AN EXPERIMENTAL STUDY OF PHAGOCYTOSIS IN RELATION TO TERMINAL INFECTIONS

--o--

Dissertation

Submitted to the Board of University Studies
of the Johns Hopkins University
in Conformity with the
Requirements for
the Degree
of

Doctor of Philosophy

--o--

by

Howard B. Cross

Baltimore

1921

TABLE OF CONTENTS

AN EXPERIMENTAL STUDY OF PHAGOCYTOSIS IN RELATION TO TERMINAL INFECTIONS

--o--

INTRODUCTION

The circumstances which originally suggested this investigation were the instances of energetic phagocytosis frequently observed at autopsy. The surprising number of bacteria occasionally found within the leucocytes in individuals dying of fatal infection or intoxication suggests the possibility that the diminution of phagocytic activity sometimes associated with destructive infections may not be as nearly universal as is commonly supposed. It further seems probable that in moribund animals, "in which the resistance fails" (1), the phagocytic defense may remain unimpaired, and, occasionally at least, function at a level considerably above the normal. These observations also indicate that terminal infections can not be accounted for by assuming a collapse or a decrease in the activity of the phagocytic functions of the animal.

Fig. 1 illustrates the extent of phagocytosis which may occur in animals dying of fatal infections.

Fig. 1. -- Illustrating the extent of phagocytosis which may occur during the late stages of terminal infection. A. and B. Cells from the peritoneal cavity of a guinea pig dying of B. coli peritonitis. C. Phagocyte from lung of patient dying of influenza pneumonia. D. Leucocyte from peritoneal cavity of a guinea pig dying of staphylococcus peritonitis. E. Leucocyte from blood stream of a guinea pig dying of generalized staphylococcus infection. F. Phagocyte containing diphtheria bacilli from lung of child with terminal pneumonia following diphtheria. G. Cell containing mixed flora from lung of patient dying of terminal pneumonia. H. Phagocyte from pleural exudate of a dog dying of generalized pneumococcus infection.

HISTORICAL SKETCH

The widespread occurrence of terminal infections was strikingly expressed by Osler when he wrote that, "Persons rarely die of the diseases with which they suffer." (2) However, the nature of the rupture in the defensive mechanism of the individual which permits the rapid invasion of the body before death by even the feebly aggressive bacteria, has never been satisfactorily explained. Flexner (3) undertook a study of this condition as early as 1896. He, however, limited his investigations to an examination of the humoral defense without any attempt at ascertaining whether cellular modifications might not be a contributing cause in terminal infections. He thought that he discovered, at least in a few cases, a slight decrease in the bactericidal actions of human serum against staphylococci. The correctness of these findings, however, was soon placed in doubt by Wright, (4) who together with Windsor demonstrated in 1902 that human blood exhibits an almost total absence of any bactericidal influence against staphylococci.

Bordet (5) was one of the first to suggest that the phagocytic activity of the body might be a relatively stable function and not easily influenced by conditions which profoundly effect other vital activities. He demonstrated that deep chloroform anesthesia which "completely deadens" (8) the central nervous system has no disturbing influence upon phagocytosis. This investogator also observed that diptheria

toxin exerts little, if any, effect on phagocytic activity (6).

Tunnicliff (7), in an investigation of the opsonic index during the leucopenia resulting from measles, found that there was a slight decrease for streptococci, staphylococci, and tubercle bacilli. She suggested that the decrease in phagocytic activity against these organisms might "account for the secondary infections" commonly associated with measles.

Bartlett and Ozaki (8) in 1917 injected a dog with a massive dose of B. coli and five hours later, when the animal was in a moribund condition, inoculated it with a quantity of staphylococci. They observed no decrease in the opsonic index for staphylococcus. This experiment was not repeated and the authors themselves were not entirely convinced of the adequacy of their controls.

There is, of course, and extensive literature (Metchnikoff (9); Fevre (11); Wright (10); Bull (12); Hektoen (13); Opie (14), etc.) indicating a sharp and very considerable decline, in some instances at least, in the phagocytic activity of the body against the specific infecting organism in fatal infections. There seems to have been no organized inquiry, however, concerning the opsonic index for those bacteria which take no part in the original infection but which later invade the weakened body giving rise to the destructive phenomenon known as terminal infection.

Bacterial invasion of the wasted body immediately preceeding death is sometimes so complete and sudden that it has

been assumed the process is unopposed. The collapse of the defensive mechanism against bacteria in terminal infections seems to be thorough and complete. Zinsser (15) expressed this idea in a discussion of fatal infections when he wrote: "The infectious process becomes rapidly generalized, the bacteria enter the blood stream and lymphatics, and the defensive powers are overwhelmed." The possibility of a distince part of this protective adaptation of the body remaining intact with unimpaired function.has not been believed and awaits confirmation. It is proposed, therefore, in this research to undertake an investigation of the phagocytic activity in animals after kataphylaxis* has occurred, and to determine what change, if any, occurs in the opsonic index for those organisms which most frequently overrun the body late in fatal infections and intoxications.

. . . . . .

*The word kataphylaxis was introduced by Bullock and Cramer (Proc. of the Royal Society, Series B, vol. 90, p. 513) and was defined by them as a rupture in the local defensive mechanism against bacteria. As used in the present paper the word will be understood to indicate a break-down in the general defensive mechanism sufficiently complete to permit the infection to lead uninterruptedly to the death of the animal.

PLAN OF INVESTIGATION

The investigation of this problem has been done almost entirely *in vitro* because there is no thoroughly dependable method of controlling results when the experiments are undertaken *in vivo*. Wright's (16) work has established that even slight changes in the phagocytic activity of the animal can be demonstrated by *in vitro* methods, so that if there is a decline in opsonic power sufficient to account, even in part, for the bacterial invasion occurring in terminal infections, there could be no difficulty in demonstrating that decrease in effectiveness by the methods employed in this investigation. The bacteria used were those most commonly associated with terminal infections -- a pyogenic coccus, *Staphylococcus aureus*, recently isolated from a case of human furunculosis, and a gram negative bacillus, *B. coli communis*. In addition, two other organisms were commonly used. *B. bronchisepticus* was selected because it is readily taken up in large numbers, making possible dependable counts in weakly phagocytic cells, while *B. typhosus* resisting engulfment to considerable extent, permits reliable enumeration when phagocytosis with other bacteria is so vigorous that even a depressed phagocytic capacity is quite sufficient to fill the cells beyond counting. Whenever possible the specific infecting organism was included in each series of experiments.

TECHNIQUE

The opsonic technique used in this research was essentially the same as that originally employed by Wright and Douglas (17). Certain modifications and refinements, however, were introduced which seemed to insure greater uniformity in the results, and to make this generally confusing technique more dependable and satisfactory.

The blood providing the cells was received into a centrifuge tube containing several volumes of citrated salt solution. Before sedimentation the cells were uniformly suspended by repeated suction and ejection with a pipette. This process was repeated before each centrifugation and is a most effective means of preventing the aggregation of the platelets around the leucocytes. The centrifugation was accomplished at a speed which permitted the sedimentation of the white cells in four to five minutes without throwing down the platelets. This is most important for it eliminates the platelets from the leucocytic layer and prevents packing of the white cells. The washings were repeated three times and the leucocytes suspended in a volume of physiological salt solution equal to one third the original volume of blood. After standing for one hour, the cells were uniformly suspended by a gentle continued agitation of the tube and all samples of any one experimental series were taken immediately. It was early observed that the ingesting capacity of phagocytes was not always as reliable and vigorous if they were used immediately after washing as when they were

allowed to stand for an hour. This is probably occasioned by a disturbance in the osmotic pressure resulting from repeated washings and centrifugation.

Confusing and contradictory results invariably accompanied a careless or hurried preparation of the bacterial suspension. The organisms used in this work were grown, whenever possible, on moist plain agar for fifteen hours. The tube was then washed out with saline to remove lint and debris. Five cubic centimeters of salt solution were next added and the tube gently agitated until the resulting turbidity was equal to or slightly in excess of that desired. This was then transferred to a second clean tube and vigorously shaken to break up possible clumps. Finally the tube was centrifuged at a speed sufficient to remove from suspension all but the single bacteria. Two and five-tenths cubic centimeters were then drawn from the upper portion and reserved for the bacterial suspension. All the samples for any experimental series were regularly taken from the same level in order to insure a more nearly uniform number of organisms. Bacteria which do not grow upon plain agar or which could not be uniformly suspended from solid medium were grown in meat infusion broth. For growing massive cultures of pneumococci, whole blood was added to the broth. The bacteria were sedimented, washed and suspended in salt solution. A turbidity was selected which presented a delicate opalescence in indirect light.

Sera were taken in the usual manner except that those

containing an excess amount of fat were, whenever possible, avoided. Those containing agglutinins for the experimental cells, although thoroughly annoying, can be used without invalidating the results. While a few hours difference in the age of the sera results in no demonstrable difference in their opsonic reactions, all sera for an experimental series were collected as nearly as possible at the same time.

The phagocytic mixtures were prepared according to the Wright technique, and incubated at 37° for twenty minutes. The smears and stains were made in the manner described by Cross (18). All smears showing gross differences in the number or distribution of the leucocytes were discarded and the preparations repeated. The organisms in fifty polymorphonuclear leucocytes were counted and these cells were always enumerated from corresponding areas on the control and experimental slides. Cells containing an excessively large number of bacteria show a tendency to collect in portions of the smear which can be predicted, and the most confusing and contradictory deviations present themselves if the selection of corresponding areas for enumeration is not rigorously observed. All preparations exhibiting marked variations from the normal or expected were repeated throughout. Whenever the phagocytes revealed unusual inequalities in the number of ingested organisms, parallel preparations were made and the average enumeration taken as the true count. Polymorphonuclear leucocytes alone were considered, and no attempt was made at enumeration in cells containing more than twenty-five organisms. In all

Fig. 2

FIG. 1.—Illustrating the appearance of phagocytes and bacteria in smears prepared according to the technique described in this paper. (A) Pus cell from a lung abscess in a multiple infection. (B) Polymorphonuclear leucocyte from the blood of a guinea pig twenty-five minutes after the ingestion of B. proteus. The bacteria are surrounded by vacuoles. One bacillus, partially digested, has lost its staining characteristics. The cell is associated with erythrocytes. (C) Smear from a lung abscess containing cocci, bacilli, and spirochetes. (D) Polymorphonuclear leucocyte containing a colon bacillus within a digestive vacuole.

counts the percentage of ingesting leucocytes as well as the total number of intracellular bacteria were determined.

Fig. 2, taken from the Johns Hopkins Hospital Bulletin (18), illustrates the appearance of the cells from which the enumerations recorded in this paper were made.

SUBJECT MATTER

The data presented in this paper are selected from a study of 85 cases of infection and intoxication which resulted in death. An effort was made to secure the widest possible variety, including both spontaneous and induced infections in animals, supplemented by a series of human cases. The period elapsing between the primary inoculation and death ranged from a few hours to several weeks, and even much longer in some of the human cases. The arrangement of subject matter and condition of each experiment are clearly set forth in the various protocols.

EXPERIMENTAL DATA

PART I

A. Pneumococcus Infections

Experiment 1. -- The dog - No. 11 - used in this experiment was an adult female weighing 15½ pounds. It was inoculated intravenously on November 27, 1920, with a sublethal dose of virulent Pneumococcus, Type I. The sublethal doses were continued on dates indicated in the protocol until the blood exhibited a high bacteriotropic action. The intravenous inocu-

lations were then gradually increased until a sufficient dose was administered to bring about the death of the animal. This result was hastened toward the end of the experiment by injection of pneumococci into the left pleural cavity. A sample of blood was taken before each injection and allowed to clot. Two hours later the serum was withdrawn and the opsonic index determined in the manner described above. The normal dogs providing the leucocytes and control serum were always bled at the same time as the experimental animal. The results of all opsonic determinations are included in the following tables:

Date	Remarks	Bacteria	Animal	No. of Cells Count	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
11/27 20	Dog #11 Before injection wt. 15½ lbs	S.aureus	#11	50	39	11	145	2.9
			Con	50	42	8	121	2.4
		Pn. I	#11	50	1	49	2	0.0
			Con	50	4	46	7	0.1
		B. coli	#11	50	41	9	86	1.7
	Inj. 25cc Pn.I		Con	50	40	10	93	1.8
		B. typhosus	#11	50	43	7	95	1.9
			Con	50	45	5	104	2.0
11/29 20	Dog #11 wt. 14½lbs Inj. 50cc Pn.I	S.aureus	#11	50	42	8	175	3.5
			Con	50	43	7	171	3.4
		Pn. I	#11	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#11	50	43	7	96	1.7
			Con	50	46	4	104	2.0
		B.typhosus	#11	50	35	15	66	1.3
			Con	50	38	12	70	1.4
12/1 20	Dog #11 wt. 13½lbs Inj. 100cc Pn.I	S.aureus	#11	50	41	9	220	4.4
			Con	50	44	6	212	4.2
		Pn. I	#11	50	26	24	101	2.0
			Con	50	5	45	6	0.1
		B. coli	#11	50	19	31	50	1.0
			Con	50	25	25	55	1.1
		B.typhosus	#11	50	13	37	21	0.4
			Con	50	15	35	20	0.4

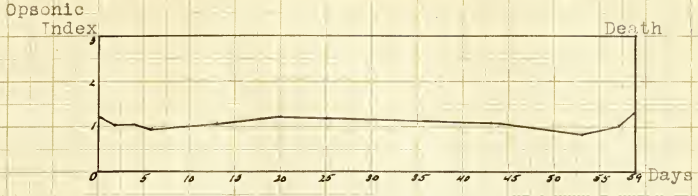
Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
12/3 20	Dog #11	S.aureus	#11	50	24	26	57	1.1
			Con	50	27	23	60	1.2
	wt. 14 lb	Pn.I	#11	50	31	19	100	2.0
			Con	50	2	48	4	0.1
	*Inj. 150cc Pn. I	B. coli	#11	50	23	27	28	0.5
			Con	50	24	26	35	0.7
		B.typhosus	#11	50	39	11	82	1.6
			Con	50	42	8	65	1.6
12/10	Dog #11	S.aureus	#11	50	41	9	166	3.3
			Con	50	38	12	145	2.9
	wt. 13 lb	Pn.I	#11	50	38	12	162	3.2
			Con	50	8	42	22	0.4
	Inj. 200cc Pn.I	B.typhosus	#11	50	21	29	30	0.6
			Con	50	20	30	45	0.9
12/17	Dog #11	S.aureus	#11	50	40	10	269	3.3
			Con	50	43	7	231	4.6
	Wt. 13 lb	Pn. I	#11	50	25	25	75	1.5
			Con	50	10	40	21	0.4
		B. coli	#11	50	32	18	77	1.5
	Inj. 250cc Pn.I		Con	50	33	17	80	1.6
		B.typhosus	#11	50	32	18	70	1.4
			Con	50	38	12	86	1.7
12/22	Dog #11	S.aureus	#11	50	33	17	121	2.4
			Con	50	25	25	104	2.0
	wt. 13 lb	Pn. I	#11	50	25	25	161	3.2
			Con	50	0	50	0	0.0
		B. coli	#11	50	43	7	113	2.2
	Inj. 250cc Pn.I		Con	50	38	12	105	2.1
		B.typhosus	#11	50	30	20	50	1.0
			Con	50	31	19	68	1.3
12/26	Dog #11 wt.13 lb Inj. 250cc Pn.I							
12/30	Dog #11 wt.13 lb Inj. 250cc Pn.I							
1/10 21	Dog #11 wt.13 lb Inj. 250cc Pn. I							

*Pneumococci were grown in meat infusion broth containing 1% whole blood. The bacteria for each injection were sedimented from the quantity indicated in the table, and then suspended in 25 cc. of broth before inoculation.

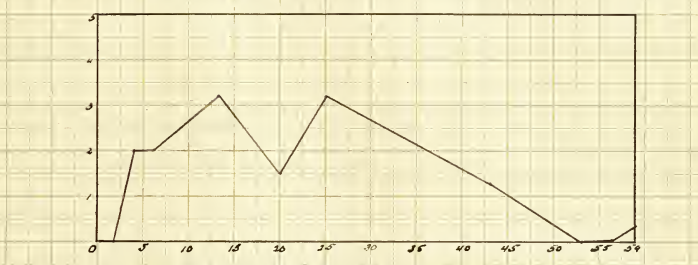
Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
1/10 21	Dog #11	S.aureus	#11	50	37	13	199	3.9
			Con	50	33	17	180	3.6
	wt. 13 lb	Pn. I	#11	50	22	28	62	1.3
			Con	50	8	42	16	0.3
		B. coli	#11	50	41	9	103	2.0
	Inj. 250cc Pn. I		Con	50	45	5	140	2.8
		B.typhosus	#11	50	26	24	27	0.5
			Con	50	23	27	38	0.7
1/20 21	Dog #11	S.aureus	#11	50	36	14	146	2.9
			Con	50	35	15	171	3.4
	wt. 13 lb	Pn. I	#11	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#11	50	42	8	93	1.9
	*Inj. 245cc Pn. I		Con	50	41	9	95	1.9
		B. bronchicep.	#11	50	27	23	65	1.3
			Con	50	42	8	73	1.4
1/22	*Inj. 50cc Pn. I							
1/24	Dog #11	S.aureus	#11	50	34	16	128	2.5
			Con	50	31	19	115	2.3
	wt. 11 lb	Pn. I	#11	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#11	50	41	9	78	1.5
	No inj.		Con	50	41	9	71	1.4
		B. bronchicep.	#11	50	37	13	136	2.7
			Con	50	38	12	137	2.7
1/25	Dog #11	S.aureus	#11	50	35	15	235	4.7
			Con	50	32	18	199	3.9
	wt. 11 lb	Pn. I	#11	50	4	46	8	0.1
			Con	50	0	50	0	0.0
	This reading made 10 hrs before death	B. coli	#11	50	15	35	45	0.9
			Con	50	14	36	50	1.0
		B. bronchicep.	#11	50	39	11	212	4.2
			Con	50	37	13	196	3.9
	No.inj.	B.typhosus	#11	50	7	43	15	0.3
			Con	50	8	42	19	0.3

*The bacteria from 25 cc. of this inoculation were injected into the left pleural cavity. The remaining quantity was given intracardially.

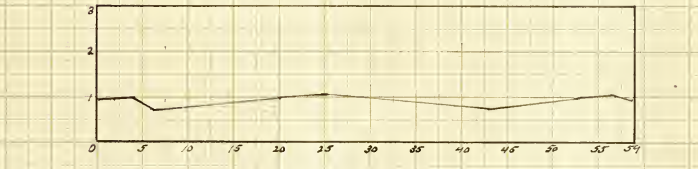
Fig. 3.
Dog No. 11--Pneumococcus Infection. Duration 59 days.



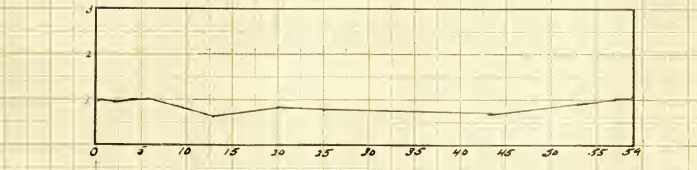
Opsonic Index Curve for Staph. aureus.



Phagocytic Index Curve for Pneumococcus, Type I.



Opsonic Index Curve for B. coli.



Opsonic Index Curve for B. typhosus.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
1/25 21	Dog #11	S.aureus	#11	50	34	16	251	5.0
			Con	50	33	17	199	3.9
	wt. 11 lb	Pn. I	#11	50	7	43	15	0.3
			Con	50	0	50	0	0.0
	This reading made	B. coli	#11	50	14	36	47	0.9
			Con	50	14	36	50	1.0
	at the time	B. bronchicep.	#11	50	33	17	226	4.5
	of death.		Con	50	37	13	195	3.9
		B.typhosus	#11	50	6	44	16	0.3
			Con	50	8	42	19	0.3

Anatomical Diagnosis:

> Slight emaciation, bronchopneumonia, purulent exudate in both pleural cavities, generalized edema. Pneumococcus, Type I, cultivated in pure culture from pleural exudate and from heart blood.

Kataphylaxis is this animal probably occurred on January 20, five days before its death, and was first indicated by the appearance of pneumococci in the blood sufficient quantity to be recovered in the culture. By referring to the curves in Fig. 3 it will be observed that there was no post-kataphylactic decline in the opsonic index for any except the specific infecting organism. Samples of blood taken a few moments before death, when the animal was in a state of complete collapse, revealed a phagocytic activity in all respects equal to the normal and for staphylococci it was even somewhat increased.

The opsonic index for the infecting organism behaved throughout in accordance with the findings published by Neufeld and Töpfer (19) and Rosenow (20). Virulent pneumococci

are not phagocyted at all by the normal animal, but after one or two inoculations of this organism the opsonins for pneumococci increase and can be still further increased by subsequent injections, provided a lethal dose is not administered. Once this quantity is given, however, there is a rapid decline in phagocytic activity, the zero mark ordinarily being reached some time before the death of the animal.

The variation in the opsonic curve for*S. aureus requires some explanation. Up to December 3 the curve for this organism had remained normal, but at this time a culture of Pneumococcus I, contaminated with*S. aureus was injected and five days later the dog's blood showed an increase in opsonins for staphylococci. This organism was almost immediately overcome, for it was never obtained in subsequent blood cultures. The increased opsonic activity for staphylococci continued a few days, but gradually decreased until at the end of a month the index was again normal. However, on January 20 pneumococci appeared in the blood and the reaction against this homologus antigen resulted in a non-specific increase in the residue of antibodies remaining from the previous staphylococcus inoculation. This was immediately reflected by a rise in the opsonic curve for staphylococcus. These observations are in agreement with the findings of Beiling (21).

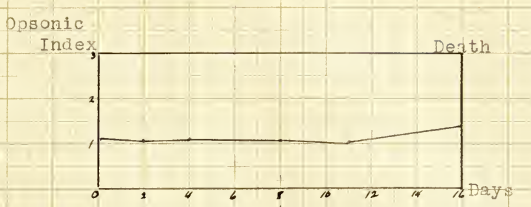
Experiment 2. -- This experiment is presented as a parallel to the preceeding experiment and was performed throughout in a similar manner, except that it was found possible to

*Staph. aureus.

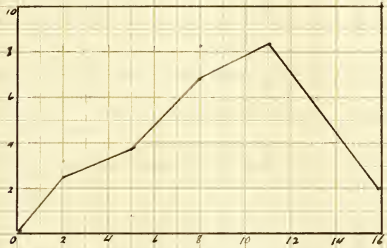
produce death by increasing intravenous injections without the supplementary pleural inoculations. Only B. coli and S. aureus were used in addition to the infecting organism, Pneumococcus, Type I.

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
8/1/20	Dog #12	S.aureus	#12	50	49	1	190	3.8
			Con	50	48	2	167	3.3
		Pn. I	#12	50	0	50	0	0.0
	*Inj. 5cc Pn.I		Con	50	0	50	0	0.0
		B. coli	#12	50	46	4	90	1.8
			Con	50	45	5	83	1.6
8/3	Dog #12	S.aureus	#12	50	50	0	162	3.2
			Con	50	49	1	150	3.0
		Pn. I	#12	50	43	7	132	2.6
	Inj. 20cc PN. I		Con	50	6	44	12	0.2
		B. coli	#12	50	47	3	100	2.0
			Con	50	42	8	105	2.1
8/5	Dog #12	S.aureus	#12	50	48	2	184	3.6
			Con	50	48	2	167	3.3
		Pn. I	#12	50	36	14	190	3.8
			Con	50	2	48	4	0.0
	No inj.	B. coli	#12	50	43	7	76	1.5
			Con	50	44	6	86	1.7
8/9	Dog #12	S.aureus	#12	50	45	5	384	7.6
			Con	50	49	1	348	6.9
		Pn. I	#12	50	48	2	265	5.3
	Inj. 200cc Pn. I		Con	50	6	44	11	0.2
		B. coli	#12	50	-	-	-	-
			Con	50	-	-	-	-
8/12	Dog #12	S.aureus	#12	50	49	1	290	5.8
			Con	50	47	3	280	5.6
		Pn. I	#12	50	44	6	415	8.3
	Inj. 250cc Pn. I		Con	50	0	50	0	0.0
		B. coli	#12	50	48	2	195	3.9
			Con	50	47	3	214	4.2
8/16	Dog #12	S.aureus	#12	50	45	5	450	9.1
			Con	50	45	5	308	6.1
	This reading was made a few minutes before death	Pn. I	#12	50	46	4	100	2.0
			Con	50	2	48	2	0.0
		B. coli	#12	50	48	2	203	4.0
			Con	50	47	3	191	3.8
		B.proteus	#12	50	50	0	553	11.0
			Con	50	47	3	445	8.9

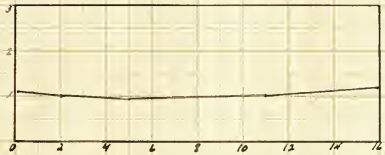
Fig. 4.
Dog No. 12--Pneumococcus Infection. Duration 16 days.



Opsonic Index Curve for Staph. aureus.



Phagocytic Index Curve for Pneumococcus, Type I.



Opsonic Index Curve for B. coli.

Anatomical Diagnosis:

> Slight emaciation; no gross lesions; no exudates. Pneumococcus, Type I, was recovered in pure culture from the heart's blood.

The results of this experiment coincide with the findings in the previous experiment so far as the heterologous organisms were concerned. There was no decrease after kataphylaxis in the phagocytic activity against any bacteria except the one responsible for the primary infection. The opsonic curve for pneumococcus, however, is not what we would expect from the work of Rosenow (22). While there was a decrease in the opsonins for this bacterium after kataphylaxis this decrease never carried the curve down to the normal level. Here, then, was an animal dying of an infection with its phagocytic defense against the specific organism not only intact but functioning with an efficiency distinctly above the normal. Whatever the explanation for the defeat of this animal in its struggle against the infection, it does not seem possible to account for it by a rupture in the phagocytic defense.

Experiment 3. -- The experimental animal used in this test was an adult guinea pig - No. 31 - weighing 375 grams. It was injected intravenously with gradually increasing amounts of Pneumococcus, Type I, and died 13 days later in a state of extreme cachexia.

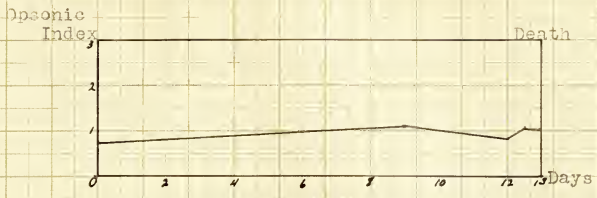
Full details of the experiment, together with the results of the opsonic determinations, are fully set forth in the accompanying tables.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
11/12	Pig #31 wt.before injection 375 grams Inj. 1.5cc Pn. I	S.aureus	#31	50	35	15	165	3/3
			Con	50	41	9	215	4.3
		B. coli	#31	50	47	3	180	3.6
			#31	50	45	5	200	4.0
		Pn. I	#31	50	0	50	0	0.0
			Con	50	0	50	0	0.0
11/21	Pig #31	S.aureus	#31	50	40	10	75	1.5
			Con	50	36	14	60	1.2
	wt. 355 g.	Pn. I	#31	50	3	47	3	0.0
			Con	50	2	48	4	0.0
	Inj. 2.5cc Pn. I	B. coli	#31	50	41	9	125	2.5
			Con	50	38	12	95	1.9
11/24 9:00 A. M.	Pig #31	S.aureus	#31	50	42	8	250	5.0
			Con	50	46	4	290	5.8
		Pn. I	#31	50	47	3	4	0.0
			Con	50	46	4	4	0.0
		B. coli	#31	50	36	14	70	1.4
			Con	50	38	12	80	1.6
11/24 6:00 P.M.	Pig #31	S.aureus	#31	50	36	14	245	4.9
			Con	50	47	3	290	5.8
	wt. 302 g.	Pn. I	#31	50	4	46	4	0.0
			Con	50	3	47	4	0.0
	No inj	B. coli	#31	50	41	9	67	1.3
			Con	50	38	12	80	1.6
11/24 10:30 P. M.	Pig #31	S.aureus	#31	50	45	5	303	6.0
			Con	50	46	4	290	5.8
	wt. 302 g.	Pn. I	#31	50	4	46	6	0.1
			Con	50	2	48	4	0.0
	No inj.	B. coli	#31	50	35	15	90	1.8
			Con	50	38	12	83	1.6
11/25	Pig #31	S.aureus	#31	50	50	0	299	5.9
	Dead		Con	50	46	4	290	5.8
		Pn. I	#31	50	2	48	3	0.0
			Con	50	3	47	6	0.1
		B. coli	#31	50	41	9	80	1.6
			Con	50	36	14	70	1.4

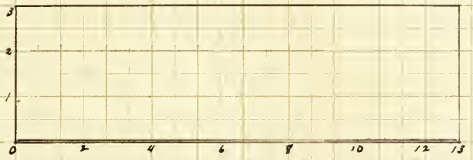
Anatomical Diagnosis:

Extreme emaciation, no macroscopic lesions. Pneumococcus, Type I, recovered in pure culture from heart's blood.

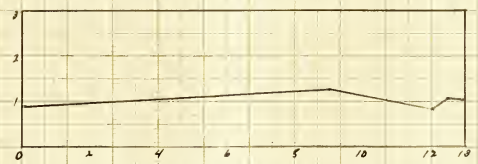
Fig.5.
Guinea Pig No.31--Pneumococcus Infection Duration 13 days.



Opsonic Index Curve for Staph. aureus.



Phagocytic Index Curve for Pneumococcus, Type I.



Opsonic Index Curve for B. coli.

Throughout this experiment there was no discoverable increase in the opsonic activity of the blood against pneumococci. This is probably due to the fact that the initial injection was so large as to closely approximate the lethal dose. The reaction to the initial inoculation was so violent that the animal lost 15% of its body weight in eight days. The subsequent injections resulted in equally violent disturbances so that the animal was practically in a kataphylactic state during the whole period of the experiment. Yet in this weakened condition, a state commonly characterized as "defenseless", the blood revealed no diminution whatever in its opsonic activity against the bacteria commonly associated with terminal infections.

In these experiments we have reviewed three types of fatal pneumococcus infections -- one in which the phagocytic index for pneumococcus was very high during the early stages of the disease, but fell rapidly after the infection began to gain on the body, and reached zero sometime before the death of the animal; in the second case there was a corresponding increase in opsonins during the early stages of the infection but the decrease following the break was gradual and the index never at any time fell to the level of the normal; the third type was one in which the character of the infection from the start was so severe that there was no increase whatever in the phagocytic activity above that observed in the normal. These three types, however, are alike in that none shows a decrease in the opsonic index for either Staph. aureus or B. coli, during the period immediately preceeding death.

B. Staphylococcus Infections

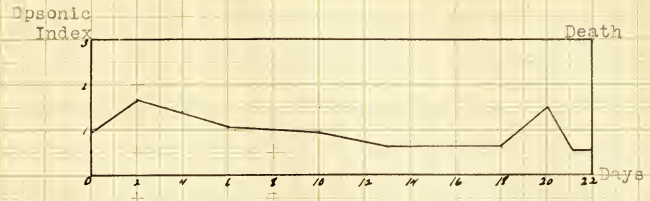
Experiment 4. -- An adult female dog - No. 14 - weighing 15 pounds was selected as the experimental animal in this test. She was given an initial intravenous injection of 1cc. of a broth culture of Staph. aureus on November 27, 1920. Gradually increasing amounts of this organism were given until the dog, in an emaciated and helpless condition, died 23 days later.

The opsonic determinations, together with other data concerning this experiment are recorded in the appended table.

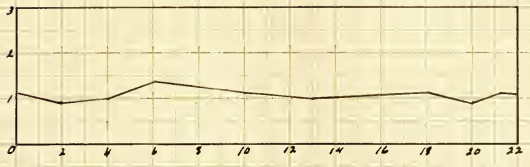
Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
11/27 20	Dog #14 wt. before injection 15 lbs Inj. 1cc S.aureus	S.aureus	#14	50	41	9	121	2.4
			Con	50	42	8	120	2.4
		Pn. I	#14	50	5	45	10	0.2
			Con	50	4	46	8	0.1
		B.typhosus	#14	50	42	8	106	2.1
			Con	50	45	5	104	2.0
11/29	Dog #14 wt. 13½lbs Inj. 2 cc. S. aureus	S.aureus	#14	50	44	6	297	5.9
			Con	50	44	6	170	3.4
		Pn. I	#14	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#14	50	38	12	96	1.9
			Con	50	46	4	104	2.0
		B.typhosus	#14	50	35	15	100	2.0
			Con	50	38	12	70	1.4
12/1	Dog #14 wt. 13½lbs No inj.	S.aureus	#14	50	50	0	309	6.2
			Con	50	44	6	212	4.2
		Pn. I	#14	50	4	46	10	0.2
			Con	50	5	45	6	0.1
		B. coli	#14	50	34	16	56	1.1
			Con	50	35	15	55	1.1
		B.typhosus	#14	50	14	36	18	0.3
			Con	50	15	35	20	0.4

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
12/3 20	Dog #14	S.aureus	#14	50	28	22	66	1.3
			Con	50	27	23	61	1.2
	wt. 13 lbs	Pn. I	#14	50	3	47	7	0.1
			Con	50	2	48	4	0.0
		B. coli	#14	50	31	19	50	1.0
	Inj. 5 cc		Con	50	34	16	35	0.7
	S. aureus	B.typho-sus	#14	50	36	14	76	1.5
			Con	50	42	8	85	1.6
12/7	Dog #14	S.aureus	#14	50	35	15	133	2.6
			Con	50	34	16	150	3.0
	wt. 12½lbs	Pn. I	#14	50	-	-	-	-
			-	-	-	-	-	-
		B. coli	#14	50	49	1	156	3.1
			Con	50	39	11	131	2.6
	No inj.	B.typho-sus	#14	-	-	-	-	-
			-	-	-	-	-	-
12/10	Dog #14	S.aureus	#14	50	41	9	76	1.5
			Con	50	39	11	115	2/3
	wt. 11 lbs	Pn. I	#14	50	-	-	-	-
			Con	50	-	-	-	-
		B. coli	#14	50	31	19	73	1.5
			Con	50	33	17	75	1.5
	No inj.	B.typho-sus	#14	50	19	31	42	0.8
			Con	50	23	27	33	0.6
12/15	Dog #14	S.aureus	#14	50	29	21	105	2.1
			Con	50	25	25	171	3/4
	wt. 11 lbs	Pn. I	#14	50	-	-	-	-
			Con	50	-	-	-	-
		B. coli	#14	50	38	12	87	1.7
			Con	50	42	8	73	1.5
	No inj.	B.typho-sus	#14	50	27	24	55	1.1
			Con	50	28	22	46	0.9
12/17	Dog #14	S.aureus	#14	50	44	6	354	7.0
			Con	50	43	7	231	4.6
		Pn. I	#14	50	9	41	22	0.4
			Con	50	10	40	21	0.4
		B. coli	#14	50	35	15	71	1.4
			Con	50	33	17	80	1.6
	No Inj.	B.typho-sus	#14	50	40	10	92	1.8
			Con	50	38	12	86	1.7

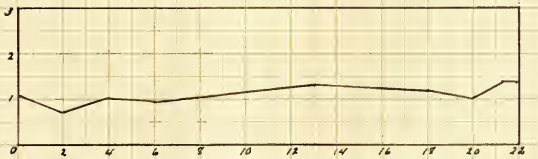
Fig.6.
Dog No.14--Staphylococcus Infection. Duration 22 days.



Opsonic Index Curve for Staph. aureus.



Opsonic Index Curve for B. coli.



Opsonic Index Curve for B. typhosus.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
12/19 20 9:00 A.M.	Dog #14 wt. 11 lbs Reading made 14 hrs before death	S.aureus	#14	50	40	10	121	2.4
			Con-	50	38	12	220	4.4
		Pn. I	#14	50	9	41	22	0.4
			Con	50	5	45	12	0.2
		B. coli	#14	50	38	12	114	2.2
			Con	50	43	17	91	1.8
		B.typho-sus	#14	50	34	16	70	1.4
			Con	50	30	20	49	1.0
12/19 11:00 P.M.	Dog #14 Reading made at time of death.	S.aureus	#14	50	36	14	121	2.4
			Con	50	38	12	219	4.4
		Pn. I	#14	50	4	46	18	0.3
			Con	50	5	45	12	0.2
		B. Coli	#14	50	41	9	111	2.2
			Con	50	42	8	91	1.8
		B.typho-sus	#14	50	29	21	72	1.4
			Con	50	30	20	49	1.0

Anatomical Diagnosis:

> Marked emaciation, Staphylococcus broncho pneumonia, multiple abscess in body wall, heart, kidneys and spleen; acute nephritis, staphylococcus cystitis. Staph. aureus in pure culture obtained from heart blood, kidneys, spleen and urine.

The variations in the opsonic index for Staph. aureus in this experiment are in agreement with the results published by Wright (23) and Neufeld (24). Under the stimulation of sub-lethal inoculation there is an increase in phagocytic activity but as soon as the lethal dose is given there is a sharp decline which often forces the index below the normal. One especially interesting feature of the opsonic index for staphylococcus in this experiment is that on December 7th the index was somewhat lower than it was at the time the animal died. Although phagocytosis is regarded as the chief defensive mechan-

ism imposed against staphylococci, this animal with an uncomplicated infection, survived the period of its lowest phagocytic activity and died when its opsonic index was only slightly lower than the normal.

Experiment 5. -- The dog - No. 15 - used in this experiment was anesthetized and an incision made in the abdominal wall. The right kidney was exposed and one-third of an agar slant of Staphylococcus aureus injected into the renal pelvis. The dog died in 52 hours with a generalized staphylococcus peritonitis and septicemia in addition to the violent reaction in the right kidney. A sample of blood was taken and the following opsonic determinations secured.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
3/11 21	Dog #15	S.aureus	#15	50	48	2	319	6.4
			Con	50	50	0	303	6.1
	2 hours after death	B. coli	#15	50	34	16	61	1.2
			Con	50	37	13	57	1.1
		B. bronchisep.	#15	50	37	13	77	1.5
			Con	50	40	7	69	1.4

Anatomical Diagnosis:

> Well nourished, generalized staphylococcus peritonitis, septicemia. Staphylococcus aureus in pure culture cultivated from the blood, right kidney and peritoneum.

Although staphylococci had already appeared in the blood there was no decrease in the opsonic index for this organism. The shock associated with deep and prolonged anesthesia and

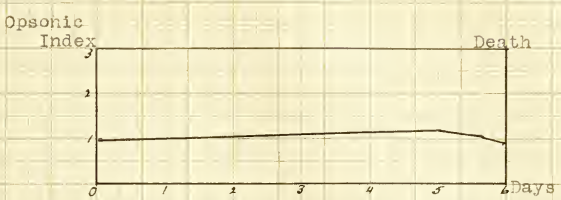
opening the peritoneal cavity did not occasion any modification in the normal phagocytic activity against any of the three bacteria tested in this experiment.

Experiment 6. -- For this experiment a large adult guinea pig - No. 16 - weighing 540 grams was inoculated intracardially with 0.5 cc. of a 24 hour broth culture of Staph. aureus on November 30, 1920. The first two days the animal exhibited no symptoms of infection but on the third day staphylococci appeared in the blood and during the next 72 hours the guinea pig suffered a phenomenal loss in weight. It died on December 4th, in a state of the most extreme emaciation and weakness.

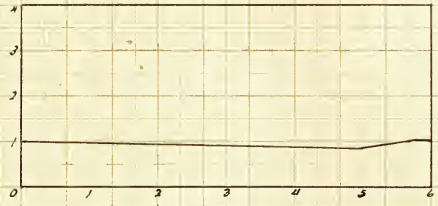
Full details of the experiment, together with the routine opsonic readings appear in the subjoined table.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
11/30 20	Pig #16 wt. before inj. 540 g.	S.aureus	#16	50	43	7	270	5.4
			Con	50	42	8	276	5.4
		Strep. hemo.	#16	50	43	7	144	2.8
			Con	50	46	4	140	2.8
	Inj. 0.5cc S. aureus	B. coli	#16	50	43	7	75	1.5
			Con	50	44	6	78	1.5
12/4	Pig #16	S.aureus	#16	50	38	12	121	2.4
			Con	50	36	14	95	1.9
	wt. 475 g.	Strep. hemo.	#16	50	40	10	108	2.1
			Con	50	40	10	125	2.5
	No inj.	B. coli	#16	50	40	10	45	0.9
			Con	50	39	11	40	0.8
12/5	Pig #16 wt. 375 g. 12 hours before death. No inj.	S.aureus	#16	50	41	9	195	3.9
			Con	50	42	8	170	3.4
		Strep. hemo.	#16	50	20	30	103	2.0
			Con	50	25	25	100	2.0
		B. coli	#16	50	46	4	225	4.5
			Con	50	45	5	220	4.4

Fig. 7.
Guinea Pig No. 16--Staphylococcus Infection. Duration 6 days.



Opsonic Index Curve for Staph. aureus.



Opsonic Index Curve for Strep. hemolyticus.

Opsonic Index Curve for B. coli.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
12/5 20	Pig #16	S.aureus	#16	50	45	5	160	3.2
			Con	50	42	8	170	3.4
	Death	Strep. hemo.	#16	50	17	33	104	2.0
			Con	50	25	25	100	2.0
		B. coli	#16	50	50	0	273	5.4
			Con	50	49	1	220	4.4

Anatomical Diagnosis:

> Marked emaciation, purulent exudate in pleural cavities; kidneys studded with small abscesses. Pelvis of each kidney and bladder contained pus. Staph. aureus in pure culture cultivated from pleural cavities, kidneys, bladder and heart's blood.

This case represents an infection of the most violent and destructive character. The animal lost 28% of its body weight in five days. The pleural cavities and urinary system were filled with pus containing abundant organisms, and the staphylococci were so numerous in the blood stream at death that 2000 colonies were cultivated from a single loop-full of blood. Yet in spite of this tremendously overwhelming infection the phagocytic system suffered practically no impairment. Only the opsonic index for the specific organism was below the normal, and this by a difference so small that it might easily be regarded as an experimental error. That the test affords an accurate estimate of conditions in the body is attested by the fact that many of the polymorphonuclear leucocytes of the blood were actively phagocytic, some containing as many as 35 cocci. Likewise, almost every polymorphonuclear leucocyte examined from the pleural exudate contained organisms. This

case is perhaps somewhat exceptional, especially the phagocytosis in the exudates, for Opie (25) has shown that exudates are often deficient in opsonins. It does, however, indicate that phagocytic collapse does not always accompany fatal infection.

Experiment 7. -- The guinea pig - No. 17 - selected for this experiment weighed 360 grams. It was injected intravenously with 2 cc. of a 24 hour broth culture of Staph. aureus. Death occurred at the end of eighteen hours.

The experimental details are summerized in the following table.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
11/12 20	Pig #17 wt. 360 g. Inj. 2 cc. S. aureus	S.aureus	#17	50	41	9	185	3.7
			Con	50	35	15	165	3.3
		B. coli	#17	50	45	5	200	4.0
			Con	50	47	3	180	3.6
11/13	Pig #17 Dead	S.aureus	#17	50	36	14	101	2.0
			Con	50	40	10	133	2.6
		B. coli	#17	50	43	7	125	2.5
			Con	50	42	8	115	2.3

Anatomical Diagnosis:

No macroscopic lesions. Heart blood yielded a pure culture of Staph. aureus.

A review of the preceeding staphylococcus infections reveals that the overwhelming of an animal by Staph. aureus may be accompanied by a sharp and subnormal decline in the opsonic index for that organism but that this is not invariably the condition. It would seem that especially in rapidly fatal

infections the phagocytic activity may not be disturbed.

In these cases, no matter how violent and destructive the cause of the infection, no matter how exhausted and wasted the animal, there was not one instance of a decrease in phagocytic activity, even in the hours immediately preceeding death, against any organism not concerned in the primary infection.

C. Typhoid Infections

Experiment 8. -- In this experiment an adult male dog - No. 13 - weighing 13½ pounds was injected intravenously on November 27, 1920, with 2 cc. of an 18 hour broth culture of B. typhosus. Sublethal inoculations were continued until the blood revealed a high opsonic content and then the injections were gradually increased until the dog died on December 28, 1920.

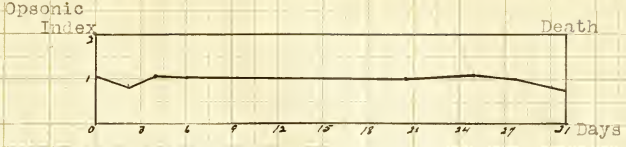
Samples of blood were taken before each injection and the usual opsonic determinations made under the conditions described in the following table.

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
11/27 20	Dog #13	S.aureus	#13	50	48	2	133	2.6
			Con	50	42	8	121	2.4
	wt. before injection 13½lbs	Pn. I	#13	50	3	47	4	0.0
			Con	50	4	46	8	0.1
		B. coli	#13	50	-	-	-	-
			Con	50	-	-	-	-
	Inj. 2 cc. B. typho.	B.typhosus	#13	50	42	8	97	1.9
			Con	50	45	5	104	2.0

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
11/29 20	Dog #13	S.aureus	#13	50	47	3	150	3.0
			Con	50	44	6	170	3.4
	wt. 13½lbs	Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	41	9	83	1.6
			Con	50	46	4	102	2.0
	No inj.	B.typhosus	#13	50	43	7	95	1.9
			Con	50	39	11	71	1.4
12/1	Dog #13	S.aureus	#13	50	43	7	218	4.3
			Con	50	45	5	212	4.2
	wt. 13½lbs	Pn. I	#13	50	4	46	8	0.1
			Con	50	4	46	6	0.1
		B. coli	#13	50	32	18	53	1.0
	Inj. 1 cc.		Con	50	25	25	55	1.1
	B. typhosus	B.typhosus	#13	50	17	33	45	0.9
			Con	50	15	35	20	0.4
12/3	Dog #13	S.aureus	#13	50	23	27	70	1.4
			Con	50	27	23	63	1.2
	wt. 13 lbs	Pn. I	#13	50	4	46	8	0.1
			Con	50	2	48	4	0.0
		B. coli	#13	50	24	26	37	0.7
	Inj. 4 cc.		Con	50	24	26	35	0.7
	B. typhosus	B.typhosus	#13	50	49	1	300	6.0
			Con	50	42	8	85	1.7
12/17	Dog #13	S.aureus	#13	50	46	4	232	4.6
			Con	50	43	7	231	4.6
	wt. 11 lbs	Pn. I	#13	50	7	43	25	0.5
			Con	50	10	40	21	0.4
		B. coli	#13	50	40	10	84	1.6
	Inj. 5 cc.		Con	50	33	17	80	1.6
	B. typhosus	B.typhosus	#13	50	42	8	211	4.2
			Con	50	38	12	86	1.7
12/22	Dog #13	S.aureus	#13	50	26	24	119	2.3
			Con	50	25	25	104	2.1
	wt. 11½lbs	Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	41	9	130	2.6
	Inj. 5 cc.		Con	50	38	12	105	2.1
	B. typhosus	B.typhosus	#13	50	40	10	211	4.2
			Con	50	31	19	68	1.3

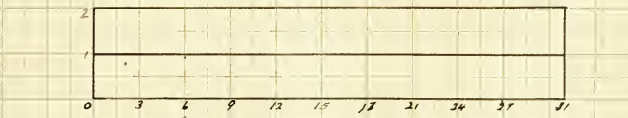
Fig.8.

Dog No.13--Typhoid Infection. Duration 31 days.

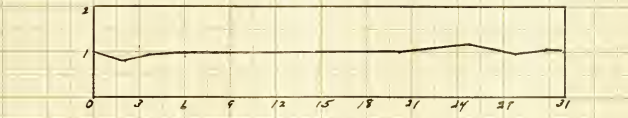


Opsonic Index Curve for Staph. aureus.

Opsonic Index Curve for B. typhosus.



Opsonic Index Curve for Pneumococcus, Type I.



Opsonic Index Curve for B. coli.

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
12/26 20 9:00 A.M.	Dog #13 wt. 9½lbs No inj.	S.aureus	#13	50	45	5	256	5.1
			Con	50	41	9	250	5.0
		Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	25	25	43	0.8
			Con	50	37	13	47	0.9
		B.typho-sus	#13	50	27	23	77	1.5
			Con	50	4	46	10	0.2
12/26 5:00 P.M.	Dog #13 wt. 9 lbs No inj.	S.aureus	#13	50	35	15	227	4.5
			Con	50	41	9	250	5.0
		Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	21	29	42	0.8
			Con	50	37	13	47	0.9
		B.typho-sus	#13	50	36	14	78	1.5
			Con	50	4	46	10	0.2
12/27	Dog #13 No inj.	S.aureus	#13	50	25	25	149	3.0
			Con	50	37	13	180	3.6
		Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. Coli	#13	50	28	22	54	1.0
			Con	50	36	16	49	0.9
		B.typho-sus	#13	50	35	15	56	1.1
			Con	50	19	31	26	0.5
12/28	Dog #13 3 hours before death.	S. Aureus	#13	50	23	27	146	2.9
			Con	50	37	13	180	3.6
		Pn. I	#13	50	2	48	2	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	36	14	62	1.2
			Con	50	36	14	49	1.0
		B.typho-sus	#13	50	32	18	50	1.0
			Con	50	19	31	26	0.5
12/28	Dog #13 wt. 8½lbs Death	S.aureus	#13	50	27	23	143	2.8
			Con	50	37	13	180	3.6
		Pn. I	#13	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		B. coli	#13	50	24	26	58	1.1
			Con	50	36	16	49	1.0
		B.typho-sus	#13	50	38	12	70	1.4
			Con	50	19	31	26	0.5

Anatomical Diagnosis:

> Emaciated; no characteristic typhoid lesions except acute splenic tumor. B. typhosus obtained from heart's blood. B. enteritidis cultivated from heart's blood and gall bladder.

Anaphylaxis in this animal probably occurred on December 26th when there was a marked decline in the opsonic index for the typhoid bacilli. The decrease, however, was not sufficient to bring the index down to the normal. The phagocytic activity of the blood against B. typhosus was at the time of death more than twice as great as that of a normal control. It is possible, too, that the test gives an inadequate measure of the real opsonic strength of the experimental animal's blood, for Harrison (26) has shown that the lytic action of anti-typhoid serum is so great against the typhoid bacilli as to lower the observed index by dissolving the bacteria before they can be taken up by the leucocytes. That this condition actually existed in this case was demonstrated when the inactivated immune serum gave a higher phagocytic index than when the serum was used unheated.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
12/18	Unhtd serum	B. typho.	#13	50	38	12	70	1.4
20	Inactvd "	B. typho.	#13	50	42	8	124	2.4

That the phagocytic cells in this dog, dying of a typhoid infection, were not functionally impaired is indicated by the following tests:

A quantity of blood was taken in citrate from dog No. 13 and from a control animal. Each lot was washed twice, using 175 volumes of salt solution. Samples from the resulting cells were then separately incubated with Staph. aureus and B. typhosus. The results are given in the table.

Date	Remarks	Bacteria	Animal	No. of Cells Count	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic index
12/28/20	30min. incubation.	S. aureus	#13	50	1	49	4	0.0
			Con	50	2	48	2	0.0
	30min. incubation.	B. typhosus	#13	50	32	18	57	1.1
			Con	50	0	50	0	0.0

Whether the energetic phagocytosis of B. typhosus by the washed cells of the immune animal, in the absence of serum can be interpreted as the action of an immune cell, or whether there was left upon the cell enough highly potent immune serum to opsonize only the specific bacteria is a matter to be decided by more detailed observations. That the last explanation, however, is probably the true one is suggested by Klein's (27) work on the dilution of sera.

A quantity of polymorphonuclear leucocytes were recovered from the urine of dog No. 13 and when incubated with sensitized bacteria were actively phagocytic, as indicated in the following results.

Date	Remarks	Bacteria	Animal	No. of Cells Count	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
12/28/20	Cells from urine dog 13	B. typhosus	#8	50	33	17	128	2.6
			Con	50	23	27	37	0.7

*Animal providing serum.

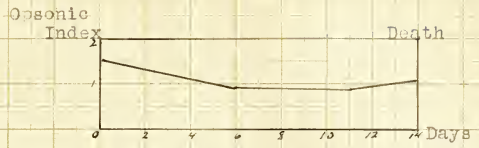
These findings, then reveal an animal dying of an infection with its phagocytic mechanism, so far at least as experimental standards can determine, functioning with an efficiency considerably above the normal against the specific organism, and without any demonstrable decrease in opsonic activity against the other bacteria used in the test.

Experiment 9. -- This experiment was intended as a duplicate of the preceeding experiment. The conditions of the test, paralleling as nearly as possible those of No. 8, are given in the accompanying table. Although this infection resulted in death it ran a much milder course than the typhoid infection recorded above. There was only slight emaciation at death, and there was a complete absence of macroscopic lesions.

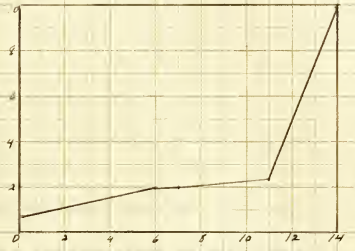
Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic index
1/18 21	Dog #19	S. aureus	#19	50	33	17	252	5.0
			Con	50	34	16	159	3.2
	wt. before injection 12½lbs. Inj. 3cc. B. typhosus	B. coli	#19	50	43	7	98	1.9
			Con	50	40	10	95	1.9
		B. typhosus	#19	50	10	40	15	0.3
			Con	50	7	43	20	0.4
		B. bronchisep.	#19	50	42	8	81	1.6
			Con	50	27	23	65	1.3
1/24	Dog #19	S. aureus	#19	50	31	19	115	2.3
			Con	50	34	16	128	2.3
	wt. 11½lbs	B. coli	#19	50	41	9	78	1.6
			Con	50	41	9	71	1.4
	Extreme weakness	B. typhosus	#19	50	8	42	19	0.3
			Con	50	6	44	16	0.3
	No inj.	B. bronchisep.	#19	50	38	12	137	2.7
			Con	50	38	12	136	2.7

Fig.9

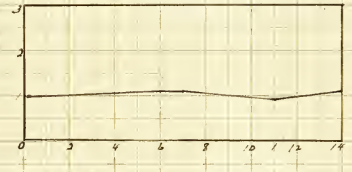
Dog No.19--Typhoid Infection. Duration 14 days.



Opsonic Index Curve for Staph. aureus.



Opsonic Index Curve for B. typhosus.



Opsonic Index Curve for B. coli.

Opsonic Index Curve for B. bronchisepticus.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No:Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
1/25 21	Dog #19	S.aureus	#19	50	-	-	-	-
			Con	50	-	-	-	-
	Wt. 11 lbs	B. coli	#19	50	14	36	50	1.0
			Con	50	15	35	48	0.9
		B.typho-sus	#19	50	8	42	19	0.3
	No inj.		Con	50	7	43	15	0.3
		B. bron-chisep.	#19	50	39	11	196	5.9
			Con	50	37	13	212	4.2
1/29	Dog #19	S.aureus	#19	50	42	8	226	4.5
			Con	50	45	5	244	4.8
	Wt. 11 lbs	B. coli	#19	50	44	6	183	3.7
			Con	50	45	5	182	3.6
		B.typho-sus	#19	50	38	12	70	1.4
			Con	50	30	20	54	1.0
	No inj.	B. bron-chisep.	#19	50	34	16	182	3.6
			Con	50	31	19	101	2.0
2/1	Inj. 5 cc. B. typhosus							
2/2	Dog #19	S.aureus	#19	50	24	26	89	1.7
			Con	50	27	23	81	1.6
		B. coli	#19	50	38	12	81	1.6
			Con	50	34	16	70	1.4
	Dead	B.typhe-sus	#19	50	44	6	157	3.1
			Con	50	13	37	18	0.3
		B. bron-chisep.	#19	50	50	0	406	8.1
			Con	50	50	0	310	6.2

Anatomical Diagnosis:

Slight emaciation; no macroscopic lesions. B. typhosus in pure culture cultivated from heart's blood.

The findings in this experiment are for the most part in agreement with the results obtained in No. 8. The characteristically high opsonic index for the specific organism suffered no decrease after the infection had overcome the general body

resistance. This may perhaps be due to the short period which elapsed between the fatal inoculation and death. Also there was, as in all preceeding tests, no discoverable change in the indices for any of the non-specific bacteria, either during the course of the infection, or within the agonal period, the time when terminal infections commonly appear.

The results of these experiments with typhoid infections challenge the validity of Bordet's (28) conception that "yielding to an infection is primarily due to an inability of the phagocytes to take up the infecting agent."

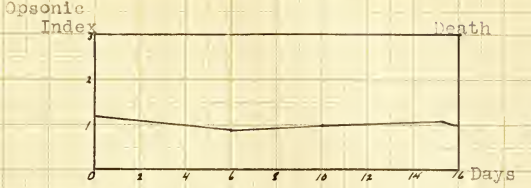
D. Streptococcus Infections

Experiment 10. -- The experimental animal in this test was a large adult guinea pig - No. 23. It was given an initial intravenous injection of 1.5 cc. of a 24 hour broth culture of hemolytic streptococci on November 24, 1920. The inoculations were repeated every second day, but were discontinued as soon as it was apparent that a fatal quantity had been given. The guinea pig died sixteen days after the first injection. During the last six days the infection assumed the most destructive character. Over this period the animal lost 29% of its body weight, and throughout the last 24 hours of its life was in a state of astonishing emaciation and weakness.

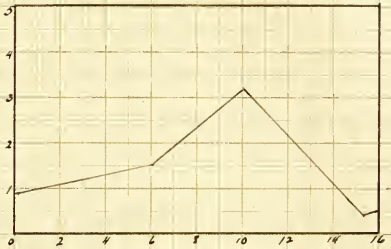
The opsonic determinations, together with other data concerning the experiment are recorded in the appended table.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
11/24 20	Pig #23 wt. before injection 555 g. Inj. 1.5cc Strep.hemo.	S.aureus	#23	50	40	10	160	3/2
			Con	50	38	12	131	2.6
		B. coli	#23	50	45	5	176	3.5
			Con	50-	47	3	150	3.0
		Strep. hemo.	#23	50	42	8	142	2.8
			Con	50	46	4	150	3.0
11/26	Inj. 1.5cc Strep.hemo.							
11/28	Inj. 1.5cc Strep.hemo.							
11/30	Pig #23 wt. 550 g. Inj. 1.5cc Strep.hemo.	S.aureus	#23	50	41	9	263	5.2
			Con	50	42	8	276	5.5
		B. coli	#23	50	41	9	80	1.6
			Con	50	45	5	76	1.5
		Strep. hemo.	#23	50	50	0	223	4.4
			Con	50	46	4	140	2.8
12/4	Pig #23 wt. 540 g. No. inj.	S.aureus	#23	50	34	16	110	2.2
			Con	50	37	13	113	2.2
		B. coli	#23	50	40	10	45	0.9
			Con	50	39	11	50	1.0
		Strep. hemo.	#23	50	42	8	320	6.4
			Con	50	25	25	100	2.0
12/6	Inj. 1.5cc Strep.hemo.							
12/8	Inj. 1.5cc Strep.hemo							
12/9	Inj. 1.5cc Strep.hemo							
12/10	Pig #23 wt. 375 g. 4 hours before death No inj.	S.aureus	#23	50	40	10	244	4.8
			Con	50	45	5	204	4.1
		B. coli	#23	50	18	32	60	1.2
			Con	50	29	21	48	0.9
		Strep. hemo.	#23	50	10	40	44	0.8
			Con	50	17	33	93	1.8
12/10	Pig #23 wt. 375 g. Death	S.aureus	#23	50	43	7	215	4.3
			Con	50	45	5	204	4.0
		B. coli	#23	50	28	22	50	1.0
			Con	50	20	30	48	0.9
		Strep. hemo.	#23	50	13	37	44	0.9
			Con	50	17	33	92	1.8

Fig. 10.
Guinea Pig No. 26--Streptococcus Infection. Duration 16 days.



Opsonic Index Curve for Staph. aureus.



Opsonic Index Curve for Strep. hemolyticus.

Opsonic Index Curve for B. coli.

Anatomical Diagnosis:

Extreme emaciation; generalized hemorrhage; purulent exudate in pleural cavities. Streptococcus hemolyticus in pure culture from pleural exudate. Streptococcus hemolyticus and B. pyocyaneus cultivated from heart's blood.

The variations in the opsonic activity against the infecting agent in this experiment coincide with the observations of Denys and Leclef (29) in their investigation of streptococcus immunity in rabbits. It is interesting in the present experiment that the marked decline in the phagocytic activity against streptococci, after the infection had overcome the general body resistance, parallels almost exactly the curve for loss in weight. There was at no time throughout the experiment any change in opsonic index for any of the non-specific bacteria examined.

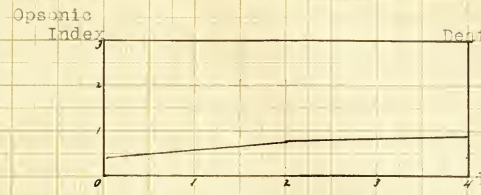
Experiment 11. -- In this experiment are presented the results of a study of four cases of fatal spontaneous hemolytic streptococcus infection in cats. The epidemic, of which these cases were a part, appeared in a room containing twenty-five cats, and ran a course so severe that of the whole number only two survived. The onset of the attack was characterized by a nasal discharge and violent sneezing. The cats lost rapidly in weight and died, generally after four or five days. The last 24 hours of the infection was especially severe. The animal was in a state of extreme emaciation and exhaustion,

being all the time unable to stand and in the last hours of life responding only to such violent stimulation as a cardiac puncture.

Samples of blood were taken from the heart at intervals indicated in the tables and the usual opsonic determinations made.

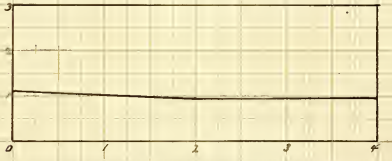
Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
2/13 21	Cat #33 at time of death	S.aureus	#33	50	43	7	210	4.2
			Con	50	46	4	188	3.7
		B. coli	#33	50	29	21	51	1.0
			Con	50	27	23	40	0.8
		B. bronchisep.	#33	50	50	0	380	7.6
			Con	50	46	4	285	5.7
		Strep. hemo.	#33	50	25	25	101	2.1
			Con	50	32	18	266	5.3
2/14	Cat #35 1 hour before death	S.aureus	#35	50	38	12	161	3.2
			Con	50	38	12	137	2.7
		B. coli	#35	50	27	23	55	1.1
			Con	50	25	25	62	1.2
		B. bronchisep.	#35	50	37	13	166	3.3
			Con	50	34	16	172	3.4
		B.typhosus	#35	50	36	14	71	1.4
			Con	50	33	17	86	1.7
2/14	Cat #36 at time of death	S.aureus	#36	50	37	13	138	2.7
			Con	50	38	12	137	2.7
		B. coli	#36	50	23	27	58	1.1
			Con	50	25	25	62	1.2
		B. bronchisep.	#36	50	43	7	216	4.3
			Con	50	34	16	171	3.4
		B.typhosus	#36	50	25	25	55	1.1
			Con	50	33	12	86	1.7
2/15	Cat #37	S.aureus	#37	50	37	13	101	2.0
			Con	50	50	0	256	5.1
		B. coli	#37	50	48	2	225	4.5
			Con	50	48	2	216	4.3
		B. bronchisep.	#37	50	50	0	294	5.8
			Con	50	42	8	250	5.0
		Strep. hemo.	#37	50	25	25	146	2.9
			Con	50	42	8	213	4.3

Fig. 11.
Cat No. 37--Streptococcus Infection. Duration 4 days.

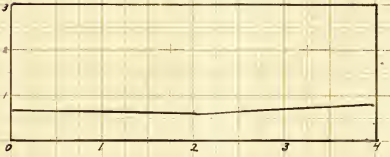


Opsonic Index Curve for Staph. aureus.

Opsonic Index Curve for B. coli.



Opsonic Index Curve for B. bronchisepticus.



Opsonic Index Curve for Strep. haemolyticus.

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
2/17 21	Cat #37	S.aureus	#37	50	39	11	145	2.9
			Con	50	46	4	188	3.7
		B. coli	#37	50	25	25	45	0.9
	48 hours before death.		Con	50	18	32	42	0.8
		B. bronchisep.	#37	50	46	4	263	5.2
			Con	50	48	2	284	5.6
		Strep. hemo.	#37	50	34	16	176	3.5
			Con	50	36	14	266	5.3
2/19	Cat #37	S.aureus	#37	50	41	9	305	6.1
			Con	50	46	4	340	6.8
	at time of death	B. coli	#37	50	42	8	216	4.3
			Con	50	29	21	159	3.2
		B. bronchisep.	#37	50	50	0	720	14.4
			Con	50	50	0	793	15.9
		Strep. hemo.	#37	50	32	18	160	3.2
			Con	50	41	9	201	4.0
2/19	Cat #37	S.aureus	#37	50	46	4	335	6.7
			Con	50	48	2	327	6.5
	1 hour after death	B. coli	#37	50	50	0	150	3.0
			Con	50	45	5	120	2.4
		B. bronchisep.	#37	50	50	0	304	6.0
			Con	50	50	0	297	5.9
		Strep. hemo.	#37	50	50	0	201	4.0
			Con	50	50	0	386	7.7

Anatomical Diagnosis:

All the cats in this experiment presented the same appearance: Marked emaciation; greenish purulent exudate filling the nasal sinuses and extending over the surfaces of the naso-pharynx and down into the trachea as far as the bifurcation. There was a complete absence of any lesions in any other organs. No. haemorrhages. Streptococcus hemolyticus in pure culture obtained from heart's blood of all four animals. This was also the predominating organism in the exudate of the upper respiratory tract.

There is, in general, agreement in the results obtained from these four cases. In all the opsonic index for the spe-

cific organism was below normal at the time of death. The opsonic indices for the heterologous bacteria, in each instance, differed from the normal by less than what can properly be regarded as experimental error.

There was in the study of cat No. 37 one feature of very special significance and interest. This animal when first examined on February 15th already revealed advanced emaciation and weakness. The blood culture on this date showed an abundance of streptococci and a few colonies of Staph. aureus. Two days later this latter organism was found abundantly in the blood, but by February 19th it had considerably decreased and had completely disappeared before the cat died on February 20th. Ten cubic centimeters of blood cultured at the time of autopsy failed to develop a single colony of Staphylococci. Streptococci were sufficiently numerous in the blood at all times to be recovered by plating a single loop of blood.

When this cat was placed under observation it was the subject of a double infection and showed evident indications of being rapidly overcome. That the streptococcus was the dominant etiological factor in the infection seems evident from the fact that the symptoms revealed by this cat were in every way similar to those in animals suffering with a pure streptcoccus infection.

Cat No. 37, then, was being overcome by a rapidly fatal infection. It had in some way become secondarily infected with Staph. aureus, and this latter organism had become so abundant

in the blood stream that there resulted a marked decrease in the opsonins for staphylococci. Yet this enfeebled animal in its losing struggle against a streptococcus infection was able to react against a secondary infection of staphylococcus in a manner so vigorous as to completely banish these bacteria from the body. During this time there was an unmistakable increase in the opsonic index for staphylococcus, and at the time of death this index was only slightly below the normal. The opsonic increase is given in the following table:

Animal	Date	Feb. 15	Feb. 17	Feb. 19
Dog #17	Op.ind.	0.40	0.79	0.89

It would seem that the body reacted against the secondary infection in an independent manner, and that the recovery from one infection and death from another occured, by chance, at the same time.

Experiment 12.--In this experiment an attempt was made to reproduce experimentally the spontaneous infections described in Experiment 11. An adult female cat, No. 38, was injected intravenously with 3cc of an 18 hour broth culture made directly from the heart's blood of a cat dying of the epidemic streptococcus infection. The cat came down with the characteristic symptoms and died after 72 hours. The extent of emaciation and collapse was not as marked as in the preceeding cases.

The routine determinations are recorded in the accompanying table

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
2/18 21	Cat #38	S.aureus	#38	50	40	10	167	3.3
			Con	50	46	4	188	3.7
	Before Injection	B. coli	#38	50	29	21	50	1.0
			Con	50	18	32	42	0.8
		B. bronchisep.	#38	50	47	3	301	6.1
	Inj. 3cc Strep. hemo.		Con	50	48	2	284	5.6
		Strep. hemo.	#38	50	35	15	306	6.1
			Con	50	36	14	266	5.3
2/20	Cat #38	S.aureus	#38	50	39	11	400	8.0
			Con	50	46	4	341	6.8
		B. coli	#38	50	45	5	155	3.1
			Con	50	28	22	159	3.2
	No inj.	B. bronchisep.	#38	50	50	0	850	17.0
			Con	50	50	0	790	16.0
		Strep. hemo.	#38	50	29	21	191	3.8
			Con	50	42	8	202	4.0
2/21	Cat #38	S.aureus	#38	50	40	10	313	6.2
			Con	50	47	3	328	6.6
	Death	B. coli	#38	50	39	11	59	1.2
			Con	50	45	5	120	2.4
		B. bronchisep.	#38	50	50	0	320	6.4
			Con	50	50	0	297	5.9
		Strep. hemo.	#38	50	33	17	224	4.5
			Con	50	20	30	286	7.7

Anatomical Diagnosis:

Slight emaciation; Streptococcus hemolyticus in pure culture cultivated from heart's blood.

The findings in this experiment are throughout similar to the results obtained in the spontaneous streptococcus infections.

E. Miscellaneous Infections

Experiment 13. -- This experiment was conducted on an adult rabbit - No. 41. It was injected intravenously with influenza bacilli washed from a slant of cooked-blood agar. The rabbit died 18 hours later without any macroscopic lesions, but with a tremendous number of influenza bacilli in the blood. The opsonic results are given below.

Date	Remarks	Bacteria	Animal	No. of cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
2/4/21	Rabbit #41 wt. before injection 2500 g.	S. aureus	#41	50	41	9	97	1.9
			Con	50	37	13	93	1.9
		B. coli	#41	50	40	10	79	1.6
			Con	50	37	13	74	1.5
		B. influ.	#41	50	17	33	22	0.4
			Con	50	10	40	25	0.5
2/5	Rabbit #41	S. aureus	#41	50	36	14	94	1.9
			Con	50	40	10	105	2.1
		B. coli	#41	50	41	9	108	2.1
			Con	50	40	10	95	1.9
		B. influ.	#41	50	13	37	13	0.3
			Con	50	17	33	29	0.6

Although this animal was overcome by a rapidly fatal infection, aided perhaps by the intoxication resulting from a large initial infection, its opsonic defense, except for the specific infecting bacteria, remained intact up to the very hour of its death.

Experiment 14. -- The two cases presented below are cats which died of a spontaneous infection of B. bronchisepticus. the infection in each case had lasted for three days and both animals presented moderate emaciation. During the last 24

hours of life the cats were in a state of extreme weakness, being unable to stand and responding only to violent stimulation. Samples of blood were taken from the heart shortly before death. The routine determinations are given in the appended table.

Date	Remarks	Bacteria	Animal	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	phagocytic Index
1/9/21	Cat #27	S.aureus	#27	50	33	17	125	2.5
			Con	50	35	15	116	2.3
	at time of death	B. coli	#27	50	50	0	306	6.1
			Con	50	48	2	264	5.2
		B. bronchisep.	#27	50	50	0	337	6.7
			Con	50	50	0	363	7.2
		Pn. I	#27	50	14	36	42	0.8
			Con	50	8	42	30	0.6
		B.typhosus	#27	50	44	6	96	1.9
			Con	50	45	5	94	1.9
1/9/21	Cat #28	S.aureus	#28	50	38	12	136	2.8
			Con	50	35	15	116	2.3
		B. coli	#28	50	48	2	271	5.4
			Con	50	48	2	264	5.2
		B. bronchisep.	#28	50	50	0	254	5.1
			Con	50	50	0	363	7.2
		Pn. I	#28	50	10	40	20	0.4
			Con	50	8	42	30	0.6
		B.typhosus	#28	50	44	6	90	1.8
			Con	50	45	5	94	1.9

Anatomical Diagnosis:

Moderate emaciation. Marked lobular pneumonia. B. bronchisepticus from blood and lungs in both cases.

Experiment 15. -- The guinea pig - No. 20 - of this experiment was inoculated on November 8, 1920, with the sediment

of 10 cc. of spinal fluid from a child dead of tubercular meningitis. By December 3 the pig had reached an advanced stage of generalized infection and was extremely weak and emaciated. The pig was sacrificed and a sample of blood obtained from the heart for opsonic determinations.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
12/3 20	Pig #30 at time of death	S.aureus	#30	50	45	5	305	6.1
			Con	50	41	9	273	5.5
		B. coli	#30	50	36	14	70	1.4
			Con	50	41	9	72	1.4
		Pn. I	#30	50	0	50	0	0.0
			Con	50	0	50	0	0.0
		Strep. hemo.	#30	50	48	2	600	12.0
			Con	50	46	4	625	12.5

Anatomical Diagnosis:

> Advanced generalized tuberculosis. Inguinal glands easily palpable; extensive infiltration, haemorrhage, necrosis at site of inoculation. Omentum, studded with tubercles, was rolled into a characteristically snarled mass. There was an extensive distribution of tubercles over the diaphragm and peritoneal wall. Smears from the enlarged glands and omentum revealed the presence of a large number of tubercle bacilli.

Notwithstanding the destructive changes which the infection had produced in this animal, there was no lowered opsonic efficiency for any of the non-specific organisms examined.

Experiment 16. -- The dog studied in this experiment had, when first seen on December 27, already developed charac-

teristic symptoms of "distemper." There was a continual nasal discharge associated with frequent sneezing and dyspnea. The animal was emaciated and staggered about when forced to its feet. A small gram-negative bacillus, probably Pasteurella canis, was present in enormous numbers in both the nasal secretion and blood stream. However, on December 30 the dog began to improve and continued to gain in strength until January 12. On this date it suffered an overwhelming and paralysing collapse. After this, the dog was unable to move and lay in a continual stupor. It died January 14, in a state of weakness so profound as at once to set it apart from any other animal observed during this investigation.

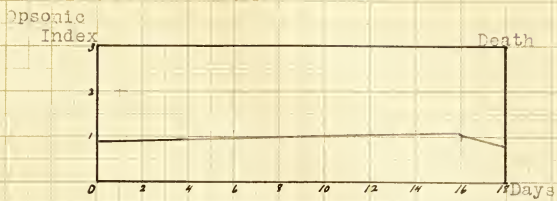
It does not seem possible that an animal could attain a more defenseless condition than this dog presented during the last 36 hours of its life, and yet throughout this period of extreme physiological depression there was no discoverable rupture in its phagocytic defense.

Full details of the experiment are set forth in the accompanying table.

Date	Remarks	Bacteria	Animal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Pact.	Phagocytic Index
12/27 20	Dog #47 wt. 5.7 Kg.	S.aureus	#47	50	33	17	180	3.6
			Con	50	37	13	199	3.9
		B. coli	#47	50	45	5	140	2.8
			Con	50	42	8	102	2.0
		B. bronchisep.	#47	50	23	27	38	0.7
			Con	50	26	24	27	0.5

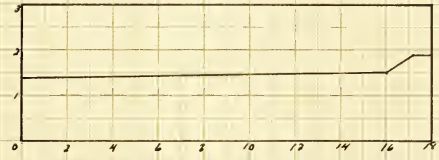
Fig. 12.

Dog No.47--Pastuerella Infection. Duration (observed) 18 days

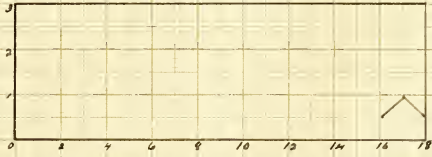


Opsonic Index Curve for Staph. aureus.

Opsonic Index Curve for B. coli.



Opsonic Index Curve for B. bronchisepticus.



Opsonic Index Curve for B. typhosus.

Date	Remarks	Bacteria	Ani-mal	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phago-cytic Index
1/12 21	Dog #47	S.aureus	#47	50	42	8	205	4.1
			Con	50	46	4	202	4.0
	wt. 5 Kg.	B. coli	#47	50	40	10	132	2.6
			Con	50	47	3	156	3.1
		B. bron-chisep.	#47	50	38	12	94	1.9
			Con	50	44	6	62	1.2
		B.typho-sus	#47	50	5	45	6	0.1
			Con	50	10	40	10	0.2
1/13	Dog #47	S.aureus	#47	50	36	14	162	3.2
			Con	50	44	6	202	4.0
	wt. 4.8Kg.	B. coli	#47	50	46	4	146	2.9
			Con	50	47	3	156	3.1
		B. bron-chisep.	#47	50	48	2	110	2.2
			Con	50	44	6	62	1.2
		B.typho-sus	#47	50	11	39	12	0.2
			Con	50	10	40	10	0.2
1/14	Dog #47	S.aureus	#47	50	41	9	163	3.2
			Con	50	46	4	202	4.1
	wt. 4.6Kg	B. coli	#47	50	47	3	163	3.2
			Con	50	47	3	156	3.1
	Death	B. bron-chisep.	#47	50	46	4	112	2.2
			Con	50	44	6	62	1.2
		B.typho-sus	#47	50	5	45	6	0.1
			Con	50	10	40	10	0.2

Anatomical Diagnosis:

Marked emaciation; purulent exudate in nasal cavity and pharynx; extensive broncho-pneumonia. Pasteurella canis cultivated from blood and from naso-pharyngeal exudate.

F. Infections in Man

It is desired to submit a study of the following human cases as a supplement to the animal experiments presented earlier in this investigation. While the number presented is small, the

exact agreement throughout with the results previously obtained endows these findings with considerable significance.

The method of procedure was the same as that employed in previous experiments. A sample of blood was obtained from the patient, or if from autopsy, as soon after death as possible, and after two hours the serum was separated and the usual opsonic determinations made. The control serum and leucocytes were obtained from a normal individual at the same time the experimental sample was taken. In some instances leucocytes were also obtained from the patient and here the "cytophagic" as well as the opsonic index was determined. These results are given in a subsequent table.

The experimental conditions and results of the study of human cases are tabulated below.

Case No. 1.

Clinical History: *

White female; age 69 years

Duration of present illness: July 1920 until March 8, 1921.

Entered hospital January 22, 1921; died March 8, 1921.

Diagnosis: Arteriosclerosis, hypertension, chronic nephritis, hemiplegia, acute bronchopneumonia..

Bacteriology: Blood culture repeatedly negative.

. . . . . .

*The clinical and autopsy data of the following human cases were secured from the Johns Hopkins Hospital records.

The complete opsonic data in this, as in the remaining cases, are condensed into the tables following the clinical histories.

Date	Remarks	Bacteria	Person	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
3/2/21	Patient #17	S.aureus	#17	50	45	5	335	6.7
			Con.	50	47	3	297	5.9
		B. coli	#17	50	32	18	77	1.5
			Con.	50	36	14	81	1.6
		B. bronchisep	#17	50	50	0	423	8.5
			Con.	50	50	0	361	7.2
		Pn. I	#17	50	9	41	16	0.3
			Con	50	5	45	8	0.2
3/3	Patient #17	S.aureus	#17	50	50	0	432	8.6
			Con	50	47	3	381	7.6
		B. coli	#17	50	32	18	51	1.0
			Con	50	35	15	80	1.6
		B. bronchisep	#17	50	50	0	356	7.1
			Con	50	48	2	345	6.9
		Pn. I	#17	50	19	31	63	1.2
			Con	50	10	40	56	1.1
3/5	Patient #17	S.aureus	#17	50	50	0	436	8.7
			Con	50	50	0	408	8.1
		B. coli	#17	50	25	25	45	0.9
			Con	50	28	22	46	0.9
		B. bronchisep	#17	50	50	0	236	4.7
			Con	50	49	1	221	4.4
		Pn. I	#17	50	0	50	0	0.0
			Con	50	0	50	0	0.0

Anatomical Diagnosis:

Autopsy not permitted.

Case No. 2. (Autopsy No. 6473)

Clinical History:

White male; age 50 years.

Duration of present illness: December 1920 to January 31, 1921.

Entered hospital January 7, 1921; died January 31, 1921.

Diagnosis: carcinoma of bladder; hydroureters; pyonephrosis; arteriosclerosis; with occlusion of coronary arteries; chronic myocarditis; jaundice.

Date	Remarks	Bacteria	Person	No.of Cells Count.	No.Cells with Bacteria	No. Cells Empty	Total No.of Bact.	Phagocytic Index
1/30 21	Patient #23	S.aureus	#23	50	50	0	390	7.8
			Con	50	42	8	248	4.9
	2 hours after death	B. coli	#23	50	43	7	159	3.2
			Con	50	50	0	116	2.3
		B. bronchisep	#23	50	50	0	600	12.0
			Con	50	50	0	297	5.9
		B.typhosus	#23	50	22	28	38	0.8
			Con	50	25	25	46	0.9

Anatomical Diagnosis:

"Carcinoma of bladder with extension to perivesical structures. Acute cystitis. Hydroureters and pyonephrosis. Arteriosclerosis with marked thickening and occlusion of coronary arteries. Chronic fibrous myocarditis. Jaundice. Anisocoria. Bilateral hydroceles. Healed tuberculosis of lung and bronchial nodes."

Case No. 3. (Autopsy No. 6486)

Clinical History:

Colored female; age 32 years.

Duration of present illness: May 1920 to January 10, 1921.

Entered hospital November 8, 1920; died February 10, 1921.

Diagnosis: Carcinoma of cervix, with metastasis to brain; pyelitis; bronchopneumonia; siphylis.

Panhysterectomy, January 10, 1921. Died 8 hours later.

Date	Records	Bacteria	Person	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
2/11 21	Patient #13	S.aureus	#13	50	42	8	298	6.0
			Con	50	48	2	316	6.3
		B. coli	#13	50	11	39	14	0.2
			Con	50	12	38	17	0.3
		B. bronchisep.	#13	50	48	2	250	5.0
			Con	50	46	4	196	4.0
		B.typhosus	#13	50	10	40	18	0.3
			Con	50	10	40	15	0.3

Anatomical Diagnosis:

"Unhealed operative wounds of abdominal wall. Adhesions about spleen, liver and intestine. Retroperitoneal abscess involving right iliopsoas muscle; abscesses in left kidney; dense adhesions (bilateral); acute bronchitis; mitral endocarditis; acute splenic tumor. Thrombosis of longitudinal sinus and cerebral veins. Thrombosis of pelvic veins."

Case No. 4. (Autopsy No. 6428)

Clinical History:

Colored male baby; age 10 days.

Diagnosis: Congenital syphilis; jaundice; osteochondritis.

Date	Remarks	Bacteria	Person	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
12/12 20	Patient #11	S.aureus	#11	50	41	9	213	4.2
			Con	50	40	10	204	4.1
	32 hours after death	B. coli	#11	50	48	2	167	3.3
			Con	50	49	1	160	3.2
		B.typhosus	#11	50	18	32	20	0.4
			Con	50	13	37	17	0.3
		B. pyoc.	#11	50	50	0	298	5.9
			Con	50	48	0	224	4.5
		Strep. hemo.	#11	50	7	43	12	0.2
			Con	50	12	38	32	0.6
		Strep. viri.	#11	50	7	43	13	0.2
			Con	50	11	39	21	0.4

Anatomical Diagnosis:

"Congenital syphilis; hepatitis; jaundice; osteochondritis."

Case No. 5. (Autopsy No. 6468)

Clinical History:

White male; age 39 years.

Duration of present illness: April 1920 to January 27, 1921.

Entered hospital January 13, 1921; died January 28, 1921.

Operated January 21 for removal of carcinoma of bladder. Streptococcus hemolyticus septicemia developed January 26.

Diagnosis: Carcinoma of bladder; streptococcus hemolyticus septicemia; uremia; chronic urethritis; acute prostatitis.

Date	Remarks	Bacteria	Person	No. of Cells Count.	No. Cells with Bacteria	No. Cells Empty	Total No. of Bact.	Phagocytic Index
1/28 21	Patient #5	S. aureus	#5	50	41	9	271	5.4
			Con	50	38	12	294	5.9
		B. coli	#5	50	44	6	87	1.9
			Con	50	38	12	105	2.1
		B. bronchisep.	#5	50	50	0	300	6.0
			Con	50	50	0	228	4.5
		Strep. hemo.	#5	50	3	47	9	0.2
			Con	50	16	34	81	1.6

Anatomical Diagnosis:

"Squamous cell carcinoma of bladder; gonorrhoeal urethritis; cystitis; ureteritis; pyonephrosis; pyelonephritis; prostatitis; surgical drainage of prostate; purulent peritonitis; bronchopneumonia; Adenoma of thyroid."

Bacteriological findings: Streptococcus hemolyticus in pure culture from heart's blood.

The results obtained here are in strict agreement with the findings previously reviewed. There is sometimes, though by no means in every case, a depressed opsonic activity against the specific infecting organism. Nowhere, however, even in the most chronic cases, is there any discoverable decrease in the phagocytic effectiveness against any bacteria not concerned in the primary infection. The downward variations in the opsonic index for these organisms are always within the possible range of experimental error.

--o--

PART II

FUNCTIONAL VARIATIONS OF PHAGOCYTES

While it is unquestionably true "that the essential regulating influence affecting phagocytosis rests upon the action of the serum upon the bacteria", (30) this should be weighed against the possibility that there may be variations in the functional capacities of the leucocytes themselves, independent of the action of the serum. Park and Biggs (31) pointed out that there was often a demonstrable difference in the phagocytic power of the leucocytes of normal persons, and in 1910 Glynn and Cox (32) obtained a similar result in persons suffering with staphylococcus and tuberculous infections. They introduced a new factor in opsonic determinations which they termed the "opsono-cytophagic-index," and which was obtained by determining the relative phagocytic power of the leucocytes and serum of one person compared with phagocytic activity of the leucocytes and serum of a control individual. Tunnicliff (33) demonstrated the difference in opsonic capacity exhibited by the leucocytes of a baby and those of an adult, emphasizing the necessity of obtaining cells and serum from children for opsonic determinations on babies. That there is considerable technical difficulty in comparing the phagocytic powers of two suspensions of leucocytes was demonstrated by Fleming (34) in an experiment in which he showed that given two unequal suspensions of leucocytes there was always less phagocytosis in the sample containing the greater number of leucocytes. This result was probably due to two factors, the relative number of bacteria in the sec-

ond suspension was less, and there was also a greater amount of non-specific absorption of opsonins in the suspension containing the larger number of cells.

The following tabulated results, selected from cases presented earlier in this paper, reveal the difficulty of obtaining an accurate and dependable measure of the relative phagocytic powers of leucocytic suspension obtained from different animals. As in the preceeding experiments the opsonic determinations are based upon a count of the bacteria taken up by 50 neutrophile leucocytes. The phagocytic mixtures were made by incubating the normal and experimental leucocytes separately with both the control and experimental sera. The kind of bacteria used and other conditions of the experiment are explained in the following table:

Date	Remarks	Bacteria	**Animal	Normal Leuco. *Total No.of Bact.	Normal Leuco. Phagocytic Index	*Experimen.Leuco. Total No.of Bact.	*Experimen.Leuco. Phagocytic Index
9/18	Dog #19	B. muc. capsu.	#19	1	0	120	2.4
20			Con	3	0	80	1.6
12/7	Pig #23	S.aureus	#23	133	2.6	150	3.0
			Con	150	3.0	190	3.8
		B. coli	#23	259	5.1	156	3.2
			Con	255	5.1	132	2.7
		Strep. hemo.	#23	300	6.0	198	3.9
			Con	398	7.9	196	3.9
12/10	Pig #23	S.aureus	#23	244	4.8	110	2.2
			Con	204	4.1	104	2.1
	4 hours before death	B. coli	#23	60	1.2	45	0.9
			Con	48	1.0	30	0.6
		Strep. hemo.	#23	44	0.8	20	0.4
			Con	93	1.8	17	0.3

*No. of bacteria in 50 neutrophile leucocytes.
**Animal providing serum.

Date	Remarks	Bacteria	**Animal	Normal Leuco. *Total No.of Bact.	Normal Leuco. Phago-cytic Index	Experimen.Leuco. *Total No.of Bact.	Experimen.Leuco. Phago-cytic Index
12/10 20	Pig #23	S.aureus	#23	215	4.3	102	2.0
			Con	204	4.1	104	2.1
	Death	B. coli	#23	50	1.0	42	0.8
			Con	48	1.0	30	0.6
		Strep. hemo.	#23	44	0.9	22	0.4
			Con	92	1.8	17	0.3
3/3/21	Patient #17	S.aureus	#17	432	8.6	247	4.9
			Con	381	7.6	252	5.1
		B. coli	#17	51	1.0	44	0.9
			Con	80	1.6	16	0.3
		Pn. I	#17	63	1.2	21	0.4
			Con	56	1.1	25	0.5
		B. bron.	#17	326	6.6	382	7.6
			Con	345	6.9	440	8.5
12/26 20	Dog #13	B.typho-sus	#13	78	1.5	128	2.6
			Con	10	0.2	37	0.7

*No. of bacteria in 50 neutrophile leucocytes.
**Animal providing serum.

While this table reveals a considerable variation in the "cytophagic" indices of the normal and infected animals, the nature of this variation is by no means constant. Sometimes the index for the treated animal is lower for the non-specific bacteria than in the control animal but quite as often it is higher. Altogether the variations observed are no greater than the work of Fleming (34) would lead us to expect for two lots of normal leucocytes. While experiments of this kind are exceedingly difficult to control and dangerous to interpret, it is contended that the results set forth in the above table are sufficiently definite to indicate that there is no invariable decline in the function of the phagocytes during the last stages of fatal infection.

PART III

INADEQUACY OF THE WRIGHT TECHNIQUE

The remaining experimental portion of this paper will be devoted to an analysis of the adequacy of the usual opsonic methods for extimating the phagocytic capacity of an animal. During the course of this investigation there have been several occasions when the opsonic index, determined in the accepted manner, has given not only an inaccurate gauge of the animal's phagocytic defense but has indicated a result the exact opposite of what was subsequently demonstrated to be the true condition. For these reasons it seems altogether worth while to examine the additional factors necessary to make Wright's method a dependable technique for determining, so far as it is possible to determine, the true phagocytic strength of an animal.

Wright (35) has demonstrated by a series of experiments which have been repeatedly confirmed that the stimulating effect of serum on phagocytosis is directed almost entirely against the bacteria. And it is reasonable to suppose from Bordet's (36) work that whenever one bacterium in a medium has been sufficiently opsonized to permit its being taken up by a leucocyte, all similar bacteria of comparable virulence in that environment will likewise be sufficiently sensitized to effect their engulfment. Neglecting, then, the possibility of non-specific absorption of opsonins by the lecocytes, the extent of the subsequent phagocytosis would be determined entirely by the number of phagocytic cells present. Accordingly, it would be impossible to

estimate the comparative phagocytic capacity of two animals without taking into consideration not only the white count of the blood but also a differential determination in order to obtain the number of neutrophile leucocytes, the cells most commonly concerned in the phagocytosis of bacteria.

Even by this method of determining a more exact measure of phagocytic capacity, no account is, or can be, taken of the tremendous phagocytic powers of the "fixed" cells. Metchnikoff (37) first pointed out that these cells play an important role in resistance, and in a more recent investigation Bartlett and Ozaki (8) demonstrated that the phagocytic capacity of the "fixed" cells often reveals a compensatory increase whenever there is an exhaustion of the circulating phagocytes. It would therefore seem necessary to balance any demonstrable decrease in phagocytic activity of the blood against the possibility that this loss in effectiveness might be compensated for by increased activity of the infinitely more numerous "fixed" cells.

There remains one other factor that should be considered before concluding that a lowered opsonic index, secured according to the method of Wright, indicates an absolute depression in phagocytic defense. It is reasonable to suppose that in consequence of the rapid destruction and replacement of neurtophile leucocytes during infection that these cellular elements are younger in infected than in normal animals. Hektoen (38) suggests that it is possible to account for the increased phagocytic activity which Tunnicliff (39) demonstrated in exudates

and in recovering pneumonia cases, by the fact that the cell obtained under these circumstances are younger than the leucocytes in the blood stream of normal individuals. This suggests, then, that it would not be possible to demonstrate conclusively a depression in phagocytic effectiveness without first eliminating the possibility of an increased "cytophagic" index which might even over compensate for the decline in opsonins.

The results of this investigation would seem to warrant the conclusion that with the possible exception of cases with extreme leucopenia the relative phagocytic power of an infected animal is never lower than the opsonic index indicates, but that it is sometimes, indeed it is commonly, very much higher. The opsonic index, with the exception noted above, never does more than express the minimal limitations of the animal's phagocytic defense.

While it is manifestly impossible, by any experimental means whatever, to estimate accurately the phagocytic capacity of an animal, it is quite useless to employ the Wright technique unless a correction is made for the observed variations in the number of neutrophile leucocytes present in the experimental animals. The factor by which this correction is made in the following table was obtained in each case by dividing the number of neutrophilic leucocytes present in 1 cmm. of experimental blood by the corresponding count in the control. The Wright opsonic index is then multiplied by the above factor. This new measure of relative phagocytic capacity may for convenience

be designated as the differential opsonic index.

The following data are intended to show the results of opsonic determinations made by taking into consideration the white count of the infected animals. Both the Wright and differential opsonic indices are given.

Date	Remarks	Normal		Experimen.		Bacteria	Factor	Wright Opson Index	Dif.** Opson Index
		Av. White Count	%Neu-troph. Leuco.	Av. White Count	%Neu-troph. Leuco.				
1/26 21	Dog #51 Death Bronchi. Infect.	10600	*75	27000	*85	S.aureus	2.0	1.3	2.6
						B. bron-chisep.	2.0	1.3	2.6
						B. coli	2.0	1.1	2.2
2/2	Dog #19 Death Typhoid Infect.	10600	75	3500	35	S.aureus	0.16	1.1	0.17
						B. bron.	0.16	1.2	0.19
						B. coli	0.16	1.1	0.17
						B. typho	0.16	9.0	1.4
1/24	Dog #11 36hr.be-fore death Pn.I Infec	10600	75	3000	75	S.aureus	0.28	1.0	0.28
						B. bron.	0.28	1.0	0.28
						B. coli	0.28	1.1	0.30
						Pn. I	0.28	1.0	0.28
2/21	Cat #38 Death Strepto Infect.	14800	69	50000	73	S.aureus	3.5	0.9	3.1
						B. bron.	3.5	0.9	3.1
						Str.hemo	3.5	0.5	1.7
2/20	Cat #38 39 hrs. before death	14800	69	100000	90	S.aureus	8.8	0.9	8.0
						B. bron.	8.8	0.9	8.0
						B. coli	8.8	1.3	11.4
						S. hemo.	8.8	0.8	7.0
2/18	Cat #37 15 hrs before death Strepto Infect.	14800	69	12000	88	S.aureus	1.0	1.0	1.0
						B. bron.	1.0	1.0	1.0
						B. coli	1.0	1.2	1.2
						S. hemo.	1.0	0.5	0.5

** Differential Opsonic Index. *Average counts compiled from results obtained in this laboratory, supplemented by the counts of Klieneberger and Carl (42).

Date	Remarks	Normal		Experimen		Bacteria	Factor	Wright Opson Index	Dif. Opson Index
		Av. White Count	%Neu-troph. Leuco.	Av. White Count	%Neu-troph. Leuco.				
3/2 21	Pat. #17	5500	69	11400	74	S.aureus	2.2	1.0	2.2
						B. coli	2.2	1.0	2.2
						B. bron.	2.2	1.0	2.2
						Pn. I	2.2	1.0	2.2
1/3	Pat. #23	5500	69	19300	73	S.aureus	3.8	1.5	5.7
						B. coli	3.8	1.3	4.9
						B. bron.	3.8	1.2	4.5
						B. typho.	3.8	0.9	3.4
1/28	Pat. #5	5500	69	32840	89	S.aureus	7.7	0.9	6.9
						B. coli	7.7	0.9	6.9
						B. bron.	7.7	1.3	10.0
						S. hemo.	7.7	0.12	0.9
2/11	Pat. #13	5500	69	24000	75	S.aureus	4.7	0.9	4.2
						B. coli	4.7	0.8	3.7
						B. bron.	4.7	1.2	5.6
						B. typho.	4.5	1.0	4.5

A study of the above table reveals that an actual depression in the phagocytic activity associated with infectious disease is not common, and indeed is exceedingly rare, except in individuals with a low leucocytic count. In most cases the increase in neutrophile leucocytes over compensates for the observed decrease in opsonic efficiency revealed by the Wright technique. It seems reasonable, then, to suppose that infections may triumph, not because of a rupture in phagocytic activity of the animal but in spite of a very considerable increase in the effectiveness of that defensive mechanism.

DISCUSSION

Throughout this investigation it has been the custom in each enumeration to determine not only the total number of bacteria taken up by 50 neutrophile leucocytes, but also to record the number of these cells actually taking part in the engulfment. A survey of these records reveals in general a parallelism between the variations in the phagocytic indices and fluctuations in the percentage of phagocyting cells. Commonly a high phagocytic index is associated with a large percentage of phagocyting leucocytes, while a low index generally is obtained in preparations with few ingesting cells. However, this is not uniformly true and of the two methods of determining the degree of phagocytic activity it would appear from this research that the technique of counting the number of organisms ingested is the more delicate and reliable. It has frequently happened that the experimental animal revealed the same percentage of phagocyting cells as the control but yet exhibited the utmost variation in the total number of bacteria ingested.

There is always a question whether the opsonic results obtained by incubating samples of cells and serum with indifferent suspensions of bacteria can be accepted as indicating even approximately the conditions which prevail in the animal body. It is, of course, at once recognized that a culture of organisms grown on artificial medium may differ fundamentally in their reaction to living cells, from bacteria which develop

within the body. Bordet (40) has shown, for example, that even when the most virulent organisms are injected into a susceptible animal, there is an initial phagocytosis. This he explains as a selective process. Supposing that any culture, no matter how virulent, contains numbers of feeble and weakly aggressive individuals and these the phagocytes at once select and devour. The remaining highly selected bacteria then, in some cases, extend the infection without any phagocytic interference. Zinsser (41) likewise has pointed out that it is possible to obtain agglutination of "culture" pallida but quite impossible to do so when the spirochetes are taken directly from a lesion. A certain reasonableness therefore attaches itself to the objection that with phagocytic determinations in vitro one may be dealing largely with the enfeebled non-pathogenic fraction of the cultures examined and that the findings are worthless as an index of vital phagocytic defense. It cannot be denied, however, that opsonic determinations possess some significance and diagnostic value. It is possible that such observations in a specific case may be of uncertain value, but when extended over a large series, representing a wide variety in species and conditions, a uniform result gives ample justification for conservative deductions.

Perhaps the most striking feature of this investigation has been the unfailing effectiveness of phagocytic activity against the non-specific bacteria. In not one instance has there been any appreciable decline in the opsonic index for any microorganisms not concerned in the primary infection. This

has been uniformly true even in cases of extended infection, associated during the last days with the most destructive emaciation and with what appeared to be complete collapse of the animal's vital defensive mechanism.

It has also been developed in this paper that the Wright opsonic index is useful for determining the lower limits of phagocytic capacity but may give no adequate measure of the actual extent of an animal's opsonic defense. It appears certain that the percent of infected animals dying because of a collapse in the phagocytic defense is very much smaller than is commonly supposed. Indeed, it is quite often as inaccurate and meaningless to say that an infected animal is overcome on account of a rupture in its phagocytic defense as it would be to contend that a man was run down by a train because of a break in his running technique, although at the time of the disaster he was exhibiting a speed beyond anything he had ever developed before in his life time.

CONCLUSIONS

The results obtained in this investigation would seem to warrant the following conclusions:

1. Although there is sometimes a decrease in the opsonic index for the specific infecting organism during the late stages of fatal infections, there is no decrease in the phagocytic activity against any bacteria not concerned in the primary infection.

2. Neutrophilic leucocytes from an animal in the late stages of a fatal infection are as actively phagocytic as normal cells when placed in a medium containing suitable opsonins. This indicates that there is no decrease in the phagocytic function of these cells.

3. The "differential" opsonic index is a more reliable measure of the animal's phagocytic capacity than the Wright opsonic index.

4. Absolute depression in phagocytic effectiveness against the infecting organism is not an invariable phenomenon in fatal infections.

--o--

In closing this paper I wish to thank Dr. MacCallum for his courtesy and assistance, and I desire to acknowledge a very especial debt of gratitude to Dr. Bayne-Jones for the substantial contributions he has made to the development of this problem.

Biography

Howard B. Cross was born in Conway Springs, Kansas, July 31, 1889. He graduated from the public schools of Oklahoma in 1905 and after completing three years of high school work taught for two years in a rural school. Then graduating from the Oklahoma Preparatory School, he entered the University of Oklahoma in the fall of 1911. The following year he was appointed Student Assistant in the Department of Zoology, and in September 1913 was selected as Acting-Head of the Department of Biology in the Southwestern State Normal School. After a year at this institution, Mr. Cross returned to the University of Oklahoma as a student and Assistant in Botany, and graduated in 1915. He then studied at the Marine Biological Laboratory throughout the summer of 1915, and in the fall returned to the University of Oklahoma to accept the position of Instructor in Zoology. He continued in this position until 1917. The summer of 1916 Mr. Cross spent in the University of Chicago and in 1917 he returned to that University as a graduate student and Fellow in the Department of Zoology. After serving in the Army Neuro-Surgical Laboratory, Baltimore, during 1918, Mr. Cross was appointed Assistant in the Department of Pathology and Bacteriology, Johns Hopkins University. He enrolled in the graduate school of this University in 1920 and completed the work necessary for his degree under Professor MacCallum.

1. MacCallum, W. G., A Text Book of Pathology, New York, 1920, 528.
2. Osler, Wm., Practice of Medicine, New York, 1901, 165.
3. Flexner, S., J. Exper. M., N. Y., 1896, I, 559.
4. Wright, A. E. and Windsor, F. N., J. Hyg., Cambridge, 1902, II, 385.
5. Bordet, J., Studies in Immunity, New York, 1909, 37.
6. Bordet, J., Ibid., 23.
7. Tunnicliff, R., J. Infect. Dis., Chicago, 1912, XI, 474.
8. Bartlett, C. J., and Ozaki, Y., J. Med. Research, Bost., 1917, XXXVII, 139.
9. Metchnikoff, E., Bull. Med. Par., 1893, VII, 63.
10. Wright, A. E. and Douglas, S. R., Proc. Roy. Soc., Lond., 1904, LXXIV, 147.
11. Fevre, M., Compt. rend. Soc. de biol., Par., 1919, LXXXII, 605.
12. Bull, C. G., J. Med. Research, Bost., 1917, XXVI, 7.
13. Hektoen, L., J. Infect. Dis., Chicago, 1906, III, 102.
14. Opie, E., Tr. Ass. Am. Physicians, 1907, XXII, 507.
15. Zinsser, H., Infection and Resistance, New York, 1919, 346.
16. Wright, A. E. and Douglas, S. R., Proc. Roy. Soc., Lond., 1904, LXXIV, 128.
17. Wright, A. E. and Douglas, S. R., Ibid., 156.
18. Cross, H. B., Johns Hopkins Hos. Bull., 1921, XXIII, 51.
19. Neufeld, F. and Rimpau, W., Ztschr. f. Hyg. u. Infectionskrankh., Leipz., 1905, LI, 283.
20. Rosenow, E. C., J. Infect. Dis., Chicago, 1907, IV, 285.
21. Bieling, R., Ztschr. f. Immunitatsforsch. u. exper. Therap. Jena, Original, 1919, XXVIII, 246.
22. Rosenow, E. C., J. Infect. Dis., Chicago, 1907, IV, 285.
23. Wright, A. E. and Douglas, S. R., Proc. Roy. Soc., Lond., 1904, LXXIV, 156.
24. Neufeld, F. and Topfer, H., Centralbl. f. Bakteriol., Jena, 1905, XXXVIII, 456.
25. Opie, E., Tr. Ass. Am. Physicians, 1907, XXII, 507.
26. Harrison, W., Lancet, Lond., I, 904.
27. Klein, H., Johns Hopkins Hos. Bull., Balt., 1907, XVIII, 245.
28. Bordet, J., Studies in Immunity, New York, 1909, 226.
29. Denys, J. and Leclef, J., Bull. Acad. roy. de Med. de Belg., Brux., 1895, IX, 1089.
30. Zinsser, H., Infection and Resistance, New York, 1919, 314.
31. Park, W. and Biggs, H., J. Med. Research, Bost., 1907, XVII, 77.
32. Glynn, E. and Cox, G., J. Path. Bacteriol., 1910, XIV, 90.
33. Tunnicliff, R., J. Infect. Dis., Chicago, 1910, VII, 698.
34. Fleming, A., Practioner, Lond., 1908, LXXX, 607.
35. Wright, A. E. and Douglas, S. R., Proc. Roy. Soc., Lond., 1904, LXXIII, 128.
36. Bordet, J., Studies in Immunity, New York, 262.
37. Metchnikoff, E., Ann. de l'Inst. Pasteur, Par., 1901, XV, 665.
38. Hektoen, L., J. Am. M. Ass., Chicago., 1911, LVII, 1579.
39. Tunnicliff, R., J. Infect., Chicago, 1911, VIII, 302.

40. Bordet, J., Studies in Immunity, New York, 1909, 13.
41. Linsser, H., Hopkins, J., and McBurney, E., J. Exper. Med., N. Y., 1916, XXIII, 341.
42. Klieneberger, C. and Carl, W., Die Blut-Morphologie der Laboratoriums-Tiere, Leipzig, 1912.

--o--