+++++
14	"	"	"	"	spl.li.	+++++
15	"	"	"	"	spl.li.kid.lu.	+++++
16	"	5.0 cc.			spl.li.	+++++
17	"	"	"	"	spl.	+++++
18	"	"	"	"	spl.li.kid.lu.	+++++
19	"	"	"	"	" " " "	+++++
20	"	"	"	"	" " " "	+++++
	Untreated control					
21	"	"	"	"	spl.li.kid.lu.	+++++
22	"	"	"	"	spl.li.	+++++
23	"	"	"	"	spl.li.kid.lu.	+++++
24	"	"	"	"	spl.lu.	+++++
25	"	"	"	"	spl.li.kid.lu.	+++++

Subc. = subcutaneous Intraperit. = intraperitoneal spl. = spleen li. = liver lu. = lung, kid. = kidney +++++ = complete agglutination in all dilution through 1:500 + complete aggl. P = partial aggl. T = trace aggl. These abbreviations are used in all tables. * animal died.

Table No. 3

Guinea pig No.	Method of injection	Spleenic emulsion per injection	Date last injection was given	Fed culture of Br. abortus	Date killed	Organs from which Br. abortus was recovered at autopsy	Agglutination reaction at autopsy
1	Subc. 3 injections 3 days apart	2.5 cc.	7/18/30	8/25/30	10/6/30	spl.li.kid.lu.	+++++
2	Same	"	"	"	"	spl.li.	+++++
3	"	"	"		7/21/30	not cultured	
4	"	"	"	"	10/6/30	spl.li.	+++++
5	"	"	"	"	"	spl.li.lu.	+++++
1	Subc. 5 injections 3 days apart	"	"	"	"	spl.kid.	+++++
2	Same	"	"	"	"	spl.li.lu.	+++++
3	"	"	"	"	"	spl.li.kid.	+++++
4	"	"	"	"	"	spl.li.	+++++
5	"	"	"	"	"	spl.li.kid.lu.	+++++
Control							
1	None	None				spl.li.kid.	+++++
2	"	"				spl.kid.	+++++
3	"	"				spl.li.kid.lu.	+++++
4	"	"				spl.li.	+++++

Table No. 4

Guinea pig No.	Method of injection	Formalin treated spleenic emulsion injected	Spleenic emulsion injected	Fed cultures of Br. abortus	Date killed	Organs from which Br. abortus was recovered at autopsy	Aggl.reaction at autopsy
1	Subc.	2.5 cc.	2/3/31	2/3/31	4/14/31	spl.li.	+++++
2	"	"	"	"	"	spl.li.lu.	+++++
3	"	"	"	"	"	spl.kid.	+++++
4	"	"	"	"	"	spl.li.kid.lu.	+++++
5	"	"	"	"	"	spl.	+++++
	Untreated control						
6	"	"	"	"	"	spl.li.	+++++
7	"	"	"	"	"	spl.li.kid.lu.	+++++
8	"	"	"	"	"	spl.li.kid.	+++++

Table No. 5

Guinea pig No.	Method of injection	Exudate injected each time	No. of injections	Date last injection was given	Fed culture of Br. abortus	Date killed	Organs from which Br. abortus was recovered at autopsy	Aggl. react at autop
1	Subc.	2.5 cc.	3	7/22/30	8/21/30	10/6/30	spl.li.kid.lu.	+++
2	"	"	"	"	"	"	li.lu.	+++
3	"	"	"	"	"	"	li.	+++
4	"	"	"	"	"	"	spl.li.	+++
5	"	"	"	"	"	"	spl.li.kid.	+++
	Untreated control							
6	"	"	"	"	"	"	li.	+++
7	"	"	"	"	"	"	spl.li.kid.lu.	+++
8	"	"	"	"	"	"	spl.li.kid.	+++

Table No. 6

Guinea pig No.	Inoculated with untreated infected spleen	Date injected	Date bled	Aggl. reaction	Date killed	Organs from which Br. abortus was recovered at autopsy	Aggl. reaction at autopsy
*1	3 cc.	4/8/31	5/6/31	T----			
*2	3 cc.	4/8/31	"	-----			
*3	1.5 cc.	"	"	-----			
4	1.5 cc.	"	"	++PT-	5/13/31	spl.	+++++
5	1.5 cc.	"	"	++P--	"	spl.	+++++

* The animal Nos. 1,2,and 3 were not killed.

Table No. 7

Date	Injections		Immunity or Aggl.reaction
6/20/30	Subcutaneously	5 cc.	-----
6/23/30	"	5 cc.	-----
6/26/30	"	10 cc.	-----
6/30/30	"	10 cc.	-----
7/3/30	"	1 infected spleen	-----
7/15/30			-----
7/30/30			+++PT
8/4/30			+++PT
8/15/30			+++P-
8/26/30			+PPT-
9/3/30			PPT--
10/9/30			+PT--
10/15/30	Fed cultures of Br.abortus		PPT--
9/24/30			PPT--
9/31/30			PPT--
10/7/30			PT---
10/14/30			++PT-
11/16/30			++P--
2/12/31			++---
3/18/31			T----

The blood serum was tested in dilutions of 1:25; 1:50 1:100; 1:200 and 1:500. + = complete agglutination P = partial agglutination T = trace agglutination

Table No. 8

Date	Injections	Immunity or Aggl.reaction
10/31/30	10 cc. spl. emulsion subc.	-----
11/7/30	25 cc. " " "	T----
11/14/30	1 untreated infected spl. (4 gr)	T----
11/21/30		T----
11/28/30		PT---
12/5/30		+++PT
12/12/30		++++P
1/16/31		PPT--
2/6/31		PT---
2/14/31	Exposed to Br.abortus cultures	PT---
2/27/31		+++PT
3/6/31		+++PT
3/13/31		++PT-
4/13/31		++PPT
5/15/31		++PPT

DISCUSSION

As stated before, the purpose of this experiment was to determine the value of Br.abortus infected tissues as an immunizing agent against Brucella infections. The spleens of infected guinea pigs both treated to render non-infective and un-treated, and treated fetal exudate were studied with the purpose of determining whether such agents would induce active immunity against subsequent infection. The nature of the infected tissues treated chemically to render non-infective would be that of a bacterin plus a substances in the tissues produced by the invading organism. In the case of the un-treated spleenic material the action would no doubt parallel that of a living vaccine plus a neutralizing substance in the infected tissue.

Neither a bacterin nor virulent vaccines has proven highly successful as immunizing agents. The only hope of infected tissues as such an agent lies in the possible presence of some substance which prevents the living virulent organism contained therein from infecting those treated and aid in establishing active immunity.

Untreated infected tissues harbors the live organism which when injected into the guinea pig are capable of producing infection.

Neither chloroform or formalin treated spleenic emulsion or fetal exudate have any immunizing effects upon guinea pigs. This statement is based upon the results obtained in the foregoing experiments. All of the treated guinea pigs

became infected on artificial exposure as did the untreated controls. The treated pigs even failed to show infection to a lesser degree than the untreated. The agglutination titers of the blood serum of the pigs were strongly positive in 1:500 dilution. Br.abortus was isolated from the organs of all of the pigs therefore there is no evidence of immunity.

The goat and heifer treated with the infective tissues offer encouragement for this type of material as an immunizing agent. In the case of the goat (table #7) after the subcutaneous injection of untreated spleenic material, an agglutination reaction was obtained no doubt due to the presence of living organisms in the tissue. The titer produced was not very high as there was only a trace agglutination produced in the 1:500 dilution. The titer gradually dropped off and did not show any increase after the goat was fed cultures of Br.abortus. Hence, in this case it appears that the goat was actively immunized against Br.abortus or the organisms were not virulent enough to produce infection. The agglutination titer of the goat has dropped to a slight trace in the 1:25 dilution. The goat gave birth to two healthy kids in April, 1931. Br.abortus was not found in the milk of the goat nor could specific agglutinins be detected in the milk serum.

The heifer's titer was somewhat similar before exposure to that of the goat in that titer developed to a trace in the 1-500 dilution after the injection of the untreated spleen.

In a short time the titer dropped to a trace reaction in a 1-50 dilution. After artificial exposure to Br.abortus cultures the titer increased again, but not beyond that of the original titer and has not decreased. Here again there appears to have been some immunity conferred by the untreated spleen emulsion in that the agglutination titer did not develop after exposure to a very high degree, but sufficient time has not elapsed to ascertain the absence of infection. The heifer is due to freshen in June, 1931.

SUMMARY

1. Br.abortus infected tissues treated with either chloroform or formaldehyde do not appear to have any immunizing value against Br.abortus infection in guinea pigs.

2. Untreated Br.abortus infected spleenic emulsion does not appear to infect the goat, or cow, but it may infect guinea pigs.

3. The results obtained in the goat and cow from the treatment with infected spleens warrant further study in this direction.

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