A STUDY OF BRUCELLA ABORTUS AGGLUTINATING SERA FOR THEIR BACTERICIDAL AND THERAPEUTIC VALUE

Thesis for Degree of M. S.

H. W. JOHNSON

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Ronald Austin

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Thesis

Submitted to the Faculty of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the Degree of Master of Science.

H. W. Johnson

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TABLE OF CONTENTS

INTRODUCTION.

In response to inquiries of the Medical and Veterinary professions, an evaluation of anti-sera for Brucella abortus infections was undertaken.

It is a well known fact that in infections with Brucella abortus in man or animals the manifestations of the disease are accompanied by the appearance in the blood serum of agglutinins, percipitins, or complement fixing antibodies, any or all of which may be present. Their importance, aside from serving as an indicator of infection or previous contact with the organism, apparently has never been studied in relation to Brucella abortus.

The role played by the above named antibodies against infections has long been a problem under investigation. They appear in the blood serum and tissue extracts in varying concentrations. In other words the body has produced antibodies, or its components have been altered in their physical or chemical state so as to make possible the determination that there has been contact between the animal body and the microorganism.

For a long time there has been doubt expressed among investigators as to the immunological importance of antibodies. Speaking of the agglutination test as used for standardizing anti-meningococcic serum, F. M. Huntoon and R. H. Hutchinson (1) say, "This test is recognized merely as an indication that the horses have been under immuniza-

tion for a considerable time". In reference to antipneumococcic sera they say (2), "The presence of agglutinins is no indication of the protective value of the serum, and is used only when diagnostic sera are desired". There are, however, numerous instances of immunity without a demonstrable amount of the above named antibodies. Furthermore, with a relatively high concentration of the antibody substance there is often present a chronic infection. It is also evident that the serum or tissue extracts of apparently uninfected animals never contain the specific antibodies to any appreciable extent.

The value of knowing the significance of the antibodies present in the tissues and blood serum in Brucella abortus infections is not only of scientific but of medical and economic importance as well. It was not thought advisable to determine at this time the relative importance of the individual types of antibodies, but to make a thorough study of their importance collectively. So, with the above idea in mind, this paper presents a report of the study of several Brucella abortus sera of high agglutination titre.

REVIEW OF LITERATURE

The literature reveals very little research pertaining to the standardization of antibacterial serum by either in vitro or in vivo methods. This is not only true of Brucella abortus antisera, but of other antibacterial sera as well. The presence of antibacterial antibodies has been discussed in some instances, but there has been no method developed by experimental work which would standardize their immune or therapeutic properties. Certain investigators have drawn conclusions concerning the values of agglutination, complement fixation, or precipitation tests from observations, or from a few experiments which when extended and further investigated did not prove true.

Cole and Moore (3) say, "In spite of all that has been written concerning the theoretical principles involved in the preparation of anti-pneumococcic serum, and in spite of all the reports of its therapeutic application which have appeared, it is very difficult to learn from the literature on the subject exactly how these sera have been prepared or standardized". This is also found true in relation to all antisera.

In their study of the production of anti-pneumococcic serum they emphasize the following points, (a) immunological specificity of the organism used for immunization, and (b) the serum should have the power of protecting mice against large amounts of virulent culture. Serum for type I pneumo-

coccus was the only one in which they were able to demonstrate any therapeutic value.

Weil, Richard, and Torrey (4) have shown that human serum from pneumonia patients sensitizes guinea pigs passively, but they were unable to conclude whether or not such action was due to agglutinins, complement fixing bodies, hemolysins, or precipitins. They did not attempt to demonstrate any protective value for the serum.

Amoss and Wallstein (5) and Flexner and Amoss (6) show that a rapid precipitation of anti-meningococcic serum has decided benefits. The immune bodies of the horse serum were estimated by testing its agglutinating and opsonizing power with normal and parameningococcus strains, and by determining its power to fix complement in the presence of antigens made from these strains. Its anti-infectious power was determined by incubating varying amounts with one minimum lethal dose of living meningococcic antiserum for one hour at 37° C. and injecting the mixture intraperitoneally into young guinea pigs weighing not less than 90 gm. or more than 110 gm.

Flexner and Amoss (7) found that specific anti-serum acts curatively by increasing the migration of leucocytes, by promoting phagocytosis directly, by agglutinating the meningococci, and by neutralizing endotoxin. Hence specific anti-serum seems to provide the logical therapeutic agent with which to combat epidemic meningitis.

McCoy (8), discussing the control of biological products

says, "There are but three antibacterial sera, anti-meningococcic, anti-pneumonic, and anti-dysenteric, that are subject to official tests". In each case results secured with any given serum are compared with a control serum distributed by the Hygienic Laboratory.

Thjotta (9), Brekke (10), Muir and Martin (11), and Pandit (12) demonstrated that in the quantitative estimation of immune bodies in bactericidal sera the "Phenomena of Neisser and Wechsberg" (1901) or "Complement Blocking" must be considered and examined in an endeavor to bring about satisfactory results with immune bodies.

Mazzi (13) treated one group of white rats with anti-abortus serum and another group with anti-melitensis serum. Twenty-four hours later he injected them with lethal doses of melitensis or abortus. He found that the anti-abortus serum protected the rats against melitensis as well as against abortus, and the anti-melitensis serum protected them against abortus also. Apparently there had been no other experimental investigation reported showing the antibacterial value of serum in relation to Brucella abortus infections.

MODE OF PREPARATION OF SERUM

The problems presented in respect of the preparation of an anti-abortus serum seem essentially similar to those encountered in the preparation of anti-pneumococcic serum. In each case similar factors must be dealt with; first, numerous and varying types with very low, if any, toxin production; and second, antibody specificity for the several types, as shown by agglutinin adsorption tests. Hence it was assumed that by employing a similar method to that used in the production of anti-pneumococcic and anti-meningococcic serum similar results could be expected.

The cultures used were culture 238, which was isolated July 1928 from the College Dairy Herd, and culture 8xL, isolated from a fetus from the experimental herd in 1915.

The method consists of beginning with relatively small doses of the organism in question and gradually increasing the size of the doses as rapidly as possible. Five slightly different methods of injections into goats, cows, and a horse were employed, namely:

1. Three intravenous injections of the growth from one agar slant, cultures 8xL and 238, were used with a three day interval between injections. (See Table I)
2. Subcutaneous and intravenous injections were given alternately every second day until three injections had been given. (See Table II, III, and IV.)
3. Subcutaneous and intravenous injections were given

alternately every third day until three injections had been given. (See Table VIII.) In each case five to ten days were allowed to intervene between series of injections.

4. Single injections were given subcutaneously every ten days. (See Tables V and VI.)
5. Intravenous injections were given every second day for three injections, maintaining a five day interval between each series of injections. (See Table VII.)

In the methods just outlined, slight, if any, febrile effects were noted after the first injections. The most marked reaction as a rule followed the first or second series of injections. The second or third series apparently produced an anaphylactic shock, or hypersensitization reaction (washed culture being used) which may be due to the rapid splitting of the bacterial proteins by the antibodies produced as the result of previous injections. Since individual animals vary in their susceptibility and responses, slight variations were noted.

In preparing a polyvalent serum of this type it was thought desirable to employ an avirulent strain to build up the titre and follow this with injections of virulent strains. This method was employed in the injections of horse 100, (Table I.) with slight increase in titre, following the use of the virulent strain, but a marked emaciation and loss of appetite followed until it was deemed advisable to bleed the

horse to death. In contrast to this type of injection goat 185F (Table VIII.), receiving only an avirulent strain, produced a much higher titre serum.

An attempt was made to desensitize the animals after the method of Bull (14) (using a dilute suspension of the organisms and injecting very slowly intravenously), who has observed that when bacteria are injected into the blood stream of animals immune to the same organism are clumped and deposited in the blood vessels of the brain, lungs, and in the spleen. Using this method, favorable results were obtained in cows C4 and 28 (Tables II and III.).

TABLE I. SERUM PRODUCTION CHART

Cultures used, 8xL and 238.

Horse 100.

Date of injection	Amounts	Remarks and cultures used	Date of titre test	Titre		Remarks
6/29/29	1 agar slant	8xL		1:50	+	
6/30/28		Temp. 105.6	7/ 4/28	1:100	P	
7/ 1/28	No injection			1:200	T	
7/ 4/28	1 agar slant	8xL		1:1000	x	
7/ 5/28	1 agar slant	8xL	7/ 9/28	1:2000	P	
7/ 6/28	1 agar slant	8xL		1:4000	T	
7/ 9/28	1 agar slant	8xL		1:1000	x	
7/10/28	1 agar slant	8xL	7/ 9/28	1:2000	P	
7/11/28	1 agar slant	8xL		1:4000	T	
7/15/28	1 agar slant	8xL		1:1000	x	
7/16/28	1 agar slant	8xL	7/20/28	1:2000	P	
7/17/28	1 agar slant	8xL		1:4000	T	
7/20/28	3 agar slants	238	7/25/28	1:1000	x	
				1:2000	P	
				1:4000	T	
7/21/28	3 agar slants		8/ 7/28	1:2000	x	
				1:4000	P	
				1:8000	T	
7/22/28	3 agar slants		8/11/28	1:4000	x	
				1:8000	x	
				1:10000	T	
			6/26/28	1:25	+	Before injection
				1:50	P	
			8/12/28	1:1000	P	After bleeding out
				1:2000	T	

All injections were given intravenously.
x = strong reaction (complete).
P = partial reaction (moderate).
T = trace of a reaction (slight).

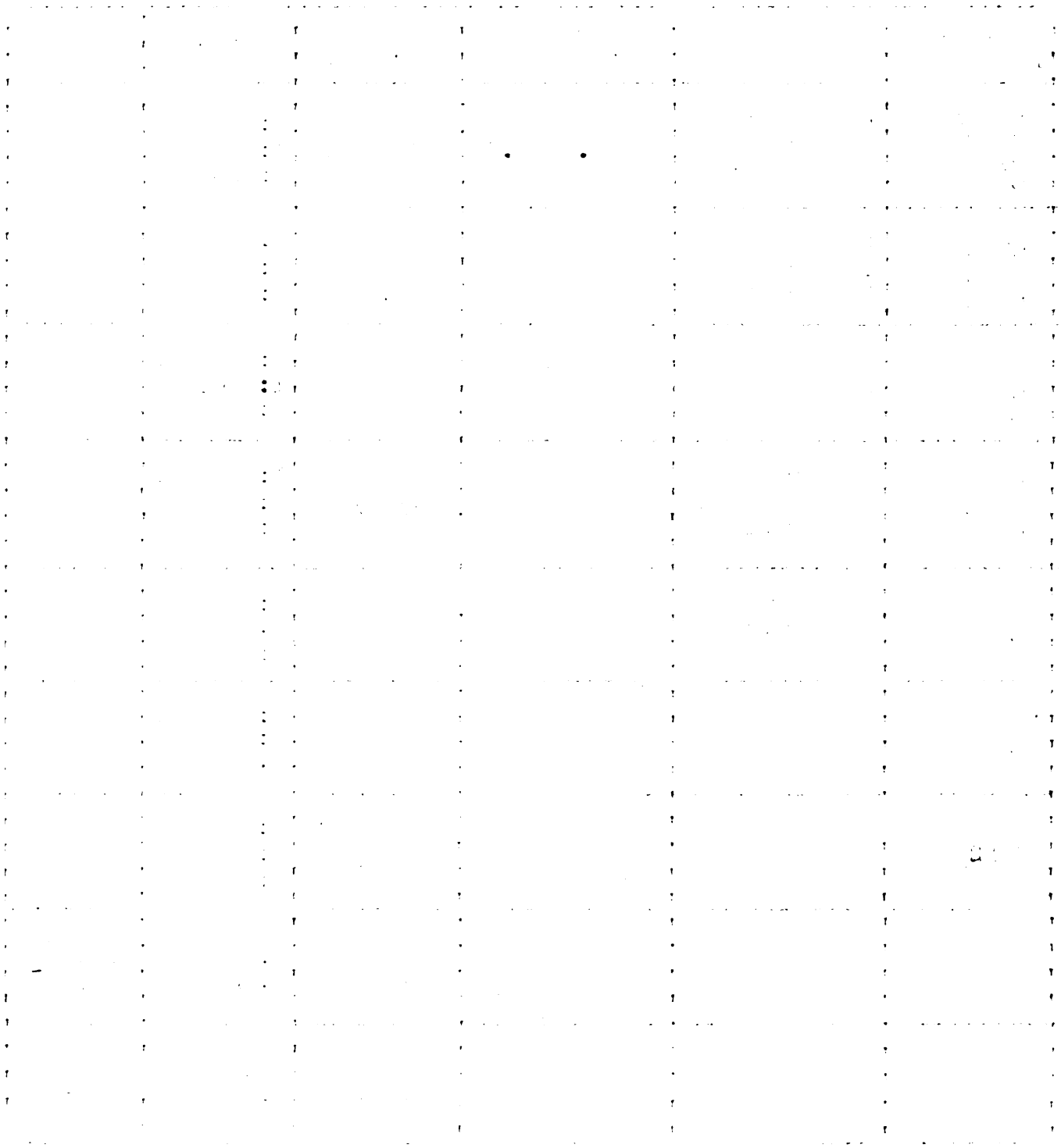


TABLE II. SERUM PRODUCTION CHART

Culture used, 8xL.

Goat 100.

Date of injection	Amounts	Injections given	Date of titre test	Titre	Remarks
11/1/28	.5 agar slant	Subcutaneous		1:1000 x	
11/3/28	.5 agar slant	Intravenous	11/7/28	1:2000 P 1:4000 T	
11/5/28	.5 agar slant	Subcutaneous	11/12/28	1:4000 P 1:8000 T	
11/12/28	.5 agar slant	Intravenous	11/23/28	1:2000 P	
11/14/28	.5 agar slant	Subcutaneous		1:4000 T	
11/16/28	.5 agar slant	Intravenous	11/23/28	1:4000 P 1:8000 T	
11/23/28	.5 agar slant	Subcutaneous		1:2000 P	
11/25/28	1 agar slant	Intravenous	12/5/28	1:4000 P	
11/27/28	1 agar slant	Subcutaneous		1:8000 T	
12/5/28	.5 agar slant	Intravenous			
12/7/28	1 agar slant	Subcutaneous	12/18/28	1:4000 P 1:8000 T	
12/9/28	1 agar slant	Intravenous			
12/18/28	2 agar slants	Subcutaneous			
12/20/28	2 agar slants	Intravenous	12/29/28	1:4000 P 1:8000 P	
12/22/28	2 agar slants	Subcutaneous			
12/29/28	2 agar slants	Intravenous			
12/31/28	2 agar slants	Subcutaneous	1/9/29	1:4000 x 1:8000 P	
1/ 2/29	2 agar slants				
1/ 9/29	3 agar slants	Subcutaneous	1/20/29	1:4000 P	
1/11/29	3 agar slants	Intravenous		1:8000 T	
			11/1/28	1:25 x 1:50 P	Before injection

x = Strong reaction
P = Moderate reaction
T = Slight reaction

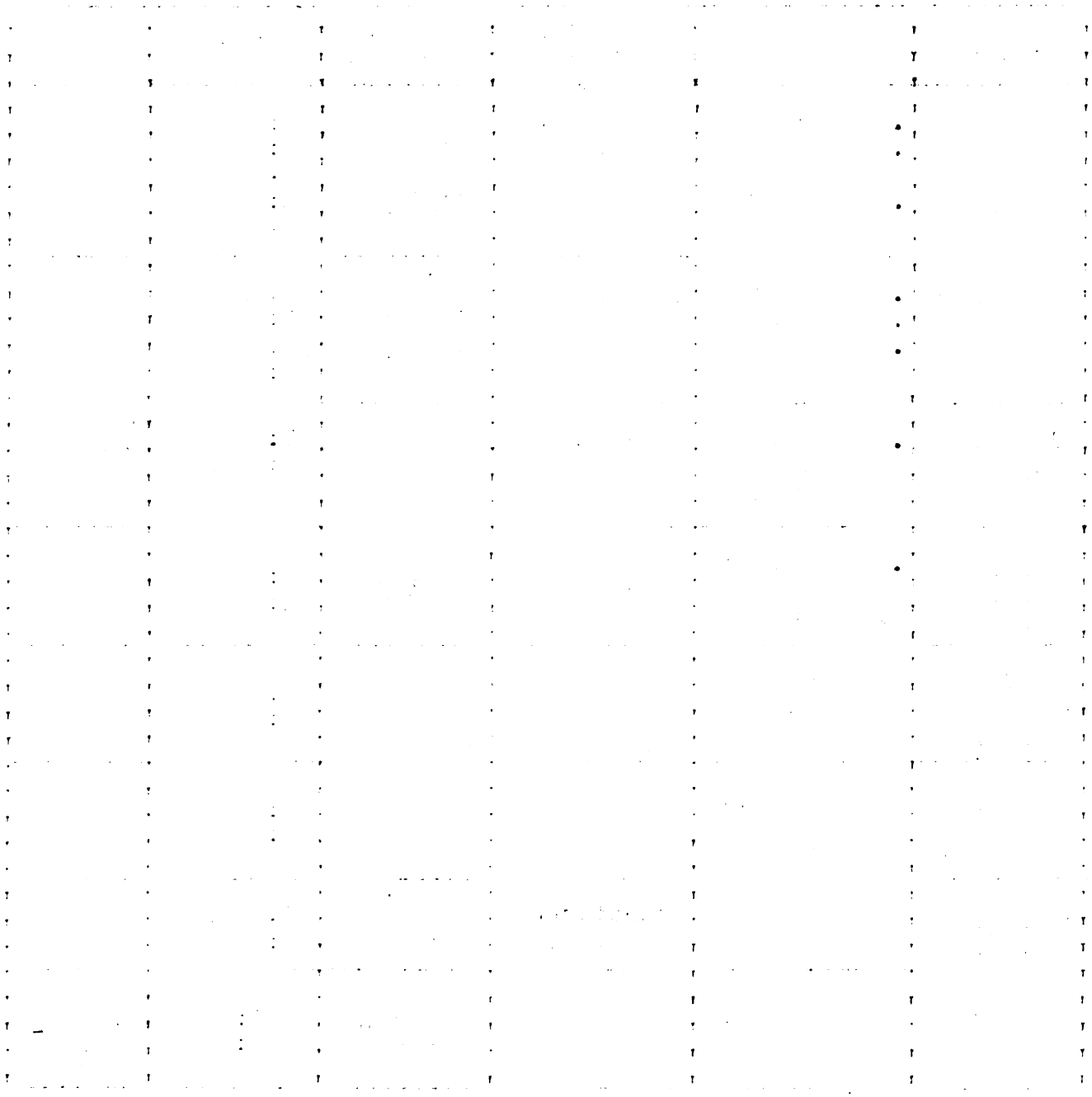


TABLE III. SERUM PRODUCTION CHART

Culture used, 8xL.

Cow C4.

Date of injection	Amounts	Injections given	Date of titre test	Titre	Remarks
11/ 3/28	.5 agar slant	Intravenous		1:1000 P	
11/ 5/28	.5 agar slant	"	11/12/28	1:2000 T	
11/ 7/28	.5 agar slant	"		1:4000 T	
11/12/28	.5 agar slant	Intravenous		1:2000 P	
11/14/28	.5 agar slant	"	11/23/28	1:4000 P	
11/16/28	.5 agar slant	"		1:8000 T	
11/23/28	.5 agar slant	Intravenous		1:2000 x	
11/25/28	.5 agar slant	"	12/ 4/28	1:4000 T	
11/27/28	1 agar slant	Subcutaneous		1:8000 T	
12/ 4/28	.5 agar slant	Intravenous		1:1000 P	
12/ 6/28	1 agar slant	Subcutaneous	12/15/28	1:2000 P	
12/ 8/28	2 agar slants	Intravenous		1:4000 T	Slight shock
12/15/28	2 agar slants	Subcutaneous		1:1000 P	
12/17/28	2 agar slants	Intravenous	12/26/28	1:2000 P	
12/19/28	2 agar slants	Subcutaneous		1:4000 T	
12/26/28	2 agar slants	Intravenous		x x x x	
12/28/28	2 agar slants	Subcutaneous	1/ 7/29	1:1000 T	
12/30/28	3 agar slants	Intravenous			
1/7/ 29	3 agar slants	Subcutaneous	1/17/29	1:1000 P	
1/9/ 29	3 agar slants	Intravenous		1:2000 T	
				1:25 x	Before injec-
			11/13/28	1:50 P	
				1:100 T	tion
			1/20/29	1:500	

x = Strong reaction
P = Moderate reaction
T = Slight reaction

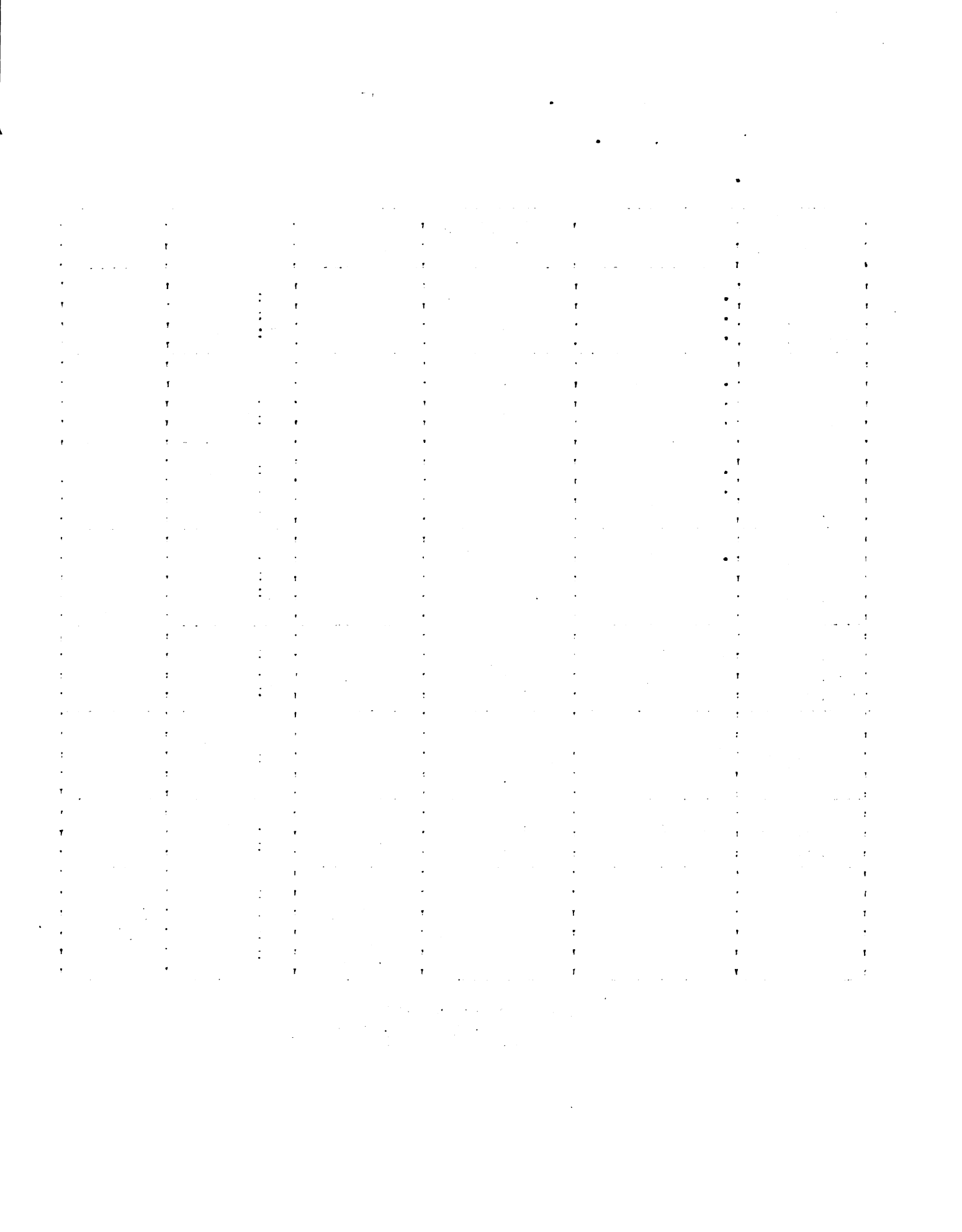


TABLE IV. SERUM PRODUCTION CHART

Culture used, 8xL.

Goat 490.

Date of injection	Amounts	Injections given	Date of Titre test	Titre		Remarks
8/27/28	1 agar slant	Intravenous	8/13/28	1:500	x	
9/ 4/28	1 agar slant	"	9/ 2/28	1:10000	T	
9/19/28	1 agar slant	"	9/ 4/28	1:10000	T	
9/25/28	3 agar slants	"	9/17/28	1:8000	T	
			9/24/28	1:8000	T	
10/ 4/28	3 agar slants	"	10/ 4/28	1:8000	T	
10/13/28	3 agar slants	"	10/13/28	1:8000	P	
			10/19/28	1:8000	x	
10/23/28	5 agar slants	Subcutaneous	10/30/28	1:8000	P	
11/ 1/28	.5 agar slant	Subcutaneous				
11/ 3/28	.5 agar slant	Intravenous	11/ 7/28	1:8000	P	
11/ 5/28	.5 agar slant	Subcutaneous	11/12/28	1:8000	x	
11/12/28	.5 agar slant	Intravenous				
11/14/28	.5 agar slant	Subcutaneous	11/19/28	1:8000	T	
11/16/28	.5 agar slant	Intravenous	11/23/28	1:8000	P	
11/23/28	.5 agar slant	Subcutaneous				
11/25/28	.5 agar slant	Intravenous	12/ 7/28	1:8000	P	
11/27/28	.5 agar slant	Subcutaneous				
12/ 7/28	.5 agar slant	Intravenous				
12/ 9/28	1 agar slant	Subcutaneous	12/17/28	1:8000	P	
12/11/28	1 agar slant	"				
12/18/28	1 agar slant	Subcutaneous				
12/20/28	1 agar slant	Intravenous	12/29/28	1:8000	T	
12/22/28	1 agar slant	Subcutaneous				
			8/26/28	1:25	x	Before injection
				1:50	x	

x = Strong reaction
P = Moderate reaction
T = Slight reaction

TABLE V. SERUM PRODUCTION CHART

Culture used, 8xL.

Goat 101.

Date of injection	Amounts	Injections given	Date of titre test	Titre	Remarks
11/23/28	.5 agar slant	Subcutaneous	12/ 3/28	1:200 x 1:500 P	
12/ 3/28	.5 agar slant	Subcutaneous	12/18/28	1:1000 T	
12/18/28	2 agar slants	Subcutaneous	12/29/28	1:1000 T	
12/28/28	2 agar slants	Subcutaneous	1/ 8/29	1:1000 P 1:2000 T	
1/ 8/29	3 agar slants	Subcutaneous	1/18/29	1:2000 P 1:4000 T	
1/18/29	3 agar slants	Subcutaneous	1/28/29	1:1000 T	
			11/23/28	1:100 x 1:200 P 1:500 T	Before injection

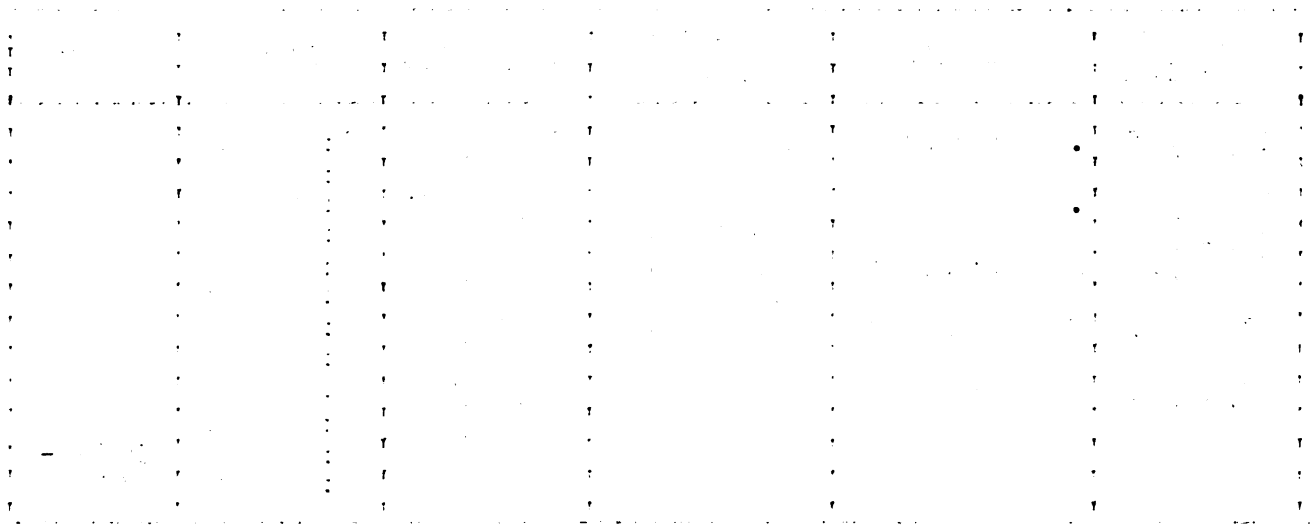
TABLE VI. SERUM PRODUCTION CHART

Culture used, 8xL.

Cow 28.

Date of injection	Amounts	Injections given	Date of titre test	Titre	Remarks
11/25/28	2 agar slants	Subcutaneous	12/ 5/28	1:500 x	
12/ 5/28	2 agar slants	Subcutaneous	12/18/28	1:1000 T	
12/18/28	2 agar slants	Subcutaneous	12/28/28	1:1000 T	
12/28/28	2 agar slants	Subcutaneous	1/ 8/29	1:1000 P 1:2000 T	
1/ 8/29	2 agar slants	Subcutaneous	1/18/29	1:1000 T	
1/18/29	2 agar slants	Subcutaneous	1/26/29	1:1000 T	
			11/25/28	1:200 x 1:500 P	Before injection

x = Strong reaction
P = Moderate reaction
T = Slight reaction



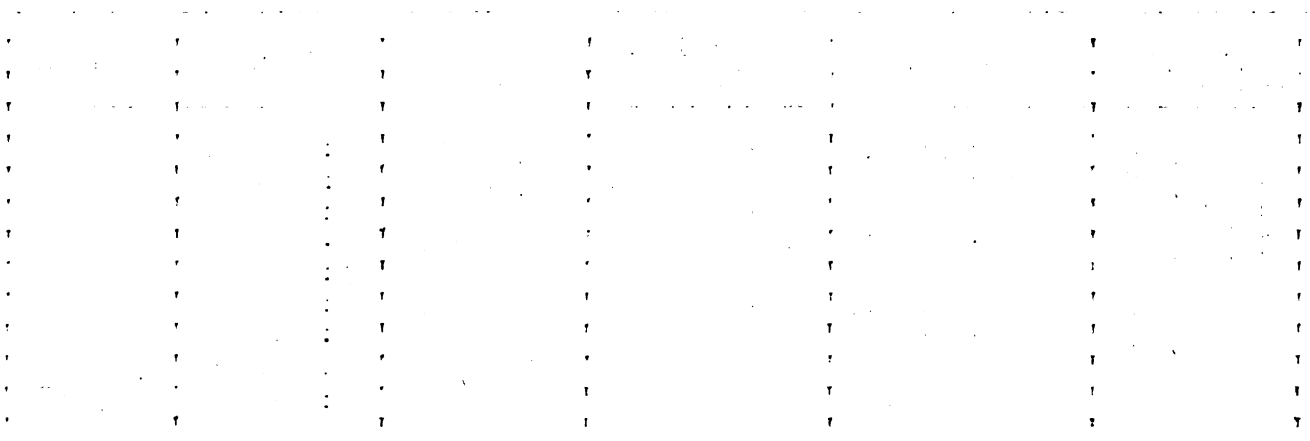


TABLE VII. SERUM PRODUCTION CHART

Culture used, 8xL.

Goat 185.

Date of injection	Amounts	Injections given	Date of titre test	Titre	Remarks
10/13/28	.5 agar slant	Intravenous	10/20/28	1:1000 P 1:2000 T	
10/15/28	.5 agar slant	Intravenous			
10/17/28	.5 agar slant	Intravenous			
10/23/28	1 agar slant	Intravenous	10/ 3/28	1:200 P 1:500 T	Died 10/28/28
10/25/28	1 agar slant	Intravenous			
10/27/28	1 agar slant	Intravenous			

x = Strong reaction

P = Moderate reaction

T = Slight reaction

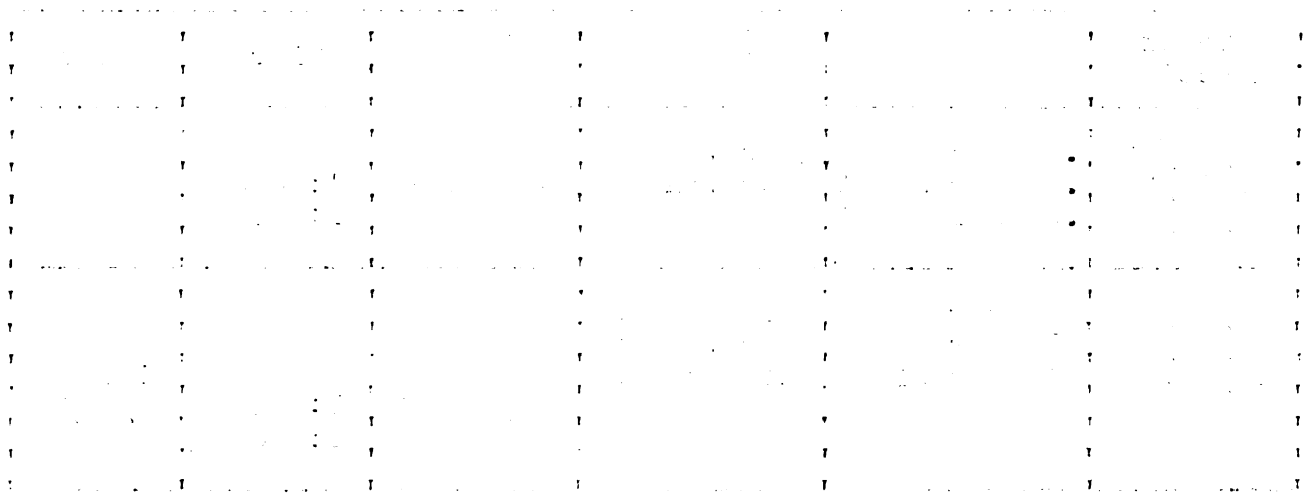


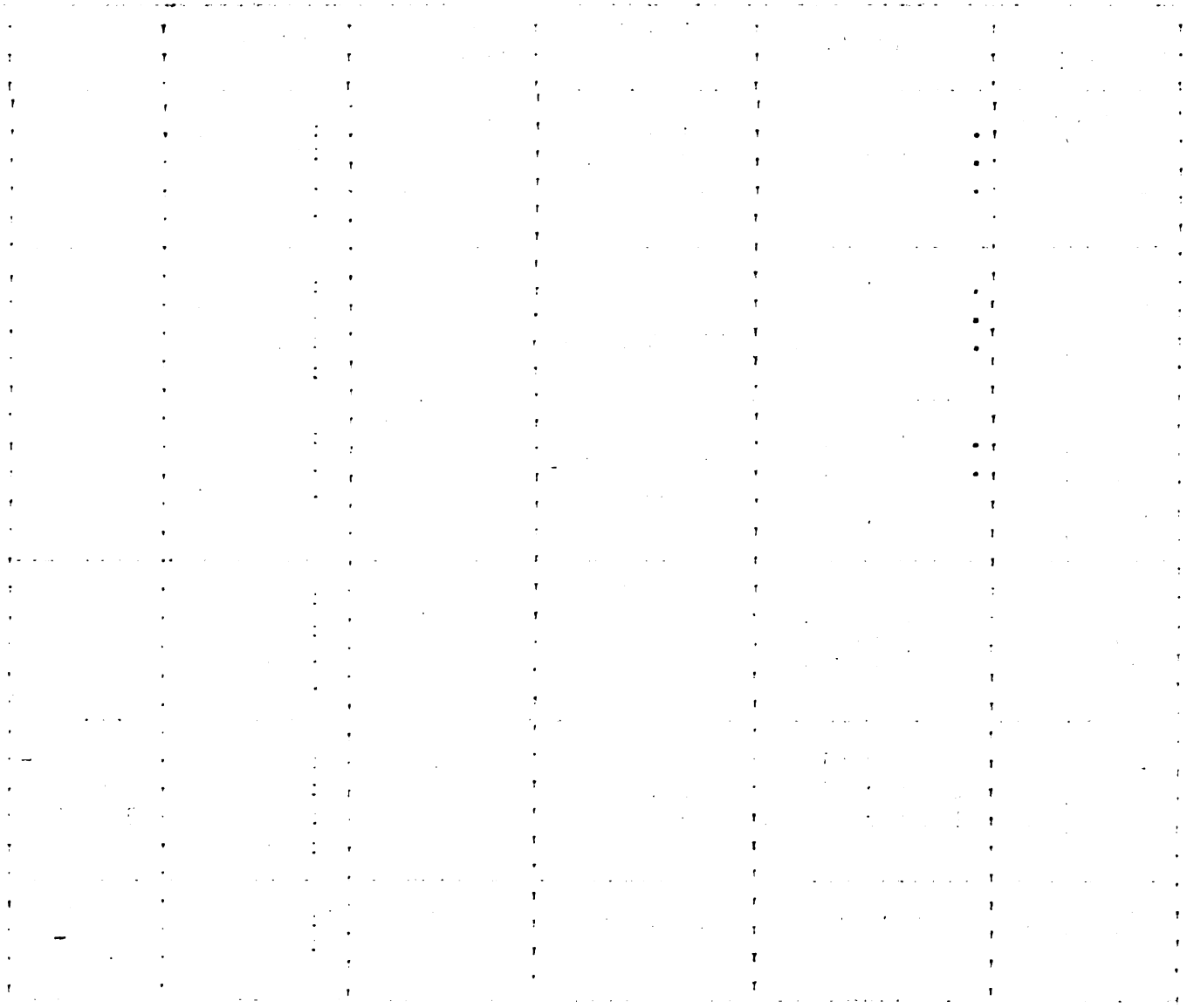
TABLE VIII. SERUM PRODUCTION CHART

Culture used, 8xL.

Goat 185F.

Date of injection	Amounts	Injections given	Date of titre test	Titre		Remarks
11/ 1/28	.5 agar slant	Subcutaneous	11/ 8/28	1:2000	x	
11/ 4/28	.5 agar slant	Intravenous		1:4000	P	
11/ 7/28	.5 agar slant	Subcutaneous	11/12/28	1:4000	P	
				1:8000	T	
11/12/28	.5 agar slant		11/19/28	1:4000	P	
11/15/28	.5 agar slant			1:8000	T	
11/18/28	.5 agar slant	Slight shock	11/23/28	1:4000	P	
				1:8000	T	
11/23/28	.5 agar slant		12/ 5/28	1:8000	x	
11/26/28	.5 agar slant	Slightly increased shock		1:10000	P	
				1:20000	T	
11/29/28	1 agar slant					
12/ 6/28	1 agar slant	Marked shock	12/14/28	1:8000	x	
12/ 9/28	no injection			1:10000	P	
12/12/28	no injection			1:20000	T	
				1:30000	T	
12/29/28	1 agar slant	Subcutaneous	12/29/28	1:1000	x	Test after large bleeding
12/31/28	1 agar slant	Intravenous		1:2000	P	
1/ 2/29	1 agar slant	Subcutaneous		1:4000	T	
				1:8000	T	
1/ 9/29	3 agar slants	Intravenous	11/ 1/28	1:25	P	Before injection
				1:50	T	

x = Strong reaction
P = Moderate reaction
T = Slight reaction



TECHNIC OF METHOD FOR DETERMINING THE BACTERICIDAL ACTIVITY OF THE SERA

Four methods were employed to determine the relative potency of the various sera prepared. Three cultural methods were used and one in vivo.

The agglutinating sera from the human and animal sources were tested from time to time by the rapid agglutination method developed by Huddleson and Carlson (15). The results are shown in Tables I to VIII inclusive. The agglutination test was employed for the purpose of demonstrating its value as an indicator of the bactericidal or therapeutic properties of the serum in question when compared with in vivo bactericidal tests.

The method used to demonstrate the presence of bactericidal substances in the sera is as follows: a standard suspension of living organisms was prepared from a 48 hour growth on liver infusion agar slants so that a turbidity of one, two, and five centimeters by the Gate's nepholometer was obtained. Dilutions of 1:25, 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16,000, 1:32,000, 1:64,000, 1:128,000, 1:256,000, 1:512,000, and 1:1,024,000 of the high agglutinating serum were prepared with sterile physiological salt solution (pH 6.8). To one cubic centimeter of each of the above dilutions was added one drop of the standard bacterial suspension. The drops were delivered from capillary pipettes of 12, 16, and 20 gauge, standardized by the use of

the United States wire gauge. Dilutions of the serum and suspensions of like turbidities of the living organisms were used as controls. The tubes of serum dilutions and bacterial suspensions were incubated at 37 degrees Centigrade and seeded with a 4 mm. wire loop after 2, 10, and 24 hours incubation in portions of gentian violet liver agar plates as described by Huddleson (16). The results were noted at varying intervals, and final reading taken 72 hours after the 24 hour plating.

The final readings were tabulated in accordance with the amounts of growth, as shown in Tables IX to XIX inclusive. An even, dense growth was charted as three plus, a seeded area with moderate but not dense growth was charted as two plus, one with but scattered growth was charted as one plus, and no growth was charted as negative. Contamination and modifications of the above discussed method of charting are explained on the individual charts.

This so-called "quantitative technic" was employed in place of the plate counting method in an endeavor to avoid such errors as the clumping of organisms by the high agglutinin content of the serum, and dead organisms present in the standard suspension.

7/10/28 Horse serum Agglutination titre 1:1000 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
12	2 hr.	- / -	+++ / +++	- / -	- / -	- / -	- / -	‡ / -	- / -	- / -	- / -	- / -	- / -	+++	Fresh serum used
	10 hr.	- / -	+++ / +++	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	+++	
	24 hr.	- / -	+ / +	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	+	
16	2 hr.	- / -	+ / ‡	- / ‡	‡ / +	‡ / -	‡ / -	‡ / ‡	- / -	- / -	- / -	- / -	- / -	+++	
	10 hr.	- / -	‡ / +	- / -	‡ / +	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	+	
	24 hr.	++ / ++	‡ / +	- / -	‡ / +	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	+	
22	2 hr.	‡ / -	‡ / ‡	+++ / ‡	‡ / ‡	‡ / ‡	- / ‡	- / -	- / -	- / -	‡ / -	- / -	- / -	+++	
	10 hr.	‡ / ‡	‡ / ‡	+++ / ‡	‡ / ‡	- / +	- / ‡	- / -	- / -	- / -	- / -	- / -	- / -	++	
	24 hr.	+++ / ++	+++ / +	+++ / +	- / ‡	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	-	

TABLE IX

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
‡ = Slight scattered growth
0 = No growth on one plate

(Note - pH change)
pH 7.0 of Salt sol. used.
pH 7.8 after 72 hours incubation

Standard antigen - 2 cm. turbidity

Growth on plates plated after 72 hours, incubation only in 1:50 in 16 and 1:25, 1:50, and 1:100 in 22.

7/15/28 Horse serum Agglutination titre 1:4000 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	Check	Remarks
12	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Readings made after 72 hrs. Incubation
	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	
	24 hr.	+++	+++	+++	++‡	+++	+++	++	+++	++	+++	++	+++	
16	2 hr.	0 +++	+++	+++	+++	+++	+++	+++	+++	+++	+++	0 +++	+++	Fresh serum used
	10 hr.	+++	+++	+++	+++	0 +++	+++	0 +++	+++	++ +++	+++	++	+++	
	24 hr.	0 +++	++	+++	0 ++	0 ++	0 ++	0 ++	++	0 ++	0 +	-	+++	
22	2 hr.	+++	+++	+++	+++	+++	+++	‡ ++	+++	+	+++	0 +	+++	
	10 hr.	+++	0 +++	+++	+++	+++	+++	‡ +++	+++	0 ‡	+++	0 ‡	+++	
	24 hr.	++	0 ++	+++	+ +++	0 +++	0 ++	-	+	-	‡ ++	0 -	+++	

+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
- = No growth
‡ = Slight scattered growth
0 = No growth on one plate

(Note - pH change)
pH 7.0 of Salt sol. used.
pH 7.45 after 72 hours incubation

Standard antigen - 1 cm. turbidity

Growth on all plates plated after 75 hours, incubation before plating.

TABLE X

7/15/28 Horse serum Agglutination titre 1:4000 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
12	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Fresh serum used
		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++		
	10 hr.	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
		++	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++		
	24 hr.	++	++	+++	+++	++	+++	++	+++	+++	+++	+++	+++	++	
		++	++	+++	++	++	+++	+	+++	+++	++	+	++		
16	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++		
	10 hr.	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
		++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++		
	24 hr.	++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	
		*	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++		
22	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
		+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++		
	10 hr.	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	
		+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++		
	24 hr.	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	
		+	‡	+++	+++	+++	+++	++	+++	+++	+++	++	++		

TABLE XI

* = Contaminated
\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
0 = No growth on one plate
‡ = Slight scattered growth

pH 7.0 before and after 72 hours incubation.

Standard turbidity 2 cm.

Plates plated after 72 hours incubation.

7/20/28 Horse serum Agglutination titre 1:4000 Bacterial titre

Serum Dilutions

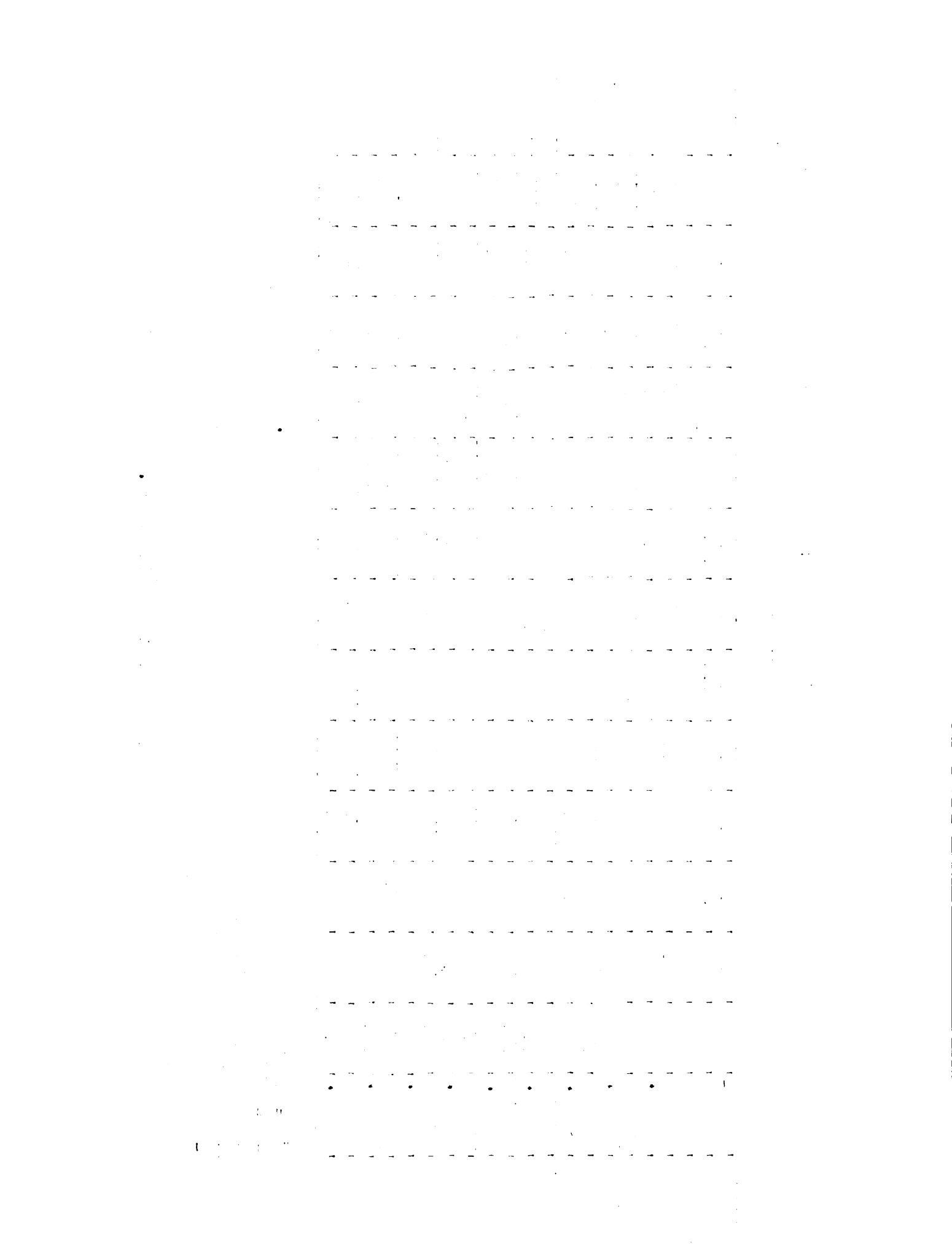
Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
12	2 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++	Fresh serum used
	10 hr.	+++ ++	++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	++ +++	+++ +++	+++ +++	++ +++	+++ +++	+++	
	24 hr.	+++ +	+ 0	+ +	++ ++	+ +	++ +++	+++ +++	+++ +++	++ ++	++ +++	+++ ++	+++ +++	+++	
16	2 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++	
	10 hr.	+++ ++	+++ +++	+++ +++	+++ +++	+++ ++	+++ +++	+++ +++	+++ +++	++ ++	++ ++	+++ ++	+++ ++	+++	
	24 hr.	+ +	+ ǂ	+++ 0	+++ +++	+++ +	+++ +++	+ +	+++ +++	++ ++	++ ++	+++ ++	+++ +++	+++	
22	2 hr.	+++ +++	+++ ++	+++ ++	+++ +++	+++ +++	+++ +++	+++ ++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++	
	10 hr.	+++ +++	+++ +	+++ +	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +	+++ +++	+++	
	24 hr.	+ +	+ 0	+++ 0	+++ ++	+++ +++	+++ ++	+++ ++	+++ ++	+++ ++	+++ ++	+++ *	+++ +++	+++	

* = Contaminated
\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
0 = No growth on one plate
ǂ = Slight scattered growth

pH 7.0 before and after 72 hours incubation

Standard turbidity 2 cm.

TABLE XII



The data in Tables IX to XII inclusive show the in vitro bactericidal effect of horse serum containing specific agglutinins. The agglutination test was employed to evaluate the height of antibody production in all sera used in this test. Several normal sera were used with no bactericidal effect demonstrated. Inconsistencies may be observed in Tables IX and X which were believed to be due to the change in the pH of the salt solution. It may also be observed as shown in Table IX that with a marked change in pH a decrease in the growth is evident in both serum dilutions and controls. While in Table X only a slight change in the pH of the salt solution was evident, no decrease in the controls and only a slight decrease in the higher dilutions could be noticed.

The results charted in Tables XI and XII show the only positive bactericidal results obtained by the use of specific horse serum. It may be observed that bactericidal effect is exemplified to only a very limited degree in the twenty-four hour seeding of the lower dilutions. The few slight variations can not be accounted for.

2/18/29 Human serum-Brown

1 = Serum of 2/15/29 + G. pig complement.
1a = Serum of 2/15/29.

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	1/128000	1/256000	1/512000	1/1024000	Check
22	2 hr. # 1	+++	+++	-	*	*	+++	+++	+++	+++	+++	+++	+++	+++	*	*	+++	+++
	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2 hr. # 1a	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	++	+++	+++	+++	+++
	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
22	10 hr. # 1	+++	+++	-	*	*	+++	+++	+++	+++	+++	+++	+++	++	+++	*	+++	+++
	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	++	+++	+++
	10 hr. # 1a	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	++	++	++	++	++	+++	+++
	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	++	+++	+++	+++
22	24 hr. # 1	+++	+++	-	*	*	+++	+++	+++	+++	+++	+++	++	-	++	*	+++	+++
	24 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	++	++	+++	+++	+++
	24 hr. # 1a	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	++	-	-	-	+	+++	+++
	24 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	+	+	+++	+++

TABLE XIII

+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
- = No growth
* = Contamination

Standard Antigen - 5 cm. Turbidity

2/15/29 Human serum - Brown Agglutination titre 1:2000 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check
22	2 hr.	+++	+++	+++	+++	++	+++	+++	+++	+++	+	-	-	+++
	2 hr.	*	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	+++
	2 hr.	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	-	+++
	2 hr.	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	-	++
22	10 hr.	+++	++	+++	+++	+++	+++	+++	+++	++	+	-	-	+++
	10 hr.	*	+++	+++	+	+++	+++	++	++	++	-	-	-	++
	10 hr.	+++	+++	+++	+++	+++	+++	++	++	++	-	-	-	+++
	10 hr.	+++	+++	+++	++	+++	+++	++	+	++	-	-	-	+++
22	24 hr.	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	-	+++
	24 hr.	*	+++	+++	+++	+++	++	++	++	+	-	-	-	+++
	24 hr.	+++	+++	+++	*	+++	++	++	++	+	+	-	-	+++
	24 hr.	+++	+++	+++	++	+++	+++	++	++	♦	-	-	-	+++

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
* = Contamination
♦ = Not to exceed 10 colonies

Standard antigen - 5 cm. Turbidity

TABLE XIV

1/25/29 Human serum - Brown Agglutination titre 1:8000 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	Check	Remarks
22	2 hr.	+++	+++	+++	+++	+++	+	+	+	+++	Fresh serum employed
	2 hr.	+++	+++	*	+++	+	+	*	+	+++	
	2 hr.	+++	+++	+++	+++	‡	‡	+	+	+++	
	2 hr.	+++	+++	+++	+++	‡	‡	+	+	+++	
22	10 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	10 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	10 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	10 hr.	+++	+++	+++	+++	-	-	-	-	+++	
22	24 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	24 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	24 hr.	+++	+++	+++	+++	-	-	-	-	+++	
	24 hr.	+++	+++	+++	+++	-	-	-	-	+++	

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
* = Contaminated
‡ = Slight scattered growth
Standard antigen - 5 cm. Turbidity

TABLE XV

2/8/29 Cow serum - C4 Bacterial titre

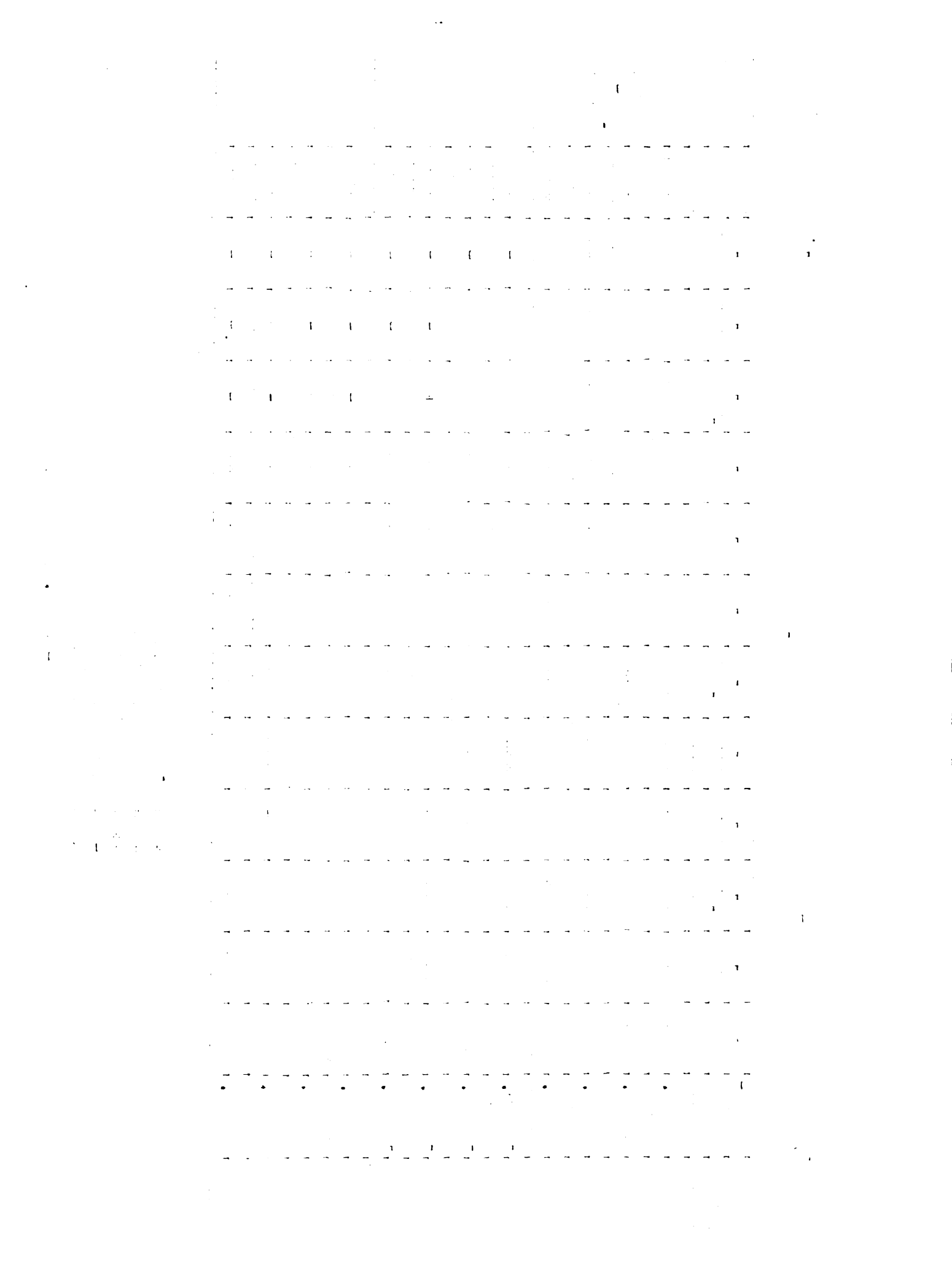
Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
22	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	+++	Fresh serum employed
	2 hr.	+++	+++	+++	++	+++	+++	++	+++	+++	++	++	++	+++	
	2 hr.	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+++	
	2 hr.	+++	+++	+++	+++	++	+++	+++	+++	++	+++	++	++	+++	
22	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	+++	
	10 hr.	+++	+++	+++	+++	+++	+++	++	+++	++	++	+	-	+++	
	10 hr.	+++	+++	+++	+++	+++	+++	++	+++	++	+	-	-	+++	
	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	+++	
22	24 hr.	+++	+++	+++	+++	+++	++	+++	+++	++	-	-	-	+++	
	24 hr.	+++	+++	+++	+++	+++	++	++	++	+	+	-	-	+++	
	24 hr.	+++	+++	+++	+++	+++	+++	++	++	+	-	-	-	+++	
	24 hr.	+++	+++	+++	*	+++	++	++	++	++	-	,-	-	+++	

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
* = Contamination

Standard antigen - 5 cm. Turbidity

TABLE XVI



2/15/29 Cow serum - C4 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
22	2 hr.	++	+++	+++	+++	+	+++	+++	+++	+++	+++	+	++	+++	Fresh serum employed
	2 hr.	++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+++	
	2 hr.	+++	+++	+++	+++	+++	+++	++	++	+++	++	-	-	+++	
	2 hr.	+++	+++	+++	+++	+++	+++	+++	++	+++	++	-	-	+++	
22	10 hr.	*	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	+++	
	10 hr.	+++	+++	+++	++	+++	+++	++	+++	++	-	-	-	+++	
	10 hr.	+++	*	+++	+++	+++	+++	+++	++	++	-	-	-	+++	
	10 hr.	+++	+++	+++	+++	+++	+++	+++	+++	++	-	-	-	*	
22	24 hr.	*	+++	++	+++	+++	+++	+++	+++	++	-	-	-	+++	
	24 hr.	+++	+++	+++	*	+++	+++	+++	+	++	+	-	-	+++	
	24 hr.	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	-	++	
	24 hr.	+++	+++	+++	+++	+++	+++	+	++	*	-	-	-	+++	

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
* = Contamination

Standard antigen - 5 cm. Turbidity

TABLE XVII

7/16/28 Cow serum - C4 Bacterial titre

Serum Dilutions

Guage of Pipette	Incubation time	1/25	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Check	Remarks
12	2 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ ++	+++ +++	++ +++	- ++	- -	- -	- -	+++ +	Fresh serum employed
	10 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	++ +++	++ +++	++ ++	- +	- +	- -	- -	++	
	24 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	- -	- -	- -	- -	- -	++	
16	2 hr.	+++ +++	+++ +++	++ +++	+++ +++	+++ +++	+++ +++	++ +++	++ ++	++ ++	++ ++	- -	- -	+++	
	10 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	- -	- -	- -	- -	- -	++	
	24 hr.	+++ +++	+ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	- -	- -	- -	- -	- -	++	
22	2 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ ++	+++ +	+ -	+ -	- -	- -	+++	
	10 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	- -	- -	- -	- -	- -	+++	
	24 hr.	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ ++	- +	* -	- -	- -	- -	+++	

\+ = Slight growth
++ = Moderate growth
+++ = Abundant growth
\- = No growth
* = Contamination

Standard antigen - 2 cm. Turbidity

TABLE XVIII

It may be noted from results shown in Table XIII when 72 hour old specific human serum inactivated with guinea pig complement was employed there appeared a zone in the 1:16,000 to 1:512,000 dilutions of the serum; and from results in Tables XIV, and, XV when fresh specific human serum was employed a zone is seen in the 1:500 to 1:64,000 dilutions of the serum. While in 72 hour old specific human serum and normal human serum no bactericidal action was observed. The above results were checked and found to be constant with only a few slight variations.

It may also be observed from results shown in Tables XVI, XVII, and XVIII when specific cow serum was employed there appeared a zone in the 1:400 to 1:64,000 dilutions of the serum. While no bactericidal action was observed when specific cow sera inactivated with guinea pig complement were tested. Three day old specific cow sera were also observed to show no bactericidal effect when the in vitro method was employed. Nor was the bactericidal effect inhanced by defibrinating or citrating the blood.

It was also observed that with an increase in the concentration of the bacterial suspension there was no bactericidal effect. The same was true when the specific sera were inactivated with guinea pig complement and employed with an increase in the bacterial suspension.

SLIDE CELL DETERMINATION OF THE BACTERICIDAL POWER OF WHOLE BLOOD

Maitland (17), Wright, (18), and Wright, Colebrook, and Stores (19) describe a technic in which whole blood mixed with a known quantity of bacteria was incubated in slide cells as an experimental method for the investigation of certain phases of immunity. In the above cited experiments the expression of bactericidal value was obtained in terms of the number of colonies which developed in a given volume of blood (the number of implanted organisms being known) and represents the combined action of the cells and serum over a period of 24 to 48 hours at 37° C. The results of which were compared with those of serum alone.

The technic described by Heist and Solis-Cohen (20) and later applied to experiments on several organisms by Parks (21) and Kolmer and Borow (22) was somewhat similar in purpose and results to the method mentioned above, but employed capillary tubes in place of slide cells.

The following observations were made using a modification of the technic described by Maitland. A standard turbidity of living organisms was prepared by the use of the Gate's nephelometer; three complete slide cell groups were prepared and incubated for varying intervals, namely 2, 10, and 24 hours, at 37° C. and the growth was charted in reference to the amount, three plus indicating abundant growth,

two plus moderate growth, and one plus only slight growth. No growth at all was charted as negative.

No bactericidal effect was observed when specific horse, cow, and goat sera were employed.

TABLE XIX. SET UP FOR WHOLE BLOOD EXPERIMENT. 8/12/28.

Cow # C4.

	Whole Blood	Organisms	Serum dilutions
1	12 guage	none	12 guage 1:50
2	none	12 guage	12 guage 1:50
3	none	12 guage	none
4	12 guage	12 guage	none
1:25	12 guage	12 guage	1:25
1:50	12 guage	12 guage	1:50
1:100	12 guage	12 guage	1:100
1:200	12 guage	12 guage	1:200
1:500	12 guage	12 guage	1:500
1:1000	12 guage	12 guage	1:1000
1:2000	12 guage	12 guage	1:2000
1:4000	12 guage	12 guage	1:4000
1:8000	12 guage	12 guage	1:8000

Standard turbidity 5 cm. (organisms)

Serum from Cow C4, 8/7/28

PREPARATION OF INFECTIOUS MATERIAL AND METHOD OF EXPOSURE

Four virulent organisms from different sources were used in the in vitro portion of this experiment. Culture 238 was isolated July 1928 from the College Dairy Herd. Culture 231 was isolated from the College Dairy Herd. Culture 196 was isolated from the fetal membranes from a cow in the College Dairy Herd on January 14, 1929. Culture 12 was obtained from Mathew's, Purdue University.

The virulent strains of Brucella abortus were grown on liver infusion agar slants for 48 hours, in an atmosphere of 10 % CO_2 and aerobically. The growth was removed with physiological salt solution, and sprinkled over the feed of the guinea pigs, or injected intraperitoneally in standard doses. When administered by feeding one third to one agar slant per guinea pig was used. The varying amounts given intraperitoneally are shown in each table. In each case the exposures were several times the infecting dose.

METHOD OF DETERMINING INFECTION

The exposed guinea pigs were under observation for a period of six weeks after exposure to infection. This time is sufficient for lesions to develop. At the end of this period they were bled for an agglutination test. The agglutination test was run in accordance with the method

developed by Huddleson (15).

The guinea pigs were then killed and autopsied and a search was made for any anatomical changes. An attempt was made to culture Brucella abortus from the lungs, liver, spleen, kidneys, and testicles. The organs were smeared on gentian violet liver infusion agar plates as described by Huddleson (16). The plates were placed in sealed jars containing 10 % CO_2 and incubated at 37° C. for three or four days. At this time the plates were examined for the presence of Brucella abortus.

DISCUSSION AND CHARTS OF IN VIVO METHOD

At the end of the six weeks period following the exposure to infection the guinea pigs were normal in external appearance and had gained weight in a majority of cases. At autopsy the spleen appeared to be the organ most commonly showing infection, as may be observed in both anatomical changes and cultural findings. The spleen was usually but not always several times enlarged and showed numerous grayish white nodules on the surface. The liver was the next most common focus of infection and was usually congested and contained few or numerous grayish white foci, varying from the size of a pin point to about two millimeters in diameter. These foci were usually raised and glistening. The lungs in several cases were pneumonic or hemorrhagic and showed small

grayish foci. In the kidneys in a few cases there were small pin-point hemorrhages and cysts one to three millimeters in diameter filled with sanguinous fluid. One or both testicles sometimes were abscessed. The epididymus was more often involved. The lymph glands were involved to some extent in several pigs. The tables which follow will be discussed by groups somewhat in detail.

Pigs Exposed to Infection and Treated with Horse Serum. (Agglutination titre 1:30,000).

Intraperitoneal Injections

Pig No.	Weight before exposure	Agg. titre	Amount culture used	Date culture injected	Amt. serum	Date serum used	Weight after 6 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre at autopsy
1	410gm.	-	1 agar slant	7/30/28	3cc.	7/30/28	525gm.	9/1/28	Small gray foci on liver with several small necrotic areas. Lungs filled with foci. Spleen twice normal. Right sachreal lymph gland slightly enlarged. Superficial inguinal gland (left) slightly enlarged.	B. abortus + in liver and spleen	1:500
2	370gm.	-	1 agar slant	7/30/28	3cc.	7/30/28	450gm.	9/1/28	Lungs show scattered pin-point hemorrhagic areas. Spleen twice normal size with few small necrotic areas. Right sachreal lymph gland enlarged.	B.abortus not found	1:500
3	445gm.	-	1 agar slant	7/30/28	1cc.	7/30/28	440gm.	9/1/28	Very few scattered pin-point hemorrhagic areas in lungs. Spleen twice normal. Hepatic lymph gland slightly enlarged.	B.abortus not found	1:500
4	310gm.	-	1 agar slant	7/30/28	1cc.	7/30/28	365gm.	9/1/28	Lungs show few scattered hemorrhagic foci. Four small pin-point grayish foci on liver. Spleen 3x normal size. Sachreal lymph glands slightly enlarged.	B.abortus not found	1:500
5	430gm.	-	1 agar slant	7/30/28	0.1 cc.	7/30/28	460gm.	9/1/28	2 visible foci on liver.	B.abortus not found	1:500
6	380gm.	-	1 agar slant	7/30/28	0.1	7/30/28	395gm.	9/1/28	All organs normal.	B.abortus not found	1:500

TABLE XX GROUP # 1.

Pigs Exposed to Infection and Treated with Serum (Horse # 1). (Agglutination titre 1:30,000).

Intraperitoneal Injection

Pig No.	Weight before exposure	Agg. titre	Amount culture used	Date culture injected	Amt. serum	Date serum used	Weight after 6 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre at autopsy
7	395gm.	-	1cc. of 5cm. turbidity	7/30/28	3cc.	7/30/28	455gm.	9/1/28	Lungs showed scattered hemorrhagic foci. Spleen about 50% enlarged. Hepatic lymph glands slightly enlarged.	B.abortus + in lung, liver, kidney, spleen.	1:500
8	375gm.	-	1cc. of 5cm. turbidity	7/30/28	3cc.	7/30/28	445gm.	9/1/28	Lungs showed scattered foci. Liver normal. Spleen twice normal size. Sacchreal lymph glands and superficial inguinal lymph glands enlarged.	B.abortus not found	1:500
9	395gm.	-	1cc. of 5cm. turbidity	7/30/28	1cc.	7/30/28	455gm.	9/1/28	Lungs showed few scattered foci. Liver showed grayish foci (6). Spleen twice normal size with 3 grayish foci about 3 mm. Superficial lymph glands enlarged.	B.abortus + in kidney	1:500
10	360gm.	-	1cc. of 5cm. turbidity	7/30/28	1cc.	7/30/28	475gm.	9/1/28	Spleen twice normal size. Left superficial lymph gland slightly enlarged.	B.abortus + in liver, 1 colony from spleen	1:500
11	320gm.	-	1cc. of 5cm. turbidity	7/30/28	0.1 cc.	7/30/28	465gm.	9/1/28	Lungs showed marked grayish foci. Spleen 50% enlarged	B.abortus + in kidney	1:500
12	410gm.	-	1cc. of 5cm. turbidity	7/30/28	0.1 cc.	7/30/28	505gm.	9/1/28	Lungs showed marked number of foci. Liver showed 7 grayish foci. Spleen slightly enlarged. Superficial inguinals greatly enlarged (4-5 times)	B.abortus not found	1:500

TABLE XXI GROUP #1.

Pigs Exposed to Infection and Treated with Serum (Horse # 1). (Agglutination titre 1:30,000).

Intraperitoneal Injection

Pig No.	Weight before exposure	Agg. titre	Amount culture used	Date culture injected	Amt. serum	Date serum used	Weight after 6 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre at autopsy
13	400gm.	-	1 agar slant	8/3/28	3cc.	8 1/2 hr. after injection	455gm.	9/5/28	Lungs showed several pneumonic areas with grayish foci. Liver showed several areas of grayish foci. Spleen about 3 x normal size with enormous number of large (2-4 mm.) grayish foci. Hepatic lymph gland slightly enlarged. Superficial inguinals slightly enlarged with grayish foci. Sachreal glands slightly enlarged.	B. abortus + in lungs, liver, spleen, and kidney	1:500
14	400gm.	-	1 agar slant	8/3/28	3cc.	8 1/2 hr. after injection	465gm.	9/5/28	Scattered hemorrhagic (pneumonic) areas with grayish foci in lungs. Spleen about 3 x normal size with scattered grayish foci. Hepatic lymph glands 2 x normal size with grayish foci (2 mm.) Superficial inguinals and sachreal glands slightly enlarged.	B. abortus + in lungs	1:500
15	405gm.	-	1 agar slant	8/3/28	1cc.	8 1/2 hr. after injection	450gm.	9/5/28	Scattered hemorrhagic (pneumonic) areas with grayish foci in lungs. Spleen about 2 x normal with few foci about 3-5 mm. in diameter. Hepatic lymph glands, Sachreal glands, and superficial inguinals slightly enlarged.	B. abortus not found	1:500
16	395gm.	-	1 agar slant	8/3/28	1cc.	8 1/2 hr. after injection	410gm.	9/5/28	Lungs showed few pneumonic areas. Spleen 3-4 x normal with abundant grayish foci. Hepatic lymph glands slightly enlarged. Superficial inguinals greatly enlarged (5-8 x) with large cheesy foci and very hemorrhagic. Sachreal glands 3-4 x normal.	B. abortus not found	1:500
17	390gm.	-	1 agar slant	8/3/28	0.1 cc.	8 1/2 hr. after injection	350gm.	9/5/28	Three small areas (1/8 - 1/4") areas of pneumonia in lung. Few small grayish foci in lungs.	B. abortus not found.	1:500
18	425gm.	-	1 agar slant	8/3/28	0.1 cc.	8 1/2 hr. after injection	385gm.	9/5/28	Lungs showed scattered hemorrhagic areas. Left kidnay small (2-4 mm.) necrotic area.	B. abortus not found	1:500

Pigs Exposed to Infection and Treated with Serum (Horse # 1). (Agglutination titre 1:30.000)

Intraperitoneal Injection

Pig No.	Weight before injection	Agg. titre	Amount injected	Date of exposure	Weight after 6 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre at autopsy
19	375gm.	-	2 cc. serum	8/3/28	385gm.	9/5/28	Very small hemorrhagic areas in lungs. (1-2 mm.)	B.abortus not found	- T---- 1:25
20	445gm.	-	3 cc. serum	8/3/28	575gm.	9/5/28	Few small (1-2 mm.) hemorrhagic areas in lungs.	B.abortus not found	-----

DISCUSSION OF RESULTS IN TABLES XX, XXI, XXII, AND XXIII.

Group one shows the results obtained from guinea pigs injected intraperitoneally with varying amounts of hyper-immune horse serum and microorganisms (strain 238). The agglutination titre was determined at the end of a six weeks period after injection. Guinea pigs one to eighteen inclusive showed an agglutination titre of 1:500, while guinea pigs # 19 and 20 showed only a slight agglutination reaction. The last two were treated with two cubic centimeters of serum only. This group of guinea pigs gained weight in the majority of cases. Typical Brucella abortus lesions were found in all excepting guinea pigs # 6, 19, and 20. Although typical lesions were found in all but three of the pigs, there is a noticeable decrease in the apparent systemic reaction as the amount of serum was decreased. In every case in which the administered serum was decreased Brucella abortus colonies were not obtained from seedings of the organs, although there were macroscopic changes which were characteristic of Brucella abortus infection. It was also noted that in Table XX where the largest infecting dose was given and one tenth cubic centimeter of serum was given simultaneously, one guinea pig showed foci in the liver while the organs of the other guinea pig were normal. Brucella abortus was not cultured from either guinea pig.

TABLE XXIV GROUP # 2

Brown's Serum. Agglutination titre 1:1000

Each Pig Received 1/3 Agar Slant (48 hrs.) of Culture 198 and Serum Dilutions.

Pig No.	Weight before exposure	Agg. titre before	Serum Dil. used with culture	Date injected	Weight after 5 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre after autopsy
1	565gm.	-	1:25	3/1/29	550gm.	4/1/29	Liver several scattered grayish foci.	No growth	1:500
2	785gm.	-	1:25	3/1/29	790gm.	4/1/29	Lungs grayish foci, also liver. Spleen 50% enlarged.	B. abortus found in liver, spleen, and kidney.	1:500
3	450gm.	-	1:50	3/1/29	390gm.	4/1/29	Lungs - grayish foci and pin-point hemorrhages. Liver - grayish foci.	B. abortus found in spleen and kidney.	1:500
4	495gm.	-	1:50	3/1/29	545gm.	4/1/29	Lungs - pin point hemorrhages, Liver - grayish foci. Spleen 50 % enlarged showed grayish foci.	B. abortus found in spleen.	1:500
5	480gm.	-	1:100	3/1/29	470gm.	4/1/29	Lungs - pneumonic. Liver - grayish foci. Spleen - many x normal with grayish foci.	B. abortus found in lung, liver, spleen and kidney.	1:500
6	635gm.	-	1:100	3/1/29	535gm.	4/1/29	Liver - grayish foci, numerous. Spleen 50 % enlarged. Left testicle abscessed.	B. abortus found in liver and kidney.	1:500
7	420gm.	-	1:500	3/1/29	460gm.	4/1/29	Liver - grayish foci. Spleen 50 % enlarged.	B. abortus found in spleen and kidney.	1:500
8	450gm.	-	1:500	3/1/29	435gm.	4/1/29	Liver - grayish foci. Spleen 5-6 x normal with grayish foci. Blind in one eye.	-	1:500
9	525gm.	-	1:1000	3/1/29	550gm.	4/1/29	Liver numerous gray foci. Spleen 6-8 x normal with gray foci.	B. abortus found in lungs and liver	1:500
10	725gm.	-	1:1000	3/1/29	705gm.	4/1/29	Lungs pneumonic. Liver enlarged with grayish foci.	B. abortus found in liver.	1:500
11	680gm.	-	1:4000	3/1/29	565gm.	4/1/29	Liver numerous gray foci. Spleen 6-8 x normal. Both testicles abscessed.	B. abortus found in Kidney and spleen.	1:500
12	820gm.	-	1:4000	3/1/29	800gm.	4/1/29	Lungs - pneumonic. Liver - gray foci. Spleen 4-5 x normal with gray foci.	B. abortus found in liver, spleen, and kidney.	1:500
13	780gm.	-	1:8000	3/1/29	615gm.	4/1/29	Liver - few scattered gray foci. Spleen twice normal.	B. abortus found in spleen.	1:500
14	695gm.	-	1:8000	3/1/29	620gm.	4/1/29	Lungs - pneumonic. Liver - gray foci. Spleen 50 % enlarged.	B. abortus found in lung, liver, spleen, and kidney.	1:500
15	705gm.	-	1:16000	3/1/29	680gm.	4/1/29	Liver - numerous gray foci. Spleen 6-8 x normal with gray foci. Superficial inguinals large and congested. Both testicles abscessed.	B. abortus found in spleen.	1:500
16	6.5gm.	-	1:16000	3/1/29	615gm.	4/1/29	Liver - gray foci. Spleen 50 % enlarged.	B. abortus found in liver and spleen.	1:500
17	765gm.	-	1:32000	3/1/29	530gm.	4/1/29	Lungs pneumonic. Liver gray foci. Spleen twice normal.	B. abortus found in lungs, liver, and spleen.	1:500
18	655gm.	-	1:32000	3/1/29	570gm.	4/1/29	Lungs - pin point hemorrhages, Liver - gray foci. Spleen 4-6 x normal with gray foci. Superficial inguinals enlarged and congested.	B. abortus found in spleen.	1:500
19	670gm.	-	1:64,000	3/1/29	575gm.	4/1/29	Lungs pin point hemorrhages. Liver numerous gray foci. Spleen 6-7 x normal with gray foci. Superficial inguinals and hepatic enlarged and congested.	B. abortus found in spleen and kidney	1:500
20	585gm.	-	1:64,000	3/1/29	490gm.	4/1/29	Lungs pin-point hemorrhages. Liver gray foci. Spleen 5-6 x normal. Superficial inguinals slightly enlarged.	B. abortus found in spleen and kidney.	1:500
21	425gm.	-	1:128,000	3/1/29	395gm.	4/1/29	Lungs - few scattered gray foci. Spleen 2-3 x normal. Blind in right eye.	B. abortus found in spleen and kidney.	1:500
22	540gm.	-	1:128,000	3/1/29	510gm.	4/1/29	Lungs pin-point hemorrhages. Liver gray foci. Spleen twice normal.	-	1:500
23	475gm.	-	1:512,000	3/1/29	510gm.	4/1/29	Liver - gray foci. Spleen twice normal.	B. abortus found in kidney.	1:500
24	470gm.	-	1:512,000	3/1/29	470gm.	4/1/29	Liver - scattered gray foci. Spleen - twice normal with adhesions.	B. abortus found in liver and spleen.	1:500
25	430gm.	-	1:1,024,000	3/1/29	425gm.	4/1/29	Lungs pneumonic. Liver scattered gray foci. Spleen 3 x normal with gray foci.	B. abortus found in spleen.	1:500
26	445gm.	-	1:1,024,000	3/1/29	500gm.	4/1/29	Lungs pin-point hemorrhages. Liver - numerous gray foci. Spleen slightly enlarged. Superficial inguinals enlarged and congested.	B. abortus found in spleen and kidney.	1:500
27	405gm.	-	No serum	3/1/29	Dead	-	Lungs pin-point hemorrhages. Liver numerous gray foci. Spleen slightly enlarged.	No cultures made	1:500
28	620gm.	-	No serum	1/2 dose spilled thus not injected	650gm.	4/1/29	Liver - numerous gray foci. Spleen twice normal with gray foci.	B. abortus found in lungs, liver, and spleen.	1:500

TABLE XXIV GROUP #2.

Cow Serum # C4. Agglutination titre 1:1000

Each Pig Received 1/3 Agar Slant (48 hrs.) of Culture 198 and Serum Dilutions.

Pig No.	Weight before exposure	Agg. titre before	Serum Dil. used with culture	Date injected	Weight after 5 wks.	Date of autopsy	Autopsy findings	Bacteriological findings	Agg. titre after autopsy
29	620gm.	-	1:25	3/1/29	650gm.	4/2/29	Half of dose spilled. Liver numerous gray foci. Spleen twice normal size.	B. abortus found in lungs, liver, and spleen.	1:500
30	620gm.	-	1:25	3/1/29	680gm.	4/2/29	Lungs pin-point hemorrhages. Liver gray foci. Spleen twice normal. Right testicle abscessed.	B. abortus found in spleen	1:500
31	450gm.	-	1:50	3/1/29	460gm.	4/2/29	Lungs - pneumonic. Liver few gray foci. Kidney - numerous large gray foci.	B. abortus not found	1:500
32	510gm.	-	1:50	3/1/29	540gm.	4/2/29	Lungs - pin-point hemorrhages Liver - gray foci. Spleen 4-5 x normal.	B. abortus found in liver, spleen, and kidney.	1:500
33	500gm.	-	1:100	3/1/29	535gm.	4/2/29	Lungs - pin point hemorrhages Liver very few gray foci. Spleen 4-5 x normal.	B. abortus found in spleen and kidney	1:500
34	385gm.	-	1:100	3/1/29	450gm.	4/2/29	Liver - very few gray foci. Spleen 4-6 x normal with gray foci.	B. abortus found in spleen and kidney.	1:500
35	370gm.	-	1:500	3/1/29	453gm.	4/2/29	Lungs - pin point hemorrhages. Liver few small gray foci. Spleen 3-4 x normal with gray foci. Superficial inguinals congested.	B. abortus found in liver and spleen	1:500
36	400gm.	-	1:500	3/1/29	370gm.	4/2/29	Lungs pneumonic. Liver few gray foci.	B. abortus found in spleen.	1:500
37	460gm.	-	1:1000	3/1/29	530gm.	4/2/29	Liver few gray foci.	B. abortus: few colonies from lungs, liver, spleen, and kidney	1:500
38	425gm.	-	1:1000	3/1/29	450gm.	4/2/29	Lungs pin point hemorrhages. Liver few gray foci.	B. abortus found in spleen.	1:500
39	525gm.	-	1:4000	3/1/29	665gm.	4/2/29	Lungs pneumonic. Liver gray foci. Spleen twice normal. Kidney gray foci (several).	B. abortus found in spleen and kidney.	1:500
40	640gm.	-	1:4000	3/1/29	600gm.	4/2/29	Lungs pneumonic. Liver numerous gray foci. Spleen 4-5 x normal with gray foci. Right testicle abscessed.	B. abortus found in spleen, kidney, and testicle.	1:500
41	520gm.	-	1:8000	3/1/29	570gm.	4/2/29	Lungs pneumonic. Liver numerous gray foci. Spleen 4-5 x normal - gray foci. Kidney petechial hemorrhages. Both testicles abscessed and seminal vesicles filled with gray fluid.	B. abortus found in spleen and testicle.	1:500
42	465gm.	-	1:8000	3/1/29	620gm.	4/2/29	Lungs gray foci. Liver gray foci. Spleen twice normal.	B. abortus found in liver and spleen.	1:500
43	555gm.	-	1:16,000	3/1/29	520gm.	4/2/29	Lungs pin-point hemorrhages. Liver gray foci. Spleen 3-4 x normal with gray foci.	B. abortus found in liver and spleen.	1:500
44	520gm.	-	1:16,000	3/1/29	610gm.	4/2/29	Lungs - pneumonic. Liver - gray foci. Spleen gray foci with 3-4 x normal. Kidneys pin-point hemorrhages.	B. abortus found in spleen	1:500
45	445gm.	-	1:32,000	3/1/29	495gm.	4/2/29	Liver - gray foci (few). Spleen 50% enlarged with 2 large (2 mm.) gray foci.	B. abortus found in kidney and liver	1:500
46	445gm.	-	1:32,000	3/1/29	520gm.	4/2/29	Liver scattered gray foci.	B. abortus found in kidney.	1:500
47	325gm.	-	1:64,000	3/1/29	335gm.	4/2/29	Kidney small gray foci	B. abortus found in liver, spleen, and kidney.	1:500
48	385gm.	-	1:64,000	3/1/29	460gm.	4/2/29	Normal	B. abortus found in liver, kidney, and spleen.	1:500
49	350gm.	-	1:128,000	3/1/29	455gm.	4/2/29	Liver 5 light gray areas.	B. abortus found in liver and lung	1:500
50	375gm.	-	1:128,000	3/1/29	555gm.	4/2/29	Lungs pneumonic. Spleen 2 x normal.	-	1:500
51	380gm.	-	1:512,000	3/1/29	400gm.	4/2/29	Liver 1 gray foci on margin. Spleen 50 % enlarged.	B. abortus found in liver.	1:500
52	455gm.	-	1:512,000	3/1/29	510gm.	4/2/29	Lungs pneumonic. Spleen 50% enlarged.	-	1:500
53	365gm.	-	1:1,024,000	3/1/29	340gm.	4/2/29	Lungs pneumonic.	-	1:500
54	360gm.	-	1:1,024,000	3/1/29	430gm.	4/2/29	Liver - gray foci. Spleen 2 x normal.	-	1:500
55	430gm.	-	No serum	3/1/29	495gm.	4/2/29	Lungs pneumonic. Liver gray foci. Spleen 5-6 x normal with gray foci.	B. abortus found in lung and liver,	1:500
56	390gm.	-	No serum	3/1/29	460gm.	4/2/29	Lungs pneumonic. Liver gray foci. Spleen 3-4 x normal - gray foci. Lymph gland 3-4 x normal.	B. abortus found in lung and liver,	1:500

The data in Tables XXIV and XXV show results from guinea pigs injected intraperitoneally with varying amounts of serum simultaneously with one third of an agar slant of microorganisms, culture 198. Serum from human and bovine sources were employed. It may be observed that each guinea pig had an agglutination titre of 1 to 500 at the end of a five weeks period following injection. It is also apparent that there was a slight loss in weight in the guinea pigs treated with human serum, and an increase when the bovine serum was employed. This may have been due to the fact that older pigs were employed when the human serum was used. In all excepting one pig (number 48) gross anatomical changes were observed, although pigs 46 to 54 inclusive showed a marked decrease in the extent of the gross anatomical changes. It may also be observed in the higher dilutions of bovine (pigs 50, 52, 53, and 54) serum no growth was obtained from cultures of the organs. One may also observe a zone in the gross pathological findings from the guinea pigs treated with a dilution of 1 to 64,000 to 1 to 1,024,000 of bovine serum (Table XXV). As both the decrease in gross pathological findings and the failure to culture the organism from the organs of the infected guinea pigs are overlaping, it would indicate that there is a dilution factor necessary in obtaining beneficial results. When human serum was employed, the results were uniformly negative

DISCUSSION

It is interesting if not instructive to compare the results of the "in vitro" and the "in vivo" methods. The results presented in this paper, based upon the "in vitro" bactericidal study of 11 sera from human, bovine, equine, and caprine sources indicate that the hyperimmune bovine sera and the immune human sera show a slightly varying bactericidal zone in dilutions between 1 to 500 and 1 to 64,000. While the "in vivo" study of 5 sera, employing 235 guinea pigs, indicates that the bovine sera has a therapeutic value in dilutions between 1 to 32,000 and 1 to 1,028,000.

From these observations the inference may be drawn that in a specific serum for Brucella abortus the bactericidal effect may exhibit itself in a zone brought about by dilution. It is not yet apparent how the dilutions of the sera and the bactericidal action are related to one another.

It is clear that the agglutinin production and the agglutinin titre are not a measure of the serum's lytic power. Whatever be the chemical nature of the various products derived from the body tissues and fluids, which bring about the bactericidal action under experimental conditions, their concentration in the serum is certainly not in direct proportion to the agglutination titre; nor is the concentration of the lytic substance of the sera in direct relation with the therapeutic value, as was shown by the presence of the lytic action in very high dilutions of the sera following exposure to infection, are not quantitatively related.

SUMMARY

1. Observations on hyperimmune bovine, equine, and caprine sera and immune human sera show a zone of bactericidal action.
2. This zone appeared in the hyperimmune bovine sera and the immune human sera, and was independent of the agglutination titre.
3. The zones in the "in vitro" and the "in vivo" tests do not coincide.
4. Cow sera appeared to give the more satisfactory results.

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