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ON BACTERICIDAL SUBSTANCES EXTRACTED FROM NORMAL LEUCOCYTES.*

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The present research was begun as a direct continuation of the work done upon the curative action of extracts of leucocytes by Hiss[1] and Hiss and Zinsser,[2] and published in November, 1908. In this work it was found that aqueous extracts of normal rabbit leucocytes exerted an unmistakably favorable influence upon a variety of infections in rabbits, and that the same substances apparently influenced favorably the outcome of disease in human subjects afflicted with pneumonia and meningitis. Since the publication of these results, similar observations have been published upon localized staphylococcus infections[3] in man, and numerous similar results have been achieved by the writers as well as by others upon pneumococcus and streptococcus infections in man. Especially extensive observations upon erysipelas have been made by Dr. Adrian V. S. Lambert, whose work is in press at the present writing.

The results achieved by Hiss in his animal experiments and by the subsequent work upon the human subject were, at that time, tentatively attributed to an endotoxin neutralization exerted by the leucocytic substances upon various infectious agents, and experiments directly aimed at the demonstration of this point, but not yet completed, were

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begun. Meanwhile, it was deemed advisable to investigate again in more detail the various immune bodies which appeared in extracts of rabbit leucocytes prepared by simple aqueous extraction. The work was afterward extended to substances extracted in salt solution after repeated freezing and thawing. The experiments recorded below were aimed primarily at the investigation of the bactericidal bodies present in the leucocytes, though a few experiments were done to ascertain the presence of agglutinins and hemolysins, as will be seen in the text.

That the leucocytes are the seat of origin of serum alexin, or complement, is of course an idea which has been held by many of the earlier writers, and the views of Metchnikoff on this subject are too well known to require elucidation. Buchner,[4] in his experiments in 1894, had noticed that aleuronat exudates produced intrapleurally in rabbits and dogs possessed a bactericidal value for B. coli which exceeded the bactericidal power of the blood serum itself. The influence of active phagocytosis could be excluded by the fact that the leucocytes of the exudate had been killed by repeated freezing and thawing. Similar results were obtained by Hahn[5] with B. typhosus. In both of these cases, however, it must be remembered that the bactericidal values of the leucocytes plus serum were investigated, and not that of leucocytes and leucocyte extracts alone.

Denys,[6] working along similar lines, found that the pleural exudates of rabbits, obtained by the injection of dead staphylococci and freed of cells by centrifugalization, were more highly bactericidal for staphylococci than the blood serum of the same animals, but found also that the inactivated exudate could not be reactivated by the addition of leucocytes. Denys offered as an explanation for these phenomena that the living leucocytes in the original exudate secreted alexin or complement which enhanced the bactericidal activity of the exudate, that the leucocytes, subsequently added to inactivated exudate, however, had lost vitality during the process of isolation and washing, and no longer possessed secretory power.

In the foregoing cases the investigators had worked with combinations of blood serum and leucocytes and had encountered interesting problems regarding the interaction of these substances upon which the work here recorded has no direct bearing.

Schattenfroh,[7] writing in 1897, worked not only with combinations of serum and leucocytes, but also with leucocytes suspended and extracted in physiological salt solution. With the latter substances he obtained results which warranted the conclusion that normal rabbit and guinea-pig leucocytes contained bactericidal substances for staphylococci, but did not contain such substances for Spirillum choleræ asiaticæ nor for Bacillus typhosus. He found, furthermore, that these substances were more thermostable than the alexin of blood serum, being uninjured by temperatures of 60° C., and being destroyed only upon heating to 75° to 80° C. for half an hour.

Moxter,[8] whose work occupied itself chiefly with the question of the origin of the bactericidal substances in the blood serum, came to the conclusions that the bactericidal substances detectable in leucocytes and leucocytic extracts were slight in quantity, and that leucocytes could not be regarded as the source either of the bactericidal immune body of the serum or of alexin or complement. Moxter worked entirely with cholera spirilla.

Petterson,[9] who had made thorough and invaluable investigations into the nature of the bactericidal leucocytic substances, and who worked chiefly with B. proteus and B. anthracis, found such substances in the leucocytes of dogs, rabbits, and guinea-pigs active against the bacteria mentioned above, but failed to find them active, at least in guinea-pig and cat leucocytes, against B. typhosus or the cholera vibrio. He expresses the beliefs that bactericidal leucocytic substances are normally given up to the blood in minute quantity only or not at all, and that these substances hold no definite relationship to the bactericidal substances found in blood serum. The theoretical conceptions in which he applies his results to the explanation of certain phenomena

of immunity in general, however interesting, have no direct bearing upon the subject of this paper.

In a later communication, Petterson[10] has shown that the endolysins, as he now calls the leucocytic bactericidal substances, may, like many enzymes and serum bacteriolysins, be precipitated out of solution with alcohol and ether; but he separates them absolutely from serum lysins and complément. These latter, while they may be, in part at least, secreted by the leucocytes, are, according to Petterson, induced easily to come out of the cells during life by slight injury or other stimulation, while the endolysins, with which we are dealing in this communication, are abstracted from the cells only after extensive extraction or maceration.

Recently Watabiki,[11] working with rabbit leucocytes extracted by freezing in salt solution, has concluded that polynuclear leucocytes of rabbits contain no bactericidal substances for B. typhosus, B. coli, B. dysenteriæ (Shiga), and Spirillum choleræ. In one experiment only did he observe such action upon B. typhosus and the cholera spirillum. On the rather insufficient basis of ten experiments he expresses a doubt of the existence of endolysins in the sense of Petterson.

Korschun[12] working with rabbit leucocytes, extracted by repeated freezing and thawing, comes to conclusions differing from those of his predecessors in regard to bacillus typhosus. He concludes that such extracts do have distinct bactericidal action upon typhoid bacilli.

An extensive investigation of the bactericidal substances which can be extracted from leucocytes has recently been made by Schneider.* This author reaffirms the statement of other workers that these bactericidal substances, for which he coins the word "Leukines," are distinct from the complement or the bactericidal antibodies of blood serum. His emphatic denial of the identity of the leucocytic extractives with the complement of serum seems hardly necessary in view of the

* Schneider. Arch. f. Hyg., 1909, lxx.

repeated assertion of the distinct characters of these bodies by Schattenfroh, Petterson, and others.

Our own work was done entirely with extracts of the washed leucocytes of rabbits, obtained by aleuronat injections, as described in our previous papers. The experiments which are described in the following protocols were aimed at the following problems:

1. Do normal rabbit leucocytes contain bactericidal substances for certain bacteria?

2. What is the nature of these substances as regards their thermostability?

3. May these substances after inactivation be reactivated by fresh leucocytic extracts or by serum?

4. How does the bactericidal power of these substances compare with that of the serum of the same animals?

5. Do substances extracted from leucocytes contain complement or alexin — either for bactericidal immune bodies of serum or for hemolytic amboceptors?

6. Are the bactericidal leucocytic substances specifically increased during the course of immunization?

Technic. — In carrying out the experiments described below, a uniform technic was developed and adhered to throughout. The substances in which bactericidal action was to be determined were carefully measured in definite volumes into sterile test-tubes with pipettes.

To these tubes were added, in each experiment, equal volumes of a bacterial emulsion in salt solution and the contents thoroughly mixed. Equal volumes of the mixtures were then immediately added to agar melted and cooled to 45° C. and plates poured. The mixtures were then incubated at 37.5° C. and platings made in a similar way, with the same capillary pipette, after varying intervals as indicated in the experiments. Both in adding the bacteria to the tubes originally, and in taking equal volumes from these tubes for the purpose of plating, the old platinum loop method was discarded for the use of a capillary pipette on which a mark had been made with a grease pencil about one and one-half

inches from the end and fitted with a rubber nipple. When such a pipette was carefully handled, washed out in hot water between platings, and dried over a flame, surprising uniformity of results was obtained. During the first experiments platings were done in sets of five or more to avoid error. As greater expertness in the technic was acquired, and after its reliability had been well demonstrated, duplicate plates and even single platings were regarded as sufficient. The experiments were rigidly controlled throughout.

The leucocyte extracts were obtained, at first, by washing the sediments from centrifugalized pleural aleuronat exudate from rabbits in .85 per cent salt solution, and then extracting in a volume of distilled water equal to the original volume of the exudate. The leucocytes were thoroughly emulsified in the water, and the emulsions left in the incubator for from six to eight hours and then for twenty-four hours or more in the ice-chest. The clear fluid was then pipetted off after final centrifugalization.

For purposes of more thorough extraction the leucocytes were emulsified in the salt solution and repeatedly frozen in a brine-ice mixture. They were then placed in the incubator for six to eight hours, and extraction, after this, continued in the refrigerator for twenty-four hours or more.

While absolute uniformity of the leucocytic concentration in the extracts is, of course, desirable, it is extremely difficult to obtain and was only roughly attempted in the experiments here recorded.

Rabbit leucocytes have been used throughout the experiments.

Experiment I.

(*a.*) To determine the bactericidal power of aqueous leucocyte extract upon Staphylococcus pyogenes aureus. Controlled with distilled water to exclude possibility of simple destruction of bacteria by hypotonicity of medium.

Aleuronat exudate. Leucocytes isolated by centrifugalization. Washed four times in salt solution. Extracted

in distilled water five hours at 37.5° C., twenty-four hours in refrigerator.

Staphylococcus pyogenes aureus "Pr." Throughout the experiment twenty-four-hour cultures on agar slant were used. One loopful of the growth was emulsified in ten cubic centimeters of salt solution and a definite quantity of this transferred to tubes with a capillary pipette.

	Leucocyte Extract 5 cc.	Sterile Control Distilled Water 5 cc.
Transplants made immediately.	6709	7123
	7441	6711
	6996	8033
	6527	7796
	5628	5523
	8605	
After two hours.	1297	4547
	1735	3843
	1303	5151
	2016	4070
	1431	
	1123	
After twenty-four hours.	1800	20
	1226	0
	1381	5
	988	
	1012	
After forty-eight hours.	17450	0
	18856	0
	17998	

There was a distinct diminution of staphylococci in the leucocyte extract, more rapid than in the distilled water, thus excluding the possibility of destruction by hypotonicity alone. Later the death of the organisms in water progresses to sterility, while the organisms, having exhausted the germicidal substances, begin to grow in the extract.

(*b.*) Comparison of bactericidal values of leucocyte extract, normal rabbit serum and supernatant fluid from aleuronat exudate after centrifugalizing out the leucocytes.

Aqueous extract of leucocytes of same lot as in preceding.

Staphylococcus pyogenes aureus "Pr."

	Leucocyte Extract 5 cc.	Fresh Normal Rabbit Serum 5 cc.	Supernatant Fluid of Exudate 5 cc.	Distilled Water Control 5 cc.
Transplanted immediately.	6678 7377 6868 6922 8204	5992 5724 6951	6360 5469 7186	6400 6252 5893
Two hours at 37.5°.	326 444 412 392 426	55 49 46 73 63	202 218 260 160 258	3279 4298
Four hours at 37.5°.	186 220 290 248 186	13 10 24 12 16	33 41 30	2736 3243
Nineteen hours at 37.5°.	390 648 660 768	10000 + 10000 +	20000 + 20000 +	1 26
Twenty-eight hours at 37.5°.	280 190 290 210	+ + + + + + + +	+ + + + + + + +	0 0

Destruction of bacteria in leucocyte extract distinct though less rapid or marked than in serum or in the serous exudate taken from pleuræ. Again the destruction is seen to be more rapid than in distilled water.

(*c.*) Comparison of leucocyte extract and normal serum. Aqueous leucocyte extract as before.

	Leucocyte Extract 2.5 cc.	Normal Rabbit Serum 2.5 cc.	Control Distilled Water 2.5 cc.
Transplanted immediately.	1144 880	1017 1272	950 890
After five hours at 37.5° C.	220 98	20 18	600 580
After twenty-four hours at 37.5° C.	1000 1200	20000 + 20000 +	0 12

Results correspond to Experiment I. (*b*). There were fewer organisms in comparison to the amount of serum and extract used, hence the difference in the number of organisms alive after five hours is less marked.

(*d.*) Comparison of bactericidal power of aqueous leucocyte extract upon various strains of Staphylococcus pyogenes aureus.

Aqueous leucocyte extract as before.

Staphylococcus pyogenes aureus "Pr." old laboratory strain. Staphylococcus pyogenes aureus "Al." isolated from a boil two weeks previously, and

Staphylococcus pyogenes aureus "El." isolated from a boil two days previously.

	Aqueous Leucocyte Extract 2 cc. S.P.A. "Pr."	Aqueous Leucocyte Extract 2 cc. S.P.A. "Al."	Aqueous Leucocyte Extract 2 cc. S.P.A. "El."	Distilled Water 2 cc. S.P.A. "Pr."
Transplanted immediately.	2544	3307	4452	4752
After three hours at 37.5° C.	25 32	636 550	2106 1820	1908 1400
There were killed.....	2516	2814	2492	

Considering the differences in quantity of bacteria in the platings made immediately, no differences in bactericidal action upon the various strains can be deduced.

(*e.*) Comparison of bactericidal action of aqueous leucocyte extract upon two different strains of Staphylococcus pyogenes aureus.

Aqueous leucocyte extract new lot.

Staphylococcus pyogenes aureus "Pr." an old laboratory strain.

Staphylococcus pyogenes aureus "El." isolated from a boil three days previously.

	Aqueous Leucocyte Extract 2 cc. S.P.A. "El."	Aqueous Leucocyte Extract 2 cc. S.P.A. "Pr."	
Transplanted immediately.	4324	3434	
After three hours at 37.5° C.	1081 850	445 380	
There were killed	3359	3022	

Again there is no noticeable difference in bactericidal effect of the leucocyte extract upon the various strains.

Experiment II.

(*a.*) Comparison of bactericidal effect of normal rabbit serum with leucocyte extract produced by repeated freezing and thawing.

Salt solution extract of leucocytes.

Staphylococcus pyogenes aureus "Pr."

Emulsion prepared as in Experiment I.

	Leucocytes Extracted in Salt Solution 2 cc.	Normal Serum 2 cc.	Salt Solution 2 cc.
Transplanted immediately.	10000 +	10000 +	10000
After three hours at 37.5° C.	80 26	12 24	10000
After twenty-four hours at 37.5° C.	10000 + 10000 +	20000 + 20000 +	

(*b.*) Same as preceding.

Leucocyte extract of same lot as in Experiment II. (*a*).

Staphylococcus pyogenes aureus "Pr."

	Leucocyte Extract 2 cc.	Normal Serum 2 cc.
Transplanted immediately.	2316 2162	2289 1961
After three hours at 37.5° C.	140 96 188	40 52 46

(*c.*) To determine whether there is any autolysis of staphylococci in salt solution which might lead to erroneous conclusions.

Staphylococcus pyogenes aureus "Pr."

	Salt Solution 5 cc.	
Planted immediately.	20000 + 20000 +	
After three hours at 37.5° C.	20000 + 20000 +	

There is apparently no noticeable diminution of staphylococci in salt solution.

In Experiment II. (*a*) and (*b*), there was evidence of marked bactericidal action upon Staphylococcus pyogenes aureus. The control of (*a*) and II. (*c*) showed that no diminution of the bacteria took place by autolysis in salt solution.

(*d.*) Comparison of the bactericidal power of aqueous and of saline extract of leucocytes, the latter produced by alternate freezing and thawing, both prepared from the same original lot of leucocytes.

Staphylococcus "Pr."

	Aqueous Leucocyte Extract 2 cc.	Saline Leucocyte Extract 2 cc.
Transplanted immediately.	3434	3052
After three hours at 37.5° C.	445 380	508 318

There was no noticeable difference between the action of the aqueous and that of the saline extract of leucocytes.

The foregoing experiments have shown that both aqueous leucocyte extracts and those obtained by extraction in salt solution aided by repeated freezing and thawing, contained easily determinable bactericidal substances for staphylococci. These substances were less active or in smaller relative quantity than the bactericidal bodies of the serum and the supernatant fluid obtained from the pleural exudate after removal of the leucocytes.

It was now desirable to determine more accurately the nature of these substances, especially in regard to their resistance to temperature. Since in all the following experiments unheated leucocyte extracts were also examined, these protocols serve to strengthen the facts ascertained in the simpler experiments which have preceded.

Experiment III.

(*a.*) Does heating to 56° C. for twenty minutes destroy the bactericidal power of aqueous leucocyte extract?

Aqueous leucocyte extract as before.

Staphylococcus pyogenes aureus "Pr."

	Leucocyte Extract 5 cc. Unheated.	Leucocyte Extract 5 cc. Heated 56° C. 20 Minutes.	Leucocyte Extract 5 cc. Heated 56° C. 20 Minutes.	Distilled Water 5 cc.
Transplanted immediately.	890 1081 1017	1335 1070 839	800 1208 1081	1220
After four and one-half hours at 37.5° C.	10 20 32	82 68 108	58 88 112	954
After twenty-four hours at 37.5° C.	58 106 86	560 880 920	526 480 866	0 18
After forty-eight hours at 37.5° C.	445 244 262	20000 + 20000 +		

Apparently no marked diminution of bactericidal power had been caused by heating to 56° C. The slight differences between the unheated and the heated, after three hours, is not sufficient to justify conclusions. It may be explained, possibly, by slight traces of serum, though the leucocytes had been three times washed as before.

(*b.*) Is the bactericidal power of saline extract of leucocytes inactivated by heating to 56° C. for twenty minutes?

Leucocyte extract in salt solution by freezing as before.

Staphylococcus pyogenes aureus " Pr."

	Saline Leucocyte Extract 5 cc.	Leucocyte Extract Heated 56° for 20 Minutes 2 cc.	Salt Solution 2 cc.
Transplanted immediately.	636 680 768	848 670 792	910 820
After three hours at 37.5° C.	31 28 33	58 34 26	860 740

No inactivation of the leucocytic extract by 56° occurred.

(*c.*) Like (*b*) but with different lot of leucocyte extract.

Saline extract of leucocytes as before.

Staphylococcus pyogenes aureus " Al."

	Saline Extract of Leucocytes 2 cc.	Saline Extract of Leucocytes Heated to 56° 20 Minutes 2 cc.
Transplanted immediately.	7632 10000 +	10000 + 10000 +
After three hours at 37.5° C.	826 763	450 365

Apparently no reduction of bactericidal power by heating 56° C. twenty minutes.

(*d.*) Same as preceding, but with different lot of saline extract of leucocytes.

Staphylococcus pyogenes aureus " Pr."

	Leucocyte Extract 2 cc.	Leucocyte Extract. Heated to 56° C. 20 Minutes.
Transplanted immediately.	2260 1908	1780 2035
After three hours at 37.5° C.	52 38 20	75 68

Experiment IV.

(*a.*) Determination of temperature at which bactericidal power of aqueous leucocyte extract is destroyed.

Aqueous leucocyte extract as before.

Staphylococcus pyogenes aureus " Pr."

	Leucocyte Extract 2 cc. Unheated.	Leucocyte Extract 2 cc. Heated 56° C. 20 Minutes.	Leucocyte Extract 2 cc. Heated 75° C. 20 Minutes.
Transplanted immediately.	1020 980	1140 1080	1240 1360
After three hours at 37.5° C.	112 62	140 260	2226 1845

(*b.*) Determination of the temperature at which the bactericidal power of saline leucocyte extract is destroyed.

Saline leucocyte extract as before.

Staphylococcus pyogenes aureus " Al."

	Salt Solution Leucocyte Extract 2 cc.	Leucocyte Extract Heated 56° C. 20 Minutes 2 cc.	Leucocyte Extract Heated 70° C. 20 Minutes 2 cc.	Leucocyte Extract Heated 80° C. 2 cc.	Salt Solution 2 cc.
Transplanted immediately.	8268	7695	7950	7441	7722
After three hours at 37.5° C.	131 285	845 508	750 875	10000+ 7586	8268

Complete destruction of bactericidal power has taken place at a temperature between 70° C. and 80° C.

(*c.*) Is bactericidal power of salt solution extract of leucocytes destroyed by heating to 75° C. for twenty minutes?

Salt solution extract of leucocytes of different lot from that used in Experiment IV. (*b*).

Staphylococcus pyogenes aureus "Pr."

	Leucocyte Extract 2 cc.	Leucocyte Extract Heated 75° C. 20 Minutes 2 cc.	Leucocyte Extract Heated 80° C. 20 Minutes 2 cc.
Transplanted immediately.	826 890	1081 1017	763 1144
After three hours at 37.5° C.	220 180	1590 1870	2444 1760

Apparently bactericidal action was destroyed at and above 75° C., confirming preceding experiment.

From the foregoing experiments (III. and IV.) it appears that the bactericidal substances for staphylococci extracted from leucocytes by the methods employed in this work are different in structure from those contained in serum in that they are of greater thermostability, being apparently unaffected — or but slightly so — by temperatures below 70° C., but completely destroyed at temperatures at and above 75° C. This confirms the work of most other investigators.

The following experiment was done to determine whether leucocyte extract inactivated by heating to 80° C. could be reactivated by the addition of fresh unheated leucocyte extract.

Experiment V.

Can leucocyte extract inactivated by heating to 80° C. for twenty minutes be reactivated by the addition of fresh leucocyte extract?

Leucocyte extract obtained by freezing in salt solution as before.

Staphylococcus pyogenes aureus " Pr."

	Unheated Leucocyte Extract 2 cc.	Leucocyte Extract Heated to 80° C. 1.9 cc. and Fresh Extract .1 cc.	Leucocyte Extract Heated to 80° C. 1.9 cc. and Fresh Extract .1 cc.	Leucocyte Extract Heated to 80° C. 1.8 cc. and Fresh Extract .2 cc.	Leucocyte Extract Heated to 80° C. 2 cc.	Salt Solution 1.8 cc. Fresh Leucocyte Extract 2 cc.
Transplanted immediately.	1452 1500	1808 1988	1871 1621	1437	1545	1935
After three hours at 37.5° C.	85 32	2257	2607	3180	2000+ 2000+	

There was no reactivation in any of the tubes to which a small quantity of fresh unheated leucocyte extract had been added. It seemed rational to do this experiment although our knowledge of reactivable anti-bodies made it seem unlikely that reactivation after heating to 80° could take place.

The experiments now following were carried out in order to determine whether the extracts of leucocytes, obtained both by aqueous extraction and by freezing in salt solution, contained complement which would reactivate the bactericidal amboceptors of normal serum against Staphylococcus pyogenes aureus.

Experiment VI.

(*a.*) Can serum inactivated by 56° C. for twenty minutes be reactivated by aqueous leucocyte extract, *i.e.*, does leucocyte extract contain complement for the bactericidal immune body of serum?

Normal rabbit serum.

Aqueous leucocyte extract.

Staphylococcus pyogenes aureus " Pr."

	Normal Serum (Rabbit) 2.3 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. and Fresh Serum .3 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. and Leucocyte Extract .3 cc.	Distilled Water 2.3 cc.
Transplanted immediately.	1017 1272	1000 1400	1335 944	950 890
After five hours at 37.5° C.	20 18	15 44	800 840	600 580
After twenty-four hours.	20000 +	20000 +	20000 +	0

There was no noticeable reactivation by the leucocyte extract.

(*b.*) Same as (*a*). Same lot of leucocyte extract.
Normal rabbit serum.
Aqueous leucocyte extract.
Staphylococcus pyogenes aureus "Pr."

	Normal Rabbit Serum Fresh 2 cc. Salt Solution .5 cc.	Serum Heated 56° C. 20 Minutes 2 cc. Salt Solution .5 cc.	Serum Heated 56° C. 20 Minutes 2 cc. Leucocyte Extract .5 cc.	Distilled Water 2.5 cc.
Transplanted immediately.	560 640 784	636 780 680	840 680 560	580 498
After three hours at 37.5° C.	5 9 10 8	380 440 298	480 368 290	144 128

There was no noticeable reactivation by the leucocyte extract.

The slight diminution may be accounted for by the action of the leucocyte extract or by a small amount of activity of the serum.

(*c.*) Can serum inactivated by 56° C. for twenty minutes be reactivated by salt solution extract of leucocytes extracted by repeated freezing?

Normal rabbit serum.
Salt solution extract of normal rabbit leucocytes.
Staphylococcus pyogenes aureus "Pr."

	Normal Rabbit Serum 2 cc. Salt Solution .5 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. Normal Serum .5 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. Leucocyte Extract .5 cc.
Transplanted immediately.	1635 1399	1536 1780	1144 1554
After four hours at 37.5° C.	0 12	24 8	10000 10000

There was no reactivation of the serum by the leucocyte extract.

The foregoing experiments have occupied themselves with the bactericidal action of leucocyte extracts upon staphylococci. Experiments were now done with the purpose of examining these substances as to their action upon the typhoid bacillus.

EXPERIMENTS WITH BACILLUS TYPHOSUS.

Experiment I.

(*a.*) To determine the bactericidal action of aqueous extracts of normal rabbit leucocytes upon Bacillus typhosus. Comparison with normal rabbit serum.

Bacillus typhosus "65" (old laboratory strain).

	Aqueous Leucocyte Extract 2 cc.	Normal Serum 2 cc.	Distilled Water 2 cc.
Transplanted immediately.	1908 1007	1282 1399	990
After two hours at 37.5° C.	66 84	8 4	308
After ten hours at 37.5° C.	22 12	0 4	20

There was well marked bactericidal action on the part of the leucocyte extract.

(*b.*) Like preceding experiment.

Aqueous leucocyte extract of same lot as in preceding.

Bacillus typhosus "65."

	Aqueous Leucocyte Extract 2 cc.	Normal Serum 2 cc.	Distilled Water 2 cc.
Transplanted immediately.	1049 846	890 954	922
After two hours at 37.5° C.	86 28	10 12	240

There was well-marked bactericidal action by the leucocyte extract — as marked as that exerted by normal serum.

(*c.*) Does extract of normal leucocytes obtained by freezing and thawing in salt solution contain substances bactericidal for Bacillus typhosus? Comparison with normal serum.

Saline extract of leucocytes.

Bacillus typhosus "65."

	Leucocyte Extract Saline 2 cc.	Normal Serum 2 cc.
Transplanted immediately.	2744 3180	2952 4324
After three hours at 37.5° C.	436 282 348	82 48 17

There was distinct bactericidal action on the part of the saline extracts of leucocytes.

(*d.*) Like (*c*) — comparison with salt solution (.85 per cent).

Same lot of leucocyte extract.
Bacillus typhosus "65."

	Saline Leucocyte Extract 2 cc.	Salt Solution .85% 2 cc.
Transplanted immediately.	1144 1017	1221
After three hours.	76 48	1028 926

Again there was well-marked bactericidal action on the part of the leucocyte extract. The control in salt solution showed slight diminution only.

(*e.*) Like preceding — but another strain of Bacillus typhosus (St. L. 1) isolated from blood culture three months previously.

Leucocyte extract similarly prepared but with a different lot of leucocytes.

	Saline Leucocyte Extract 2 cc.	Salt Solution 2 cc.
Transplanted immediately.	10000 +	10000 +
After four hours.	150 98	10000 + 10000 +

Results comparable to preceding.

(*f.*) Is there autolysis of typhoid bacilli in salt solution? Three different strains of typhoid bacilli tested.

	Salt Solution 2 cc. Bacillus Typhosus "65."	Salt Solution 2 cc. Bacillus Typhosus "G.H. 1."	Salt Solution 2 cc. Bacillus Typhosus "J."
Transplanted immediately.	12448	10239	10684
After four hours.	15000 + 10000 +	8412 10000 +	10000 + 10000 +

Apparently there was no reduction in the numbers of bacteria in salt solution within four hours.

Experiment II.

Comparison between action of aqueous and that of saline extracts (obtained by freezing) upon Bacillus typhosus "65."

	Bacillus Typhosus "65." Aqueous Leucocyte Extract 2 cc.	Bacillus Typhosus "65." Saline Leucocyte Extract 2 cc.
Transplanted immediately.	4134	2780
After three hours.	318 210	0 0
There were killed	3870	2780

The difference apparent between the bactericidal action of the salt solution extract and the aqueous extract is slight and may well be due to experimental error.

In the preceding experiments (I.–II.) a uniform reduction of microörganisms occurred, there being no marked difference between the aqueous and the saline extracts. The latter were used more extensively in the experiments which follow for the reason that the element of possible plasmolysis in the distilled water extracts, necessitating constant control, was excluded.

Inasmuch as the results of the preceding five experiments were at variance with those obtained by some other observers, who failed to obtain bactericidal action in working with the extracts of the leucocytes of other animals — cats and guinea-pigs — and Bacillus typhosus, it seemed desirable to study other strains of this species, preferably with some more recently isolated from the human body. All the strains used in the preceding as well as the following experiments were re-tested upon differential media, both the simpler ones in

general use and the various sugar media and gave the reactions typical for Bacillus typhosus. All of the strains used, moreover, agglutinated in typhoid-immune serum in dilutions of one to two thousand. Experiments were done also to exclude the possibility of reduction of the typhoid bacilli by autolysis in salt solution, all with negative results.

The possibility of a simulation of bacterial reduction by the agglutination of the bacilli in the leucocyte extracts was also considered and no agglutination could be demonstrated in a series of macroscopic tests.

Experiment III.

(*a.*) To test the bactericidal action of saline leucocyte extracts, obtained by freezing and thawing as before, upon more recently isolated strains of typhoid bacilli.

Saline extract of rabbit leucocytes as before.

Bacillus typhosus "G.H. 1," "2," and "3" — strains isolated by blood culture from patients three months previously.

	Typhosus "G.H.1." Saline Leucocyte Extract 2 cc.	Typhosus "G.H.2." Saline Leucocyte Extract 2 cc.	Typhosus "G.H.3." Saline Leucocyte Extract 2 cc.
Transplanted immediately.	5406	8013	3816
After three hours at 37.5° C.	1208	636	445
There were killed.....	4198	7377	3371

There was well-marked bactericidal action in all three cases; the differences between the various strains was, however, too slight to warrant conclusions.

(*b.*) Comparison of the bactericidal action of saline leucocyte extract upon old laboratory strain "65" with its action upon two of the more recently isolated strains used in (*a*).

New lot of saline leucocyte extract obtained by freezing as usual.

Bacillus typhosus "65" old laboratory strain.

Bacillus typhosus "G.H. 1" obtained from blood culture three months previously.

Bacillus typhosus "G.H. 2" obtained from blood culture three months previously.

	Bacillus Typhosus "65." Leucocyte Extract 2 cc.	Bacillus Typhosus "G.H. 1." Leucocyte Extract 2 cc.	Bacillus Typhosus "G.H. 2." Leucocyte Extract 2 cc.
Transplanted immediately.	3180	5724	5215
After three hours at 37.5° C.	8 0	2385 2035	2289 2607
There were killed	3172	3524	2765

Considering the differences in the original mixtures, the actual bactericidal effect upon the three strains was approximately the same.

(*c.*) Comparison of bactericidal power of saline leucocyte extract upon Bacillus typhosus "65" and upon Bacillus typhosus "Cor" isolated from blood culture two days previously.

Saline leucocyte extract as before.

Bacillus typhosus "65."

Bacillus typhosus "Cor" isolated from blood culture two days previously.

	Typhosus "65." Leucocyte Extract 2 cc.	Typhosus "Cor." Leucocyte Extract 2 cc.	Typhosus "Cor." Leucocyte Extract 2 cc.
Transplanted immediately.	10303	11540	8776
After four hours.	110 48	180 112	200 108

No difference between action of leucocyte extract upon old strain and upon newly isolated strain.

Following experiments III. (*a*), (*b*), and (*c*), a number of experiments were done in which the action of saline leucocyte extract was tested upon various strains of Bacillus typhosus — a comparison being made between old strains and others more recently isolated. In some of these experiments it seemed as though the leucocytic substances were more powerfully active upon the older strains than upon the more recent ones. This difference was not, however, sufficiently marked to be conclusive. To settle the point the following comparative tests were made. All the strains used were transplanted to fresh agar slants daily for one week previous to the experiments, in order to obtain as nearly as possible a similarity in the vigor and vitality of the cultures. Emulsions in salt solution were then made — two loopfuls to five cubic centimeters of salt solution, and equal volumes of these emulsions were added respectively to two cubic centimeters of leucocyte extract, produced by freezing and thawing in salt solution as before.

(*d.*) Comparison between action of saline leucocyte extract upon different strains of Bacillus typhosus. New lot of saline leucocyte extract.

1. Typhosus "65" old laboratory strain.
2. "G.H. I." obtained in blood culture six months before.
3. "G.H. II." obtained in blood culture six months before.
4. "St. L. I." obtained in blood culture eight months before.
5. "St. L. II." obtained in blood culture three months before.
6. "St. L. III." obtained in blood culture one month before.
7. "J." obtained from urine three weeks before.
8. "V.K." obtained from urine four days before.

Saline Leucocyte Extract 2 cc.

	1. Typhosus "65."	2. Typhosus "G.H. I."	3. Typhosus "G.H. II."	4. Typhosus "St. L. I."	5. Typhosus "St. L. II."	6. Typhosus "St. L. III."	7. Typhosus "J."	8. Typhosus "V.K."
Transplanted immediately.	3625	4770	5088	3752	5851	5660	3862	3160
After three hours at 37.5° C.	30 0	48 36	52 38	0 0	15 8	0 0	26 36	0 10

There was no marked difference in the action of the leucocyte extract upon the various strains of typhoid bacilli.

(*e.*) Repetition of preceding but with different lot of leucocyte extract.

Saline Leucocyte Extract 2 cc.

	1. Typhosus "65."	2. Typhosus "G.H. I."	3. Typhosus "G.H. II."	4. Typhosus "St. L. I."	5. Typhosus "St. L. II."	6. Typhosus "St. L. III."	7. Typhosus "J."	8. Typhosus "V.K."
Transplanted immediately.	6000	5787	7059	6868	7632	4943	4261	4770
After three hours at 37.5° C.	0 6	25 38	15 21	0 0	12 0	5 12	80 20	0 0

Results comparable to preceding.

The preceding experiments have showed that distinct bactericidal action upon various strains of Bacillus typhosus takes place both in aqueous and the saline extracts of normal leucocytes.

There appears to have been no difference in the action of the extract upon different strains of this bacillus.

The experiments immediately following were aimed at the thermostability of these bactericidal substances for Bacillus typhosus.

Experiment IV.

(*a.*) Is the bactericidal action of aqueous leucocyte extract for typhoid bacilli destroyed by heating to 56° C. for twenty minutes?

Aqueous leucocyte extract.

Bacillus typhosus "65."

		Test in Duplicate.	
	Aqueous Leucocyte Extract 2 cc.	Aqueous Leucocyte Extract Heated to 56° C. for 20 Minutes 2 cc.	Aqueous Leucocyte Extract Heated to 56° C. for 20 Minutes 2 cc.
Transplants taken immediately.	1272	922	1452
After two hours at 37.5° C.	120 88	156 136	108 96

There was no appreciable diminution in bactericidal power by heating to 56° C. for twenty minutes.

(*b.*) Is the bactericidal power of saline leucocyte extract for typhoid bacilli destroyed by heating to 56° C. for twenty minutes?

Saline extract of leucocytes; same as preceding.

Bacillus typhosus "65."

	Salt Solution Extract of Leucocytes 2 cc.	Salt Solution Extract of Leucocytes Heated 56° C. for 20 Minutes 2 cc.
Transplanted immediately.	562 680	490 386
After three hours at 37.5° C.	2 0	5 3

There was no diminution of bactericidal power by heating to 56° C. for twenty minutes.

(*c.*) Same purpose as preceding.

Salt solution extract of leucocytes of same lot as preceding.

Bacillus typhosus "65."

	Salt Solution Extract of Leucocytes 2 cc.	Salt Solution Extract Heated 56° 20 Minutes 2 cc.
Transplanted immediately.	1971	1460
After four hours at 37.5° C.	104	8
	110	4
	96	15

No reduction of bactericidal power by heating to 56° C.

(*d.*) Same purpose as preceding.

Salt solution extract of leucocytes of different lot from preceding.

Bacillus typhosus "65."

		Test in Duplicate.	
	Leucocyte Extract 2 cc.	Leucocyte Extract Heated 56° 20 Minutes 2 cc.	Leucocyte Extract Heated 56° C. 20 Minutes 2 cc.
Transplanted immediately.	2544	2380	2416
	2989	3243	3306
After three hours at 37.5° C.	14	2	0
	12	5	5

No reduction of bactericidal power by heating to 56° C.

Experiment V.

(*a.*) To ascertain the temperature at which the bactericidal power of leucocyte extract is destroyed.

Saline leucocyte extract.

Bacillus typhosus "65."

	Leucocyte Extract 2 cc.	Leucocyte Extract Heated 56° C. for 20 Minutes.	Leucocyte Extract Heated 70° C. 10 Minutes.
Transplanted immediately.	3754	4070	3961
After four hours at 37.5° C.	61 74	40 48	58 84

Heating to 70° C. has evidently left the bactericidal substances uninjured.

(*b.*) Same purpose as preceding experiment.
Saline leucocyte extract of same lot as preceding.
Bacillus typhosus "65."

	Leucocyte Extract 2 cc.	Leucocyte Extract Heated 70° C. 10 Minutes.	Leucocyte Extract Heated 75° C. 10 Minutes.
Transplanted immediately.	5734	7059	6868
After four hours at 37.5° C.	2 12	8 32	20000 + 15000 +

A temperature of 75° C. effectually destroyed the bactericidal action of the leucocyte extract.

(*c.*) Same purpose as preceding experiment.
Saline leucocyte extract of same lot as preceding.
Bacillus typhosus "St. L. I." obtained from blood culture six months previously.

	Leucocyte Extract 2 cc.	Leucocyte Extract 2 cc. Heated 56° C. 20 Minutes.	Leucocyte Extract 2 cc. Heated 75° C. 10 Minutes.
Transplanted immediately.	10000 +	10000 +	10000 +
After four hours.	150 98	225 160	10000 + 15000 +

Heating to 75° C. has apparently destroyed the bactericidal action of the leucocyte extract.

From the preceding it is seen that the bactericidal substances for Bacillus typhosus in leucocyte extracts are destroyed at or above 75° C. — differing therein from the bactericidal substances of serum. The following experiments deal with the attempted reactivation of heated leucocytic substances by the addition of fresh extract.

Experiment VI.

(*a.*) Can leucocyte extracts inactivated by heating to 80° C. for ten minutes be reactivated by the addition of fresh leucocyte extract?

Saline leucocyte extract, new lot.

Bacillus typhosus "65."

	Leucocyte Extract 2 cc.	Leucocyte Extract 2 cc. Heated to 80° C.	Leucocyte Extract 2 cc. Heated + .2 cc. Fresh Leucocyte Extract.
Transplanted immediately.	1276	739	1081
After three hours.	21 13	1244	1272

There was apparently no reactivation.

No reactivation of leucocyte extract taking place on the addition of fresh extract, it was now desirable to ascertain whether leucocyte extract contained complement, *i.e.*, can it reactivate inactivated serum?

Experiment VII.

(*a.*) Does aqueous leucocyte extract reactivate normal serum inactivated by heating to 56° C. for twenty minutes, *i.e.*, does leucocyte extract contain complement for bactericidal immune body against Bacillus typhosus? The leucocyte extract used in all these experiments was not older than four days.

Normal rabbit serum.
Fresh aqueous leucocyte extract.
Bacillus typhosus "65."

	Normal Serum 1 cc. Salt Solution .1 cc.	Normal Serum 1 cc. Heated 56° C. 20 Minutes. Salt Solution .1 cc.	Normal Serum 1 cc. Heated 56° C. 20 Minutes. Fresh Serum .1 cc.	Normal Serum 1 cc. Heated 56° C. 20 Minutes. Leucocyte Extract .1 cc.
Transplanted immediately.	514 384	560 446	484 296	338 420
After five hours at 37.5° C.	0 8	1399 2142	1 15	4840 5620
After twenty hours.	0 0	20000 + 20000 +	20000 + 20000 +	+ + + + + +

There was no reactivation of normal serum, heated, by leucocyte extract.

(*b.*) Same purpose as preceding experiment.
Aqueous leucocyte extract.
Normal rabbit serum.
Bacillus typhosus "65."

	Normal Serum 2 cc. Salt Solution .2 cc.	Normal Serum 2 cc. Heated 56° C. 20 Minutes. Fresh Serum .2 cc.	Normal Serum 2 cc. Heated 56° C. 20 Minutes. Leucocyte Extract .2 cc.
Transplanted immediately.	1272 1399	1278 1040	1462 1244
After two hours.	66 84	90 110	2734 2544
After ten hours.	22 12	20000 + 20000 +	6000 + 8000 +

There was no reactivation of the heated serum by leucocyte extract.

(*c.*) Same purpose as preceding.
Normal rabbit serum.
Aqueous leucocyte extract, different lot from preceding.
Bacillus typhosus "65."

	Normal Serum 2 cc. Salt Solution .2 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. Leucocyte Extract .2 cc.
Transplanted immediately.	580 476	456 354
After four hours.	2 1	5650 4670

No reactivation by leucocyte extract took place.

(*d.*) Same purpose as preceding.
Aqueous leucocyte extract of same lot as preceding.
Bacillus typhosus "65."

	Normal Serum 2 cc. Salt Solution .5 cc.	Serum Heated 56° C. 20 Minutes 2 cc. Serum Fresh .5 cc.	Serum Heated 56° C. 20 Minutes 2 cc. Leucocyte Extract .5 cc.
Transplanted immediately.	10500	9700	8960
After four hours at 37.5° C.	120 84 136	80 60	6780 4800 9680

There was no reactivation by the leucocyte extract.

(*e.*) Does saline leucocyte extract, obtained by repeated freezing and thawing in salt solution, reactivate serum inactivated by heating to 56° C. for twenty minutes, *i.e.*, does saline leucocyte extract contain complement?
Saline leucocyte extract.
Bacillus typhosus "65."

	Normal Serum 2 cc. Salt Solution .5 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. Fresh Serum .5 cc.	Normal Serum Heated 56° C. 20 Minutes 2 cc. Leucocyte Extract .5 cc.
Transplanted immediately.	966	826	890
After three hours at 37.5° C.	24 9	36 21	640 580

Saline leucocyte extract failed to reactivate heated normal serum.

(*f.*) Same purpose as preceding.
Saline leucocyte extract, different lot from preceding.
Bacillus typhosus "65."

	Serum 2 cc. Heated 56° C. Salt Solution .2 cc.	Serum 2 cc. Heated 56° C. 20 Minutes. Leucocyte Extract .2 cc.	Serum 2 cc. not Heated. Salt Solution .2 cc.
Transplanted immediately.	1908	1845	2035
After four hours at 37.5° C.	10000 + 10000 +	20000 +	2 1

No reactivation by saline leucocyte extract.

In none of the preceding experiments was there any evidence of the presence of complement in either the aqueous or the saline leucocytic extract.

In connection with the experiments done to determine whether leucocytic extracts contained complement for bactericidal immune bodies of normal serum, it was deemed desirable to ascertain whether such extracts contained complement which would activate hemolytic amboceptor. For this purpose the serum of a rabbit immunized against sheep corpuscles was used. It is well known, since the work of Morgenroth and Sachs, that there is an inverse quantitative

relationship between amboceptor and complement in hemolytic relations. The larger the quantity of amboceptor used in a reaction, the less the complement required for hemolysis, and vice versa within certain limits. By using a large excess of amboceptor, therefore, minute quantities of complement can be detected.

In the experiments recorded below, a rabbit serum, having a hemolytic unit of .001 cubic centimeter for sheep's corpuscles (in the presence of .1 cubic centimeter of fresh guinea-pig complement) was used. Twenty units of amboceptor or .02 cubic centimeter was used to give a twenty-fold excess of amboceptor. A series of tubes was prepared in each of which was placed one cubic centimeter of a five per cent emulsion of washed sheep corpuscles, twenty units of amboceptor and diminishing quantities of fresh guinea-pig serum, in order to determine the minimum of guinea-pig complement which would give reaction in such a mixture. In order to avoid hemolysis because of the hypotonicity of the aqueous leucocyte extract, the volume in each tube was made up to five cubic centimeters with normal salt solution. A parallel series of tubes was prepared to which, instead of guinea-pig serum, varying quantities of leucocyte extract were added. The experiments, done in duplicate, were as follows:

Experiment VIII.

(*a*.)

1. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. } + { guinea-pig serum .1 cc. } = { complete hemolysis.

2. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. } + { guinea-pig serum .05 cc. } = { complete hemolysis.

3. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. } + { guinea-pig serum .025 cc. } = { complete hemolysis.

4. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. } + { guinea-pig serum .005 cc. } = { almost complete hemolysis.

5. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. } + { guinea-pig serum .001 cc. } = { very slight hemolysis.

6. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + guinea-pig serum .0005 cc. = no hemolysis.

7. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + guinea-pig serum .0001 cc. = no hemolysis.

Read at end of two hours at 40° C.

Guinea-pig complement, therefore, gave almost complete hemolysis in quantities of .005 cubic centimeter.

(*b.*)

1. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + aqueous leucocyte extract 1 cc. = no hemolysis.

2. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + aqueous leucocyte extract .5 cc. = no hemolysis.

3. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + aqueous leucocyte extract .3 cc. = no hemolysis.

4. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + aqueous leucocyte extract .2 cc. = no hemolysis.

5. 1 cc. sheep corpuscles 5% + 20 units amboceptor volume to 5 cc. with salt solution. + aqueous leucocyte extract .1 cc. = no hemolysis.

There was no evidence of complementary action even when one cubic centimeter of the leucocyte extract was used. Leucocyte extracts as prepared by us apparently contain no complement for hemolytic amboceptor.

Several experiments were now carried out with the purpose of ascertaining whether the bactericidal substances of leucocytes were specifically increased by immunization.

Experiment IX.

(*a.*) Saline leucocyte extract prepared by freezing and thawing from typhoid immune rabbits. The cells were thoroughly washed in salt solution (six times) and, in order to inactivate any minute traces of serum which might be present, the extracts were heated to 60° for twenty minutes upon use.

Bacillus typhosus "65."

The serum of the rabbits used, agglutinated Bacillus typhosus in dilutions of from 1 : 40,000 to 1 : 80,000.

	Immune Leucocyte Extract 2 cc.	Immune Leucocyte Extract 1 cc. Salt Solution 1 cc.	Immune Leucocyte Extract .5 cc. Salt Solution 1.5 cc.	Immune Leucocyte Extract .2 cc. Salt Solution 1.8 cc.	Immune Leucocyte Extract .1 cc. Salt Solution 1.9 cc.	Normal Leucocyte Extract 2 cc.	Normal Leucocyte Extract 1 cc. Salt Solution 1 cc.
Transplanted immediately.	890	780	820	1272	1144	636	508
After three hours at 37.5° C.	2 3	50 16	168 445	2862	1272	4 6	180 340

There was apparently no difference between the bactericidal action of the extracts obtained from immune and those from normal rabbits.

(*b.*) Same as preceding.
Bacillus typhosus "65."

	Immune Leucocyte Extract 2 cc.	Immune Leucocyte Extract 1 cc. Salt Solution 1 cc.	Normal Leucocyte Extract 2 cc.	Normal Leucocyte Extract 1 cc. Salt Solution 1 cc.
Transplanted immediately.	7632	9222	8950	8522
After three hours at 37.5° C.	150 85	960 586	225 180	2544 1440

Very slight or no difference between immune and normal.

(*c.*) Same as preceding.
Bacillus typhosus "65."

	Immune Leucocyte Extract 2 cc.	Immune Leucocyte Extract 1 cc. Salt Solution 1 cc.	Normal Leucocyte Extract 2 cc.	Normal Leucocyte Extract 1 cc. Salt Solution 1 cc.
Transplanted immediately.	2226	2544	2089	2416
After three hours at 37.5° C.	20 12	827 763	110 40	1054 699

(*d.*) Same as preceding.
Bacillus typhosus "65."

	Immune Leucocyte Extract 2 cc.	Normal Leucocyte Extract 2 cc.
Transplanted immediately.	2550	2194
After three hours at 37.5° C.	35 68	97 115

SUMMARY.

In summing up the work recorded above, we believe the following conclusions to be fully justified:

1. Extracts of normal rabbit leucocytes, both those obtained by aqueous extraction and those obtained by freezing in salt solution, have distinct bactericidal powers for pyogenic staphylococci and for Bacillus typhosus. There is considerable uniformity in the action of various lots of such extracts upon the same strain of microörganisms, and it is apparent that separate strains of the same species show no decided variations in their susceptibility to the bactericidal substances contained in the extracts.

2. In regard to thermostability, our results are uniform in confirming the researches of workers mentioned in the introduction, in showing that the endocellular bactericidal substances are not destroyed by heating to 56° C., but that temperatures of 75° C., at least, are necessary for their destruction. This justifies the conclusion that they are distinct from and differently constituted to the bactericidal substances of serum.

3. It was further shown that, in the case of both organisms studied, reactivation of these substances after heating to 80° C. does not take place upon the addition of fresh leucocytic extract.

4. Quantitatively these bactericidal substances are insignificant compared with the bactericidal powers of normal serum. It is unlikely, therefore, that the bactericidal action

of the leucocyte extracts has been responsible, except in a purely secondary way, for the curative results obtained in infection in animals and man.

5. In regard to the detection of complement in leucocytic extracts, either for the activation of bactericidal antibodies in serum or for hemolytic amboceptor, our work has been entirely negative. Our experiments show absolutely no complement in the extracts from washed leucocytes.

6. Immunization, furthermore, apparently did not enhance the bactericidal power of the leucocytic substances, at least in the case of Bacillus typhosus, in which case alone experiments were done with this point in view. It would seem that these substances, as contained in the leucocytes, may be at all times simply sufficient for the destruction of the limited numbers of bacteria which can be ingested by the cell, and have no quantitative relationship to the specific immunity acquired by animals or human beings during their reactions against spontaneous or experimental infection.

[In completing this work, the writer wishes to acknowledge the assistance and many valuable suggestions received from Prof. Philip Hanson Hiss, Jr. In fact, the work here recorded was done as a direct outcome of the work on leucocyte extracts begun by him at the College of Physicians and Surgeons.]

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