"OBSERVATIONS ON THE EFFECT OF CHICKEN BLOOD PLASMA AND SERUM ON THE GROWTH OF SALMONELLA PULLORUM"

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INTRODUCTION

The object of this experiment was to observe the bactericidal action of chicken serum and plasma. First, observations were made on the killing power of normal chicken serum and plasma and then on the killing power of infected and hyperimmune serum and plasma. For comparison, human serum and plasma were used. It was hoped that with the collective results, thus obtained, a definite contribution toward a better understanding of *Salmonella pullorum* disease in chickens would result.

Much work has been done by others on normal animal serum using various species of organisms. The bactericidal action of each serum varies with each species and strain of organisms. Since the bactericidal action varies toward each species of organisms, it is necessary to test the specific serum and plasma on specific organisms.

The first work concerning the bactericidal action of chicken serum on *S. pullorum* was done by Bahler, Hodes and Hartsell in 1941. They experimented on the bactericidal action of normal chicken serum on *S. pullorum* and found that the bactericidal property of fowl serum increased with the age of the birds.

This work had emphasis on the bactericidal action of infected and hyperimmune serum and plasma as well as the normal serum.

REVIEW OF LITERATURE

A review of the literature shows that much work on the reactions of various serums was done by Nuttall. The classical observations of of Nuttall[1] (1888) and many others first contributed to our knowledge of serum reactions. It has been recognized that the bactericidal property of serum is a variable one, differing according to the animal species and the type of organism. There has been uncertainty regarding the specificity or non-specificity of natural bactericidal effects. Muir and Browning[2] (1908) reviewed the literature on this subject and studied the specificity of these reactions by absorption methods. They found that treatment of a normal serum with increasing amounts of bacterial suspension produced first a diminution of the bactericidal action towards the homologous bacterium, and also a decrease in the effect of natural complement-fixing and agglutinating antibodies. This suggested the likelihood that the bactericidal effects of normal serum may be due to multiple specific antibodies sensitizing bacteria to the lytic action of complement.

Thjotta[3] (1919) has shown that during immunization there is produced along with the antibodies, a complement-inhibiting substance which he believes to be separate and distinct from agglutinins, precipitins and bactericidal amboceptor. If sufficient dilution and if extra complement is added, the serum will show bactericidal action, while undiluted, fresh immune serum, mixed with the homologous organisms, exhibits little if any bactericidal effect.

Gordon and Wormall[4] (1928) have shown how bacteriolysis of *Shigella dysenteriae* (Flexner) by normal guinea pig serum depends on the combined

action of complement and a thermostable factor removed from the serum by absorption with particular organisms. The question is further complicated by the fact that different mechanisms may be concerned in the bactericidal action of normal sera and that the facters involved may vary with different organisms. The killing of anthrax bacilli by normal serum was attributed by Gruber (1908) to a product of blood platelets (plakanthrakocidine) which is independent of complement. Leucocytic extracts (leukins) have been shown to possess marked bactericidal properties for certain bacteria (Ledingham 1922). Pettersson[5] (1928) has classified the bactericidal agents of serum into alpha lysins and beta lysins. The former apparently represent the complement acting along with a sensitizing agent analogous to an immune body. The latter, according to Pettersson, consist of a stable "activating" agent (resisting a temperature of 63° C. for 1/2 hour) and an activable principle which unites with the bacteria in the presence of the activing agent.

Finkelstein[6] (1931) summarized his results as follows:

1. An analytical study has been made of the mechanism of natural bactericidal action by the serum of various animals towards certain organisms exhibiting the maximum reactivity to this effect.
2. The serum-complement has no bactericidal action by itself and an antibody-like agent invariably acts as an intermediary agent, "sensitizing" the particular organism to the action of the complement and capable of being "absorbed" by it from serum at 0° C.
3. This sensitizing agent is stable at 55° C. but labile at 60°-65° C. In this respect it resembles natural hemolysins

and agglutinins, but contrasts with the more stable immune antibodies and the more labile nature complement-fixing antibodies.

4. The absorption tests demonstrate the high degree of specificity of these natural bactericidal antibodies for particular bacteria.
5. A non-specific extracellular substance occurs in bacterial cultures which may neutralize or inhibit these antibodies, and interfere with their sensitizing action even at 0° C. This substance is liberated in large amounts in cultures heated to 120° C.

In 1932, Finkelstein[7] demonstrated that the bactericidal property of normal serum towards gram-negative bacteria is labile at 55° C. for 30 minutes; the factors responsible for the corresponding effect on gram-positive bacteria are stabile at this temperature. Thus the gram-negative and gram-positive organisms are acted on by separate mechanisms, the "thermolabile" and "thermostable" bactericidins respectively. Bactericidal effects are more frequent and pronounced towards the gram-negative than the gram-positive bacteria. The "thermolabile" bactericidin consists of complement and a sensitizing antibody. The lability of the bactericidin is due to the lability of the complement. The antibody is stable at 60° C. and specific for the particular organism acted on. The "thermostable" bactericidin in undiluted serum withstands a temperature of 57.5° C. though labile at 60° C.; its lability is considerably increased in diluted serum and in slightly alkalmized serum though unaltered by slight acidity.

The work of Gordon[8] (1933) demonstrated that the absorption of both normal and heated sera by dead bacteria fails to yield any evidence of the existence of a series of specific antibodies in serum. The loss of bactericidal power consequent upon absorption is never specific for the absorbing organism but is always general.

Mudd[9] (1933) showed that sensitization by serum renders various dissimilar bacteria similar with respect to their surface properties. This convergence of surface properties is carried further by homologous immune than by heterologous or normal sera, and the homologous immune sera are effective in higher dilutions.

The work of Gordon and Johnstone[10] (1940) also showed that the absorption of a normal serum with a series of strains of one organism causes a general diminution in bactericidal power for all the strains, but there is a more striking diminution for the strain with which the serum was absorbed. Three strains of Micrococcus catarrhalis was used to absorb the guinea pig serum. They also demonstrated that the complement titer of guinea pig serum was high, of human serum low and of rabbit serum still lower. The results shows low bactericidal action of human serum on the gonococcus, whereas guinea pig serum with a higher and rabbit serum with a lower complement titer were both markedly bactericidal. In this experiment one human serum had no bactericidal action on Vibrio cholerae but another human serum killed Vibrio cholerae in one hour. The rabbit serum, which had a lower complement titer than the guinea pig serum, was again the more bactericidal, and inactivation of complement completely destroyed the bactericidal action of both sera. Gordon and Johnstone showed that many species specific antibodies can be individually absorbed or that there is a general bactericidal antibody which can be so modified by contact with a large excess of any particular organism or strain as to render it specifically inactive for that organism or strain.

Bahler, Hodes and Hartsell[11] (1941) studied the normal bactericidins of the domestic fowl. They found that bactericidins, active against a certain strain of S. pullorum, vary with the age of the bird, appearing first at 37 days of age, increasing to a maximum of 143 days and showing fluctuating activity for 164 days. The presence of sodium citrate in the plasma did not cause any reduction in the bactericidal action of most samples.

MATERIALS AND METHODS

Preparation of culture.

A smooth strain of *Salmonella pullorum* (p. 19) was used in this experiment. The organism was grown on nutrient agar slants for 24 hours at 37° C. The growth was removed by means of a sterile wire loop and suspended in sterile diluting fluid, which consisted of 0.05% tryptose peptone at 0.5% sodium chloride in distilled water. Ten ml. of this diluting fluid was usually used for each agar slant. The bacterial suspension was transferred into a sterile test tube then thoroughly mixed and then diluted to a scale of 40 on the photolometer. The standard suspension contained from 8×10^{1} to 8×10^{10} of live *S. pullorum* organisms per ml. Serial dilutions ranging from 8×10^{-2} to 8×10^{-6} were made from this suspension in the same diluting fluid. The number of organisms present was determined by plating 0.5 ml. of 10^{-9} and 10^{-8} dilutions. The pour plate method was used. The nutrient agar was melted, cooled to 45° C. and poured into the Petri dish which was rotated to mix the content well before the agar solidified. When the agar was solidified the plates were incubated for 3 to 4 days at 37° C. and then the colonies were counted. The initial number of bacteria added to the blood plasma or serum from different dilutions can be calculated by multiplying the number counted by the dilution factor.

Preparation of blood.

Each bird was tested for *S. pullorum* infection by using the stained antigen rapid whole blood test.

Blood for the bactericidal test was drawn aseptically from the heart of the bird and placed in a sterile bottle. For the tests requiring plasma 0.1 ml. of sterile saturated sodium citrate solution for each 10 ml. of blood was placed in the bottles. When heparin was used as a substitute for the sodium citrate, 0.1 ml. of heparin was used for every 10 ml. of blood.

The plasma was separated from the whole blood by centrifuging at 2,500 R. P. M. for 25 minutes. The supernatant was poured off aseptically into another sterile tube. Serum was obtained by allowing the blood to clot after which it was centrifuged if necessary.

All tests were made within 24 hours after collection of blood. During this period the blood specimens were kept in an ice box (4^o C.).

Infected chickens were hyperimmunized by injecting 1. ml. of live S. pullorum suspended in saline (8×10^{10}) intravenously. One week later 1 ml. of dead S. pullorum suspension (suspension was boiled to kill the organisms) was injected into the same chicken intravenously. Blood was drawn a week after the last injection. The purpose of using the live and dead organisms was to raise the antibody titer to the maximum.

The bactericidal test.

In order to show the maximum bactericidal activity of serum or plasma on S. pullorum, two methods of setting up the tests were used. In one the volume (1 ml.) of undiluted serum or plasma was kept constant in a series of tubes to which were added the same volume of diluting fluid containing live organisms in varying numbers. In the second, serial dilutions of serum or plasma, in 1 ml. amounts, were placed in sterile tubes to which were added the same volume of diluting fluid containing a constant number of live S. pullorum. The tubes were shaken and incubated at 37^o C.; the length of time varied with the experiment.

At the end of the period of incubation o.25 ml. of the mixture was taken from each tube and placed in a sterile Petri dish. Melted nutrient agar was cooled to 45° C. Ten ml. amounts were then poured into each Petri dish. The contents were mixed by rotation and then allowed to harden after which the plates were incubated at 37° C. for 3 days. Colony counts were made and compared with those of the control tubes. The same procedure was repeated at the end of 4 hours, 8 hours, 24 hours and 48 hours.

Table No. 1 shows how the dilutions are being prepared and the amounts of *S. pullorum* suspension used.

TABLE NO. 1

DILUTIONS OF SALMONELLA PULLORUM

SUSPENSION USED IN THIS EXPERIMENT

Tubes	1	2	3	4	5	6	7	8	9	10
Saline Added	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.	9. ml.
Pullorum Suspension Added	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →	1. ml. →
Dilutions	1-10	1-100	1-T	1-10T	1-100T	1-M	1-10M	1-100M	1-B	1-10B

Nine milliliters of saline solution (containing 0.05% tryptose peptone and 0.5% sodium chloride) were placed in all test tubes, then pipetted 1. ml. of suspension into the first tube. The content was well mixed, then pipetted 1. ml. of tube no. 1 mixture into tube no. 2 and mixed. The following tubes were carried out likewise.

T = Thousand

M = Million

B = Billion

TABLE NO. 2

AGGLUTINATION TEST

Number of Chicken	Dilutions								
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Normal									
No. 533	++								
No. 530	++								
No. 546	++								
Infected									
No. 45	++++	++++	++++	+++	++				
No. 563	++++	++++	++++	+++	++	+			
No. 591	++++	++++	++++	+++	++	+			
Hyperimmune									
No. 508	++++	++++	++++	++++	++++	++++	+++	++	+
No. 555	++++	++++	++++	++++	++++	++++	+++	+++	+
No. 588	++++	++++	++++	++++	++++	++++	+++	++	+
Human									
No. 1.	++++	+++	+						

The _Salmonella pullorum_ antigen was adjusted to pH 8.2, then 1.9 ml. of the above antigen was placed in tube no. 1 and 1. ml. in the following tubes; pipetted 0.1 ml. of the testing serum into tube no. 1 and mixed well, then transferred 1. ml. into the second tube. The following tubes were carried out likewise.

TABLE NO. 3

SODIUM CITRATE PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	7×10^8	677	29	2	0
	7×10^7	29	3	0	0
	7×10^6	2	1	0	0
	7×10^5	0	0	0	0
	7×10^4	0	0	0	0
Diluted					
1: 4	7×10^5	707	62	0	0
1: 8	7×10^5	N	161	1	0
1:16	7×10^5	N	N	N	N
1:32	7×10^5	N	N	N	N
1:64	7×10^5	N	N	N	N

Rate of Bactericidal Action of Infected Plasma

Undiluted Plasma	7×10^8	N	N	N	N
	7×10^7	N	N	N	N
	7×10^6	46	35	N	N
	7×10^5	3	8	5	0
	7×10^4	0	0	0	0
Diluted					
1: 4	7×10^5	N	N	N	N
1: 8	7×10^5	N	N	N	N
1:16	7×10^5	N	N	N	N
1:32	7×10^5	N	N	N	N
1:64	7×10^5	N	N	N	N
Control	7×10^4	N	N	N	N

The results on this table show that the normal chicken plasma has a better bactericidal action than that of the infected chicken plasma in vitro.

N = Too numerous to count

0 = No growth

TABLE NO. 4

SODIUM CITRATE PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	6×10^8	400	203	0	0
	6×10^7	15	6	0	0
	6×10^6	0	0	0	0
	6×10^5	0	0	0	0
	6×10^4	0	0	0	0
Diluted					
1: 4	6×10^5	400	159	0	0
1: 8	6×10^5	N	967	501	105
1:16	6×10^5	N	N	N	N
1:32	6×10^5	N	N	N	N
1:64	6×10^5	N	N	N	N

Rate of Bactericidal Action of Infected Plasma

Undiluted Plasma	6×10^8	N	N	N	N
	6×10^7	N	N	500	84
	6×10^6	126	121	14	7
	6×10^5	22	23	1	0
	6×10^4	4	5	0	0
Diluted					
1: 4	6×10^5	N	N	N	N
1: 8	6×10^5	N	N	N	N
1:16	6×10^5	N	N	N	N
1:32	6×10^5	N	N	N	N
1:64	6×10^5	N	N	N	N
Control	6×10^4	N	N	N	N

The results on this table show that the normal chicken plasma has a better bactericidal action than that of the infected chicken plasma in vitro also.

TABLE NO. 5

SODIUM CITRATE PLASMA

Rate of Bactericidal Action of Infected Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	8×10^8	N	N	N	N
	8×10^7	N	N	N	1016
	8×10^6	N	964	N	358
	8×10^5	963	551	181	302
	8×10^4	333	254	161	200
Diluted					
1: 4	8×10^4	N	1026	977	N
1: 8	8×10^4	N	N	N	N
1:16	8×10^4	N	N	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N

Rate of Bactericidal Action of Infected Serum

Reacting Substance	Number of Bacteria Added	4	8	24	48
Undiluted Serum	8×10^8	N	N	N	N
	8×10^7	N	N	967	N
	8×10^6	N	988	664	N
	8×10^5	699	232	433	N
	8×10^4	201	199	349	N
Diluted					
1: 4	8×10^4	N	N	N	N
1: 8	8×10^4	N	N	N	N
1:16	8×10^4	N	N	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N
Control	8×10^4	N	N	N	N

The results on the above table indicate that the infected chicken plasma has a better bactericidal action than the infected chicken serum in prolonged incubation in vitro.

TABLE NO. 6

SODIUM CITRATE PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	8×10^8	1121	987	13	11
	8×10^7	452	46	10	4
	8×10^6	42	5	4	0
	8×10^5	15	0	0	0
	8×10^4	2	0	0	0
Diluted					
1: 4	8×10^4	16	1	0	0
1: 8	8×10^4	411	62	3	0
1:16	8×10^4	N	N	671	0
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N
Rate of Bactericidal Action of Normal Serum					
Undiluted Serum	8×10^8	997	195	6	4
	8×10^7	343	36	3	1
	8×10^6	48	7	1	0
	8×10^5	11	4	1	0
	8×10^4	4	3	1	0
Diluted					
1: 4	8×10^4	12	8	18	61
1: 8	8×10^4	992	48	254	N
1:16	8×10^4	N	123	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N
Control	8×10^4	N	N	N	N

The results on this table indicate that the normal chicken plasma and the normal chicken serum have a slight variation in bactericidal action.

TABLE NO. 7

SODIUM CITRATE PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	8×10^8	976	644	22	10
	8×10^7	368	87	5	7
	8×10^6	73	17	3	6
	8×10^5	15	7	1	0
	8×10^4	6	2	1	2
Diluted					
1: 4	8×10^4	48	4	2	2
1: 8	8×10^4	300	160	5	6
1:16	8×10^4	601	939	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N

Rate of Bactericidal Action of Infected Plasma

Undiluted Plasma	8×10^8	N	N	N	N
	8×10^7	N	N	89	12
	8×10^6	1099	877	19	8
	8×10^5	382	249	7	5
	8×10^4	210	90	5	4
Diluted					
1: 4	8×10^4	485	273	27	17
1: 8	8×10^4	1110	988	N	N
1:16	8×10^4	N	N	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N
Control	8×10^4	N	N	N	N

The above results again indicate that the normal chicken plasma has a more effective bactericidal action than that of the infected chicken plasma in vitro.

TABLE NO. 8

HEPARIN PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	8×10^8	N	1126	N	N
	8×10^7	951	438	N	N
	8×10^6	139	109	N	N
	8×10^5	33	24	N	N
	8×10^4	10	20	N	N
Diluted					
1: 4	8×10^4	68	486	N	N
1: 8	8×10^4	411	N	N	N
1:16	8×10^4	N	N	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N

Rate of Bactericidal Action of Infected Plasma

Undiluted Plasma	8×10^8	N	N	N	N
	8×10^7	N	N	N	N
	8×10^6	1000	1115	N	N
	8×10^5	486	586	561	534
	8×10^4	261	230	171	160
Diluted					
1: 4	8×10^4	966	1153	N	N
1: 8	8×10^4	N	N	N	N
1:16	8×10^4	N	N	N	N
1:32	8×10^4	N	N	N	N
1:64	8×10^4	N	N	N	N
Control	8×10^4	N	N	N	N

The above results indicate that the heparin plasma has less bactericidal power than that of the sodium citrate plasma in prolonged incubation periods. The sodium citrate may have some influence on bactericidal action.

TABLE NO. 9

HEPARIN PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	9×10^8	N	1109	N	N
	9×10^7	1188	179	N	N
	9×10^6	204	53	N	N
	9×10^5	58	28	N	N
	9×10^4	39	5	911	N
Diluted					
1: 4	9×10^4	12	5	N	N
1: 8	9×10^4	82	408	N	N
1:16	9×10^4	238	1112	N	N
1:32	9×10^4	286	N	N	N
1:64	9×10^4	339	N	N	N

Rate of Bactericidal Action of Infected Plasma

Reacting Substance	Number of Bacteria Added	4	8	24	48
Undiluted Plasma	9×10^8	N	N	N	N
	9×10^7	N	N	979	1010
	9×10^6	1109	486	165	251
	9×10^5	408	237	65	76
	9×10^4	188	96	35	20
Diluted					
1: 4	9×10^4	66	118	126	300
1: 8	9×10^4	68	154	214	N
1:16	9×10^4	75	193	998	N
1:32	9×10^4	79	360	1121	N
1:64	9×10^4	87	394	N	N
Control	9×10^4	N	N	N	N

The above results indicate that the heparin plasma of the infected chicken has a better bactericidal action than that of the normal chicken plasma in prolonged incubation periods.

TABLE NO. 10

HEPARIN PLASMA

Rate of Bactericidal Action of Human Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	9×10^8	N	346	746	N
	9×10^7	1201	63	8	28
	9×10^6	246	9	4	19
	9×10^5	89	3	4	16
	9×10^4	37	1	1	2
Diluted					
1: 4	9×10^4	49	5	3	18
1: 8	9×10^4	96	9	6	28
1:16	9×10^4	182	66	53	63
1:32	9×10^4	468	445	980	N
1:64	9×10^4	551	886	N	N

Rate of Bactericidal Action of Human Serum

Reacting Substance	Number of Bacteria Added	4	8	24	48
Undiluted Serum	9×10^8	N	N	142	55
	9×10^7	1221	262	97	2
	9×10^6	236	27	12	0
	9×10^5	47	7	3	0
	9×10^4	9	1	3	0
Diluted					
1: 4	9×10^4	1	0	0	0
1: 8	9×10^4	6	1	0	0
1:16	9×10^4	6	1	1	0
1:32	9×10^4	87	13	N	N
1:64	9×10^4	119	82	N	N
Control	9×10^4	N	N	N	N

The above results show that the human plasma and serum are more bactericidal in prolonged incubation periods.

TABLE NO. 11

HEPARIN PLASMA OF THE HYPERIMMUNIZED CHICKEN

Rate of Bactericidal Action of Infected Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours			
		4	8	24	48
Undiluted Plasma	12×10^8	N	N	N	N
	12×10^7	N	N	N	N
	12×10^6	N	N	910	822
	12×10^5	1211	1112	263	301
	12×10^4	270	221	196	99
Diluted					
1: 4	12×10^4	154	156	526	600
1: 8	12×10^4	161	334	710	889
1:16	12×10^4	170	412	762	N
1:32	12×10^4	174	616	1110	N
1:64	12×10^4	216	748	N	N
Undiluted Plasma	12×10^8	N	N	N	N
	12×10^7	N	N	N	N
	12×10^6	N	N	1088	418
	12×10^5	1190	1144	892	408
	12×10^4	245	225	222	78
Diluted					
1: 4	12×10^4	144	187	449	586
1: 8	12×10^4	155	257	460	578
1:16	12×10^4	176	286	650	N
1:32	12×10^4	191	408	780	N
1:64	12×10^4	219	448	971	N
Control	12×10^4	N	N	N	N

The above results indicate that the initial bactericidal action of the hyperimmune chicken plasma is about the same as the normal plasma, but as the incubation period prolonged, the hyperimmune chicken plasma is more effective in vitro.

TABLE NO. 12

HEPARIN PLASMA

Rate of Bactericidal Action of Normal Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Plasma	9×10^{10}	N	N	N	N
	9×10^{9}	N	N	N	N
	9×10^{8}	1431	492	N	N
	9×10^{7}	422	178	1250	N
	9×10^{6}	116	32	0	226
	9×10^{5}	14	6	0	0
	9×10^{4}	6	1	1	0
	9×10^{3}	1	0	0	0
	9×10^{2}	0	0	0	0
	9×10^{1}	0	0	0	0

Rate of Bactericidal Action of Infected Plasma

Undiluted Plasma	9×10^{10}	N	N	N	N
	9×10^{9}	N	N	N	N
	9×10^{8}	N	1124	506	216
	9×10^{7}	1074	750	207	52
	9×10^{6}	275	234	101	8
	9×10^{5}	57	69	48	25
	9×10^{4}	8	11	5	0
	9×10^{3}	1	2	0	0
	9×10^{2}	1	0	0	0
	9×10^{1}	0	0	0	0

Control	9×10^{5}	N	N	N	N
Control	1×10^{1}	22	36	N	N

The hyperimmune chicken plasma indicates a better bactericidal action than that of the normal chicken plasma in vitro.

TABLE NO. 13

HEPARIN PLASMA

Rate of Bactericidal Action of Mixed Plasma

Reacting Substance	Number of Bacteria Added	Colony Count Period of Incubation in Hours 4	8	24	48
Undiluted Mixed Plasma	9×10^{10}	N	N	N	N
	9×10^{9}	N	N	N	N
	9×10^{8}	N	918	217	66
	9×10^{7}	1024	317	123	35
	9×10^{6}	211	146	66	33
	9×10^{5}	51	31	25	1
	9×10^{4}	3	6	3	0
	9×10^{3}	2	4	1	0
	9×10^{2}	0	0	0	0
	9×10^{1}	0	0	0	0
Control	9×10^{5}	N	N	N	N
Control	1×10^{1}	22	36	N	N

The above table shows the results of mixed plasma. The mixed plasma has 50% hyperimmunized chicken plasma and 50% normal chicken plasma. It shows a better bactericidal action than any one plasma acting alone.

RESULTS AND DISCUSSION

Table No. 2 shows the results obtained where normal, infected, hyperimmune chicken sera and human serum are used on S. pullorum agglutinating antigen. The purpose of the agglutination test is to find out if the agglutinating titer has any relationship to the bactericidal action of serum.

According to the results of this experiment, as shown in Table No. 3, when a limited number of S. pullorum was mixed with a definite quantity of normal chicken plasma none of the plates showed any colonies after 4 hours of incubation. The plasma either inhibited the growth or killed the organisms. Further experiments are necessary in order to prove whether the organisms were killed or merely inhibited. When the number of organisms was increased from 7×10^4 to 7×10^6 in 1 ml. of plasma it required about 8 hours of incubation before no growth occurred on the plates. As the number of organisms increased, the period of incubation required for bactericidal action lengthened. When an excess number of organisms (8×10^8 to 1 ml. of plasma) was mixed with a constant quantity of plasma many colonies were present on the plates even after 48 hours of incubation, however, the number of colonies was always declining as the incubation period lengthened.

Table No. 4 shows the results of normal and infected chicken plasma bactericidal action on S. pullorum also. The number of colonies are varied in each corresponding plate which indicates the variability of plasma in different chickens.

In Table No. 5 the results indicate the number of organisms surviving in the infected chicken plasma and serum. It indicates that the infected chicken plasma has a better bactericidal action than the infected chicken serum.

In Table No. 6 the results show that the normal chicken plasma has a slight variation in bactericidal action when comparing it with the normal serum. In some cases the normal chicken plasma seems to possess a better bactericidal action than the normal serum.

The sodium citrate added to the blood may have had some influence on the bactericidal effect. For this reason heparin was used for comparison in the latter part of this experiment. Table Nos. 7 and 8 show the results of 2 sets of tests. Table No. 7 shows that sodium citrate was used and Table No. 8 shows that heparin was used. The same number of organisms were added to each set and were treated under identical conditions. The plasma which had the sodium citrate showed a continuous decrease of organisms while the plasma in which heparin was used had about the same number of organisms after the first 4 hours of incubation, but the decrease of organisms was less than that in the tubes containing sodium citrate in the second 4 hours of incubation, however, showing a definite increase in number in the 24 and 48 hours' incubation period. This condition occurred when a large number of organisms were added to the heparin plasma tubes.

By in vitro tests it was shown that plasma of the infected chickens is less effective than plasma of normal chickens. Table No. 9 indicates

that the tubes of the infected chicken plasma showed a greater number of organisms surviving.

Human plasma showed the same potency of bactericidal action as normal chicken plasma in the first 4 hours of incubation, but it was more effective in the longer incubation period (this individual immunized against typhoid fever) as shown in Table No. 10.

The plasma from hyperimmunized chickens seems to have a better bactericidal action than the normal chicken plasma. It had a more effective and prolonged bacteriostatic action somewhat like the normal sodium citrate plasma. (The results are shown in Table No. 11.)

In Table No. 12 the results indicated that the hyperimmune chicken plasma had about the same degree of bactericidal action in the first 4 hours of incubation. As the incubation period proceeded the tubes with an excess number of organisms (9×10^8) showed a decrease in number of colonies, while those of normal chicken plasma tubes showed increased in number of colonies.

Table No. 13 shows the results when mixed plasma is used. The mixture consisted of 50% hyperimmunized plasma and 50% normal plasma. It indicates an even better bactericidal action than when either normal or hyperimmune plasma alone was used.

Chicken plasma and serum showed a noticeable bactericidal action in dilutions up to 1:8. When the dilutions were higher, the bactericidal effect diminished rapidly.

Human plasma and serum showed a noticeable bactericidal action in dilutions up to 1:16.

The agglutination test showed that the normal chicken serum agglutinated S. pullorum at 1:20 dilution.

The infected chicken serum agglutinated S. pullorum at 1:320 dilution.

The hyperimmune chicken serum agglutinated *S. pullorum* at 1:2560 dilution.

The human serum agglutinated *S. pullorum* at 1:40 dilution. (Individuals had been vaccinated against typhoid paratyphoid.)

Serum with the increase of agglutinating titer generally shows the increase of bactericidal action also, but this does not hold true with the infected chicken serum or plasma.

The failure of plasma of infected chickens to exert a more effective bactericidal action may be due to any one of the following factors: (1) its low complement content, (2) the absence of complement-binding groups on a portion of the bactericidal antibodies, or (3) the presence of anticomplement.

Although the infected chicken plasma may not kill many bacteria per volume concentration in vitro, the agglutination factor alone may aid the prevention of bacteria from spreading in vivo, which could be a protective factor or mechanism.

SUMMARY

The normal chicken serum and plasma have a better bactericidal action than the infected chicken serum and plasma.

The human serum and plasma have a more effective bactericidal action than the normal chicken serum and plasma only in prolonged incubation periods.

The hyperimmune chicken serum and plasma possess a greater bactericidal action than the normal chicken serum and plasma.

The mixed chicken plasma (50% hyperimmune chicken plasma and 50% normal plasma) has the most effective bactericidal action.

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