ON THE EFFECT OF REACTION AND OF CERTAIN SALTS ON NORMAL OPSONINS.*

H. E. EGGERS.
(From the Memorial Institute for Infectious Diseases, Chicago.)

I. ON THE EFFECT OF REACTION ON OPSONIFICATION BY NORMAL SERUM.

IT would be natural to expect that normal opsonins would display their maximum activity at the normal reaction of the blood, that is, at a reaction slightly alkaline to the ordinary indicators. Noguchi,[1] however, obtained results to the effect that the opsonins of normal serum act best in a neutral medium, and that any acidity or alkalinity

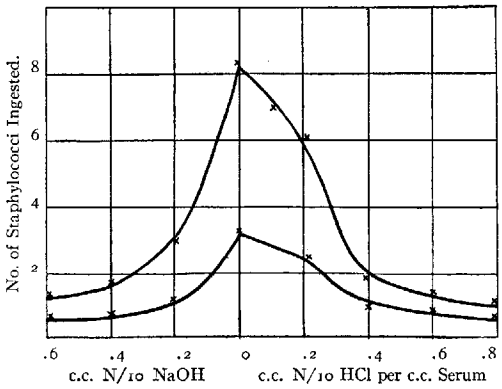

CHART 1.—Effect of reaction on opsonin of dog serum.

results in diminished opsonification. He pointed out a difference in behavior between opsonin and the complement of normal serum, the latter being most effective in an alkaline medium. For various reasons it seemed desirable to repeat some of the work of Noguchi, and this I have done, but without reaching the results that he did.

The technic used by me was as follows: 24–48 hour growths of *Staph. albus* on agar were suspended in salt solution, washed once, and resuspended in small quantities (about 1 c.c.) of salt solution. Such suspensions gave no acid reaction with lacmoid paper. Mixtures were then made by adding to equal quantities of fresh serum varying amounts of N/10 acid (HCl), or alkali (NaOH). The mixtures were then adjusted to a minimum constant volume with salt solution and incubated for half an hour at

* Received for publication July 6, 1909.

[1] *Jour. Exper. Med.*, 1907, 9, p. 453.

37°. Then to each was added a given volume of the staphylococcus suspension and the whole incubated for half an hour. At the end of that time the cocci were thoroughly removed, using an extremely rapidly rotating "haematocrit" centrifuge apparatus, washed once, and finally resuspended in salt solution. These suspensions were then mixt with washed human leukocytes and opsonic counts made after the usual fashion. As it was feared that the final suspensions of organisms might not be of uniform density, in several instances bacterial counts of these suspensions were made. Such counts, when made, are given in the following tables.

Dog, rabbit, and human sera were used. The results appear in the following tables, the figures being the average count for at least 50 leukocytes:

A. DOG SERUM.

	Phagocytosis Dog A	Phagocytosis Dog B
1. 0.5 c.c. serum+0.5 c.c. NaCl sol.	3.13	8.1
2. " " " +0.1 " N/20 HCl +0.4 c.c. NaCl sol.	....	7.0
3. " " " +0.1 " N/10 " +0.4 " " "	2.41	6.0
4. " " " +0.2 " " " +0.3 " " "	1.20	1.84
5. " " " +0.3 " " " +0.2 " " "	0.94	1.30
6. " " " +0.4 " " " +0.1 " " "	0.62	1.00
7. " " " +0.5 " " "	0.36	....
8. " " " +0.1 " " NaHO +0.4 " " "	1.10	3.0
9. " " " +0.2 " " " +0.3 " " "	0.70	1.6
10. " " " +0.3 " " " +0.2 " " "	0.54	1.3

B. RABBIT SERUM.

1

	Phagocytosis	Staph. per cu. mm.
1. 0.1 c.c. serum+0.24 c.c. NaCl sol.		
2. " " " +0.08 " N/10 HCl+0.16 c.c. NaCl sol.	11.4	510,000
3. " " " +0.16 " " " +0.08 " " "	4.2	540,000
4. " " " 0.24 " " "	3.1	600,000

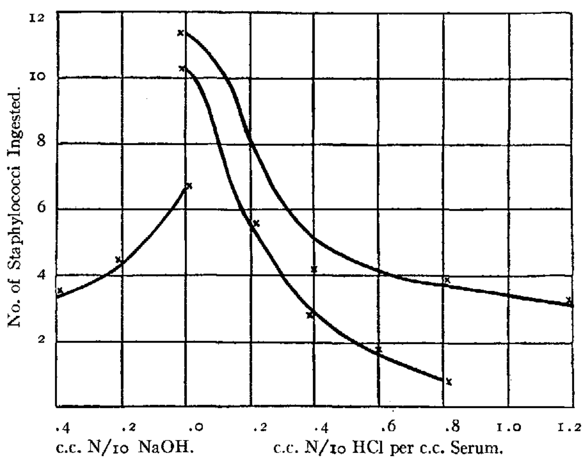

CHART 2.—Effect of reaction on opsonin of rabbit serum.

2

	Phagocytosis	Staph. per cu. mm.
1. 0.1 c.c. serum+0.08 c.c. NaCl sol.	10.2	526,000
2. " " " +0.02 " N/10 HCl+0.06 c.c. NaCl sol.	5.6	637,000
3. " " " +0.04 " " " +0.04 " " "	2.9	657,000
4. " " " +0.06 " " " +0.02 " " "	1.6	634,000
5. " " " +0.08 " " "	0.8	533,000

		3	
1.	0.1 c.c. serum + 0.08 c.c. NaCl sol.	6.6	.....
2.	" " " + 0.02 " N/10 NaHO + 0.06 c.c. NaCl sol.	4.3	.....
3.	" " " + 0.04 " " " + 0.04 " " "	3.42	.....

C. HUMAN SERUM

		Phagocytosis "E"		"M"	"J"
		1	2		
1.	0.1 c.c. serum + 0.08 NaCl sol.	4.70	3.91	27.1	18.5
2.	" " " + 0.02 " N/10 HCl + 0.06 c.c. NaCl sol.	3.12	3.56	25.2	....
3.	" " " + 0.04 " " " + 0.04 " " "	3.30	2.59	22.7	16.9
4.	" " " + 0.06 " " " + 0.02 " " "	1.20	0.70	4.34	5.72
5.	" " " + 0.08 " " "	0.90	....	....	1.76
6.	" " " + 0.09 " " "	. ..	0.52	....	....

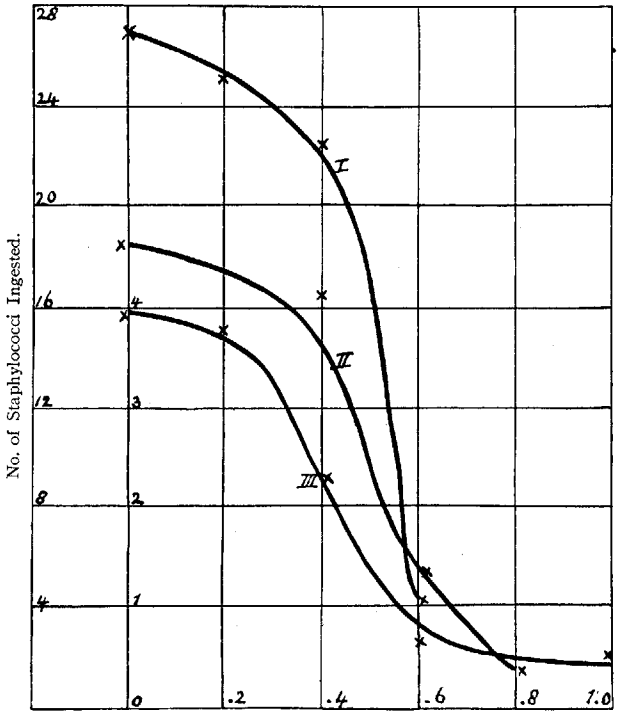

CHART 3.—Effect of reaction on opsonin of human serum: I, serum "M;" II, serum "J;" III, serum "E."

With the procedure used the results might have been influenced on the one hand by the effect of the acid (or alkali) on the organisms themselves, and on the other by the possibility of sufficient residual acidity remaining in the final suspension to unfavorably affect phagocytosis by action on the leukocytes. Hamburger and Hekma[1] have shown that a very slightly acid reaction is sufficient to inhibit phagocy-

[1] *Biochem. Zischr.*, 1908, 9, p. 275.

tosis, in this manner, to a marked degree. To ascertain to what extent these objections would apply, the following control experiments were made:

1. EFFECT OF ACIDITY ON ORGANISMS.

1. 0.18 c.c. NaCl sol. + 0.1 c.c. staph. suspension
2. 0.10 " " " +0.08 " N/10 HCl +0.1 c.c. staph. suspension
3. 0.14 " " " +0.04 " " NaHO +0.1 " " "

These mixtures were incubated 30 minutes, washed once, and the staphylococci resuspended in equal volumes of salt solution; opsonic counts were made, using for the suspension equal volumes of these bacterial suspensions, rabbit serum, and (human) leukocytes.

1. gave an average count of 11.
2. " " " " " 7.7.
3. " " " " " 8.3.

It will be seen that while some diminution in phagocytosis resulted, the effect is not nearly as striking as is the effect of altered reaction on the serum itself.

2. EFFECT OF RESIDUAL ACIDITY ON LEUKOCYTES.

Staphylococci were sensitized by incubation in serum + salt solution, just as in the first mixture of each of the tables, and were washed free from serum as in those experiments. To 0.1 c.c. of serum was added 0.08 c.c. N/10 HCl and 0.1 c.c. of salt soluion. This was placed in one of the small centrifuge tubes used in the preceding work, and enough was drawn off to have a remainder equal to that ordinarily left in the tube in this work. An amount of salt solution equal to that used in washing the organisms in the previous work was now added, and after mixing the solution was again drawn off to the same point. The remainder was then taken up in the same volume of salt solution as was used in the final suspensions of the organisms. A mixture was made up of one part of this, one part of the sensitized, washed cocci, and one part of leukocyte suspension; as a control, a mixture of salt solution, sensitized organisms, and leukocytes was used. Opsonic counts gave for the former 2.6, for the control 2.04, thus indicating that what acid may have remained in the final mixture was too slight to affect the leukocytes.

One series of determinations was made to ascertain the effect of reaction on the streptococcus opsonin of dog's serum. The procedure was exactly that used for the work on staphylococcus.

1.	0.1 c.c. serum			+0.08 c.c.	NaCl sol.					1.79
2.	"	"	"	+0.02 "	HCl	+0.06 c.c.	NaCl sol			1.66
3.	"	"	"	+0.04 "	"	+0.04 "	"	"	"	1.10
4.	"	"	"	+0.06 "	"	+0.02 "	"	"	"	0.68
5.	"	"	"	+0.08 "	"					0.48

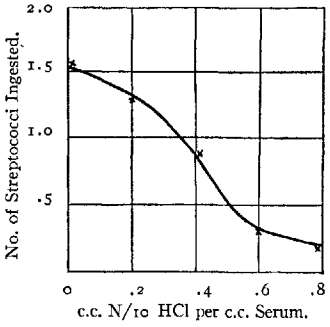

CHART 4.—Effect of reaction on streptococcus opsonin of dog serum.

To determine whether or not the effect of altered reaction on the thermolabile constituent of normal

opsonin would be similar to that on the normal opsonin as a whole, the following experiments were made:

1. Human Serum. Staphylococci were suspended in (reactivable) human serum previously heated to 60° for 10 minutes, and were incubated at 37° for half an hour. They were then centrifuged out, washed once, and resuspended in a small volume of salt solution.

Normal serum was meanwhile treated as follows:

1. 0.1 c.c. serum + 0.08 c.c. NaCl sol.
2. " " " + 0.02 " N/10 HCl + 0.06 c.c. NaCl sol.
3. " " " + 0.04 " " " + 0.04 " " "
4. " " " + 0.06 " " " + 0.02 " " "
5. " " " + 0.08 " " "

These were incubated at 37° for half an hour, then each was diluted by adding 1.8 c.c. salt solution. To 0.2 c.c. of each of these was added 0.1 c.c. of the suspension of sensitized bacteria, and these suspensions were incubated for 30 minutes. Finally, the cocci were centrifuged out, washed once, and resuspended in equal volumes of salt solution, and used for opsonic determinations with the following results:

1	1.94
2	2.10
3	1.60
4	1.12
5	1.02

2. Dog Serum. A similar experiment with reactivable dog's serum gave the following results:

Acid or Alkali per c.c. Serum	Phagocytosis
0.0	3.5
0.1 c.c. N/10 HCl	3.0
0.2 " " "	2.95
0.4 " " "	1.32
0.6 " " "	1.08
0.8 " " "	0.95
0.2 " " NaOH	2.60
0.4 " " "	1.50
0.6 " " "	0.80

Most of the results given above in tabular form are represented graphically in the following figures. It is unfortunate that Noguchi's results are not presented in such form as to permit of their graphical presentation for comparison. The results presented do not confirm the results of Noguchi to the effect that normal opsonins exert their greatest effect in a neutral medium. On the contrary, it was uniformly found that the maximum of opsonification occurred at the normal (alkaline) reaction of the serum, and that any change in this reaction, either in the direction of increased or diminished alkalinity, resulted in lessened effect.

Finally, the effect of altered reaction on normal opsonin is in large

measure at least due to its action on the thermolabile constituent of the opsonin.

II. THE EFFECT OF CERTAIN SALTS ON PHAGOCYTOSIS.

Hektoen and Ruediger[1] found that a considerable number of salts, in sufficient concentration, would inhibit phagocytosis of bacteria to a greater or less degree. In no case did they find, with the concentrations used, any evidence of stimulation to greater phagocytic activity. This inhibitory effect of salts on phagocytosis they attributed at that time to action on the opsonins rather than on the leukocytes directly.

Hamburger and Hekma,[2] in an extensive study of the influence of various factors on phagocytosis, found that various salts, notably sodium fluorid and barium chlorid, had a marked inhibitory effect on phagocytosis, this action being exerted directly on the leukocytes. With calcium chlorid, on the other hand, they found the phagocytic power of the leukocytes for finely divided carbon particles markedly increased.

My own work has been done mainly with salts normally occurring in serum, a few additional ones being studied. At first staphylococci were treated with serum to which had been added the salt studied and then washed free from the modified serum before subjecting them to phagocytosis. These experiments, however, failed to establish any effect whatever of the salts on phagocytosis, with the exception of barium chlorid and sodium fluorid, and the inhibition obtained with sufficiently large amounts of these salts could quite probably be explained on the basis of absorption of some of the salt by the cocci.

The plan was then adopted of adding the salt directly to the mixture of organisms, serum, and leukocytes. In this way, any action of the salt on any of the factors entering into the combination might be shown and the exact point of action determined later. The salts used were potassium sulfate, calcium, magnesium, barium, chlorids, and sodium fluorid in approximately m/8 solution. Barium chlorid and sodium fluorid were found to exert a marked toxic effect on the leukocytes, in even very dilute solutions, so that the numerical presentation of the results obtained with them would not be of value.

The results obtained with the other salts are given below in tabular

[1] *Jour. Infect. Dis.*, 1905, 2, p. 128.
[2] *Biochem. Ztschr.*, 1908, 9, p. 275.

form. The exact strengths of the solutions were ascertained by titration with eighth normal silver nitrate solution.

Mixtures were made of equal parts of normal human serum, suspension of human leukocytes, suspension in physiological salt solution of a 24-hour growth of staphylococci, and solution of the given salt. For the lesser quantities of the salt examined the original solution was diluted with physiological salt solution. The fractions at the head of the columns represent the degree of dilution in any given case. The results are given in ratio of organisms ingested in the mixture containing the salts studied to that of those ingested in mixtures in which the serum was diluted to the same degree with physiological salt solution.

	DILUTIONS.				
	0	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{1}$
m/8 K_2SO_4	1.0	0.81	1.02	1.01	0.93
m/8 $CaCl_2$	1.0	1.0	1.08	1.06	0.50
1.3m/8 $MgCl_2$	1.0	1.4	1.38	1.22	0.52

From these results it would appear that the only one of the three salts to have any marked effect in moderate concentrations on the phagocytosis of staphylococcus is magnesium chlorid, and that the action of this is stimulatory.

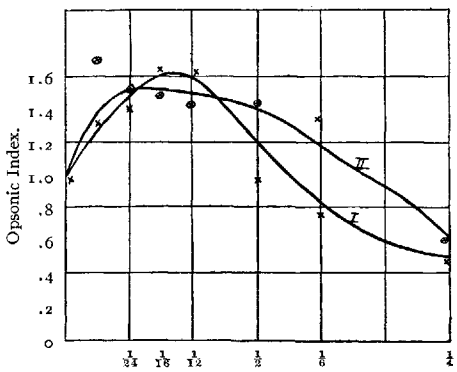

CHART 5.—Effect of MgCl on staphylococcus opsonin in human serum: I, staphylococci previously sensitized; II, staphylococci not previously sensitized.

Using magnesium chlorid again and a m/8 solution of strontium chlorid the following results were obtained:

	DILUTIONS.							
	0	$\frac{1}{8}$	$\frac{1}{6}$	$\frac{1}{4}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{1}{1}$
1.3m/8 $MgCl_2$.....	1.0	1.70	1.53	1.50	1.44	1.46	1.36	0.64
m/8 $SrCl_4$.....	1.0	0.78	0.96	0.97	0.90	1.22	1.10	....

The results here obtained with magnesium chlorid again show a stimulatory action of this salt on phagocytosis, while strontium chlorid, like the salts of the preceding series, is apparently practically inert.

To ascertain whether the action of the magnesium chlorid was on the leukocytes themselves or on the serum, the following experiment was made:

Staphylococci were sensitized by treating them with normal human serum for 30 minutes. They were then washed and resuspended in physiological salt solution. Mixtures were then made of equal parts of this suspension, of human leukocytes, of solution of the salt under examination, and of physiological salt solution. In this way the salt examined was present in dilutions corresponding exactly to those used in the preceding experiments. Magnesium and strontium chlorids were studied in this way.

	Dilutions.							
	0	$\frac{1}{8}$	$\frac{1}{6}$	$\frac{1}{4}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{1}{1}$
1.3m/8 $MgCl_2$	1.0	1.32	1.40	1.64	1.58	0.89	0.76	0.51
m/8 $SrCl_2$	1.0	1.08	1.16	1.22	1.05	1.08	1.08	1.01

Here again, the magnesium chlorid acting only on the leukocyte exerts a stimulatory effect. So that, altho the graphic representation of the two sets of results does not show an absolute parallelism between the curves, it is very probable that the action of the salt is mainly at least on the leukocyte. Strontium chlorid, as before, is apparently without much effect.

It will be noted that the concentration of the magnesium chlorid solution is such as to heighten the osmotic pressure of the medium in which the leukocytes were finally suspended. Inasmuch as Hamburger and Hekma[1] found that comparatively slight increases in osmotic tension resulted in diminished phagocytic activity on the part of the leukocytes, the explanation of the apparent stimulation by the magnesium chlorid cannot be sought on the basis of altered tension.

From these results it would appear that as regards the phagocytosis of staphylococci by normal leukocytes in normal serum, the salts tested are for the greater part inert. Exceptions to this are barium chlorid and sodium fluorid, which exert a very toxic influence, and magnesium chlorid, which would appear to have some stimulatory action on the leukocyte.

[1] *Zittingsverlag der Koninkl. Akad. v. Wetensch.*, 1907.