E X P E R I M E N T S O N P H A G O C Y T O S I S I N V I T R O

by

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I N D E X.

1.

The object of this investigation was to determine what influence was exerted upon the process of phagocytosis in vitro by the presence of small quantities of certain drugs. For this purpose a modification of Wright's method of investigating the opsonic content of blood was used. The drugs examined were the following;- Quinine, sodium salicylate, sodium cinnamate, sodium benzoate, phenol, per-chloride of mercury and alcohol. In addition a small series of commercial antitoxic sera was investigated. I have not been able to find any record of a similar series of experiments.

The experiments recorded are complete in themselves, and it is not necessary to go into the history of phagocytosis at length. It is sufficient to point out that at one time, chiefly through the elaborate investigations of Metchnikoff and his followers, great importance was attached to the process of phagocytosis as one of the chief methods of defence used by the animal body against the invasion of foreign organisms. With the development of the theory of soluble toxins and antitoxins, this conception has been materially altered and the role ascribed to phagocytosis at the present time is that of the subsidiary function of acting as a scavenger in removing altered and dead invading germs, and other foreign material such as carbon particles.

Within the last few years the work of Wright and other investigators upon the opsonic properties of serum has directed attention to certain aspects of phagocytosis, and a large number of researches bearing upon various aspects of the process have been published. It is unnecessary to go into these at any length, as the only/

only one which has any direct bearing upon the present work is that of Hektoen and Ruediger,[1] who showed that calcium chloride, lactic acid, and barium chloride exerted an anti-opsonic action.

In investigating the effect of the drugs recorded upon the process of phagocytosis in vitro I have used Wright's technique for determining the opsonic index. Wright has shown that the process of phagocytosis requires not only the presence of phagocytic cells but also, in the body fluids, of certain substances to which he gives the name of "opsonins". Without these substances phagocytosis does not take place. That is to say, phagocytic cells freed from all traces of opsonic bodies are incapable of phagocyting foreign organisms. This statement is in complete accord with the earlier observations of Denys & Leclef.[2] Working with rabbits immunized against streptococci, they showed that such animals were able to phagocyte streptococci more readily than normal untreated rabbits. This did not depend upon any alteration in the phagocytic cells since these cells, separated from the fluids of the body, were no more highly phagocytic than the cells of normal animals under similar conditions. The fluids of the immunized animals had acquired some property which permitted phagocytosis to be more readily carried out. This was shown by the fact that the cells of the normal untreated animal became as actively phagocytic as the cells of the immunized animal when brought into contact with the fluids of an immunized animal. To the substances on which this property of the body fluids is due, Wright gives the name, "opsonins", and he believes that these bodies combine with the germs and modify them in some fashion/

fashion, so that they are rendered capable of being phagocyted.

The object of the present thesis was primarily intended to determine whether the presence of definite quantities of certain drugs, commonly used as antiseptic in any way modified this process of phagocytosis. The technique of such an investigation is as follows;- Blood is drawn off and allowed to separate into serum and corpuscles by clotting and subsequent centrifugation The serum is then separated and preserved. Another sample of blood is mixed with sufficient sodium citrate solution to prevent clotting. This mixture is then centrifuged, whereupon the corpuscles separate out and the supernatant mixture of serum and citrate solution is pipetted off. The corpuscles that remain are then intimately mixed with normal saline solution and are again centrifuged. The clear fluid so obtained is removed by pipetting, and after this process of washing the corpuscles with saline solution has been repeated four times, it is found that the washed corpuscles, when brought in contact with an emulsion of an organism in saline solution, are capable of phagocyting the germs, unless the mixture is allowed to remain in contact for several hours.(3) On the addition of a small quantity of the serum the cells in the mixture become actively phagocytic. The degree in which phagocytosis takes place can be estimated either by determining the number of phagocytic cells in a stained film which have taken up germs, or as is more commonly done, by averaging the number of germs contained in a phagocytic cell. To do this at least 20 cells must be counted. To determine the effect of a drug it is necessary only to take equal volumes of washed corpuscles, serum, bacterial emulsion, and/

and the solution of the drug. These are intimately mixed and transferred to a glass tube, drawn into a capillary tube, which is then sealed by heat and incubated for 20 minutes at blood heat. The control experiment consists of mixing equal quantities of washed corpuscles, serum, bacterial emulsion and normal saline, which are similarly treated. At the end of the period of incubation films are made from both preparations and stained with Leishman's stain. Equal numbers of phagocytic cells (polymorphonuclear leucocytes) are then counted and the average number of bacteria in a cell is fixed.

This technique, though comparatively simple, is tedious, and in a number of instances it was materially simplified by omitting the repeated washing with normal saline solution. In such cases the freshly drawn blood was simply mixed with an equal portion of citrate solution (1½%). One volume of this citrated blood was mixed with equal volumes of the bacterial emulsion and the dissolved drug or (in the control experiment) normal saline, and then treated in the same fashion as before. In either case an experiment is obtained in which only one factor varies, namely, the presence of the drug. For such comparative investigations it is immaterial whether or not the blood be separated first into washed corpuscles and serum. Da Costa (4) has also dispensed with the washing with saline solution. He finds that the presence of the citrate solution is of no importance. As a rule I have used the more elaborate technique of washing the corpuscles, and in cases where this has not been done, the term "citrated blood" is used.

In all cases the control experiment was made under exactly the same conditions of time, source of blood/

blood, bacterial emulsion and period of incubation. The experiments were devised so that as far as possible only the one factor varied, viz. the addition of the drug to be tested.

As a rule the drug was dissolved in normal saline solution.(A) The addition of the drug may act in two ways; (a) by causing a specific alteration in the activity of the corpuscles, or (b) by (conceivably) exerting an action by simply modifying the molecular content of the mixture. The investigations of Hamburger (5) and his co-workers have shown that the alteration of the molecular content of the fluid in which cells are suspended materially alters the functions of the cells. Gross alterations in the appearance and mobility of the cells may be produced by altering the molecular tension of the fluid suspending them. I record, therefore, a number of experiments in which the effect of altering the quantity of saline content was investigated.

The alteration in molecular content by the addition of a drug in solution can be determined in several ways. Thus the determination of the freezing-point of the mixture gives us information on this point. As the apparatus for such investigation was not at my disposal, I was unable to avail myself of this method. I, therefore, fell back upon the simpler method of determining/

(A) The investigations of Scheurlen,(7) Paul & Krönig(8) showed that the addition of Na Cl intensified the bactericidal action of a watery solution of phenol and this statement has been found to apply to perchloride of mercury and other substances.

determining by mathematical formula what the molecular tention of a given solution represents in terms of Na Cl.

. This method is described by Hamburger and enables us to determine, e.g. what solution of Na I corresponds in osmotic tension to that of .75 per cent of Na Cl. As these two substances belong to the same chemical group, i.e. alkali salts of monobasic acids, a molecule of each substance attracts water with the same power. Consequently, the concentration of the fluid, which exerts a definite osmotic effect, is proportioned to the molecular weight. This follows from Avadogro's law that a gram molecule of any given substance dissolved in 22.34 litres of distilled water exerts, at the temperature of 0 ^{o} C, an osmotic pressure of 760 m.m. of mercury. A gram molecule means the molecular weight expressed in grams. Thus in the case of Na Cl. (mol. weight 56.5) a gram molecule means 58.5 grams; for Na I a gram molecule is 149.5 grams. Now 58.5 grams of Na Cl dissolved in 22.34 litres of water exert an equal osmotic tention to that of a solution of 149.5 grams of Na I in 22.34 litres. From which it can easily be calculated what percentage of Na I corresponds to 0.75% of Na Cl;-

$$\frac{149.5}{58.5} \times 0.75 \quad = \quad 1.49\%.$$

This simple formula of dividing the molecular weight by that of Na Cl and correcting for the osmotic coefficient, if the substances belong to different chemical groups, enables us to determine what osmotic pressure any given solution exerts. With each drug I have made the calculation and express it in terms of Na Cl. at the end of the experiments on each substance.

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I do not think the recent researches of Hamburger and Hekma (6) need be considered, as they do not directly bear upon my work. These investigators show that the addition of water, which caused a diminution of the osmotic pressure of the blood fluids, results in a distinct fall in phagocytic power. Thus the addition of 50% of water reduces the phagocytic power to 43% of its original value, but the subsequent transfer of the cells into normal serum enables them to regain more or less completely their original phagocytic power. In all my experiments the proportion of water to corpuscles remains the same in the test and control experiments so that this factor of variation in watery content does not enter into the discussion. The only varying factor in my experiments was the addition of the small quantities of the drugs in solution, and it will be found, that expressed in terms of Na Cl, this addition in most cases corresponds only to a very trifling excess of Na Cl which cannot explain the marked results that took place with certain of the drugs, notably quinine salts in weak solution. I think these changes must obviously be due rather to the specific effect of the drugs in question, and as such I interpret my results.

11.

Effect upon Phagocytosis of altering the quantity of Na Cl in the mixture.

In this series the experiment consisted in mixing equal volumes of washed corpuscles, serum, bacterial emulsion and the salt solution.

Experiment A.

Staphylococcus pyogenes aureus made up into a emulsion with .9% Na Cl. solution.

S = Serum C = Corpuscles B = Staphylococcic emulsion

1. S + C + B + .66% Na Cl = 7.5 cocci for each phagocytic cell
2. S + C + B + .9% " = 4.3 " " " " "
3. S + C + B + 1.0% " = 2.6 " " " " "
4. S + C + B + 1.4% " = 2.1 " " " " "
5. S + C + B + 1.6% " = 2.3 " " " " "

Experiment B.

Emulsion of bacterium Col.commune.

1. S + C + B + .75% Na Cl = 4.2 bacilli per cell
2. S + C + B + 1.0% " = 4.0 " " "
3. S + C + B + 2.0% " = 2.7 " " "

Experiment C.

Emulsion of staphylococcus pyogenes aureus in .9% Na Cl.

1. S + C + B + .66% Na Cl = 6.5 per cell.
2. S + C + B + .9% " = 4.7 "
3. S + C + B + 1% " = 4 "
4. S + C + B + 1.4% " = 3.7 "
5. S + C + B + 1.6% " = 1.7 "

Experiment D.

Emulsion of Staphylococcus pyogenes aureus in .83% Na Cl.

1. S + C + B + 1% Na Cl = 6.2 average cocci per cell.
2. S + C + B + .91% " = 9 " " " "
3. S + C + B + .83% " = 10.7 " " " "
4. S + C + B + .77% " = 10.4 " " " "
5. S + C + B + .71% " = 8.6 " " " "
6. S + C + B + .66% " = 9.5 " " " "

(In the next experiment no saline solution was added)

7. S + C + B = 12 cocci per cell.

Experiment E.

1. S + C + B + 1.0% Na Cl = 6.6 cocci per cell.
2. S + C + B + .91% " = 7 " " "
3. S + C + B + .83% " = 7.1 " " "
4. S + C + B + .77% " = 7.3 " " "
5. S + C + B + .66% " = 7.4 " " "

Experiment F.

Emulsion of Staph. pyog. aureus in 1.0% Na Cl. solution.

Corpuscles washed in .77% Na Cl.

1. S + C + B + .91% Na Cl = 5 cocci per cell.
2. S + C + B + .83% " = 6.1 " " "
3. S + C + B + .77% " = 7.4 " " "
4. S + C + B + .71% " = 9.1 " " "
5. S + C + B + .66% " = 9.3 " " "

(Control experiment without Na Cl. solution.)

6. S + C + B = 10.6 cocci per cell.

Experiment G.

Staph. pyog. aureus emulsified with 1% Na Cl solution

Corpuscles washed in .83% Na Cl.

1. C + S + B + 1% Na Cl = 1.4 cocci per cell.
2. C + S + B + .91% " = 2.17 " " "
3. C + S + B + .71% " = 2.24 " " "
4. C + S + B + .66% " = 2.44 " " "

(Control experiment without Na Cl solution)

5. C + S + B = 3.3 cocci per cell.

These experiments show that the quantity of salt content in the mixture affects the number of germs phagocyted. To bring this out more clearly I have constructed a table in which each experiment has been brought to a common denominator for the values of the 66% count through multiplying by a correcting factor.

CORRECTED TABLE FOR Na Cl.

	Serum alone.	.66% Nacl.	.71% Na cl.	.75% Na cl.	.77% Na cl.	.83% Na cl.	.91% Na.cl.	1% Nacl.	1.4% Nacl.	1.6% Ncal	2% Nacl
A.		9.4	5.4					3.25	2.6	2.9	
B.				6.3				6			4
C.		9.7	7					6	5.5	2.5	
D.	12	9.5	8.6		10.4	10.7	9	6.2			
E.		9.3			9.1	8.9	8.7	8.2			
F.	10.6	9.3	9.1		7.4	6.1	5				
G.	15.2	9.7	8.96				8.7	6.4			

111.

Action of Quinine.

I have not been able to discover much information about the action of quinine salts upon the phagocytic power of the blood. Quinine is the typical example of a drug which acts as a general protoplasmic poison.(9) Binz has shown that dilute solutions inhibit the active movements of vigorous Paramaeciae. A solution of 1:1000 kills them in three or four minutes, while even in so dilute a solution as 1:20,000 the organisms show signs of incipient paralysis within five minutes and are killed in two hours. The administration of quinine checks the diapedesis of white cells in the artificially induced inflammation of the mesentery of the frog.(10) Mannaberg (quoted by Binz) states " that the "effect of quinine upon the phagocytic cells is the "same as that on the amoebae of malaria".(11) Golgi has "noted a decline or impairment of the phagocytic power "of the blood as a direct consequence of the administra-"tion of quinine".(12) According to Dixon, the presence of one part of quinine in four hundred parts of human blood arrests the amoeboid movements of the white corpuscles, which becomes spheroidal in shape. Added in the proportion of 1:20,000 Englemann found that quinine noticeably checked the amoeboid movements of white cells. To kill the cells completely Binz found it necessary to use a solution of 1:200-500.(13) Uhlmann investigated the action of quinine solutions upon the activity of the leucocytes. In solutions of 1:200-2000 the amoeboid movements ceased and the cells become round, and take on a dark, apparently granular appearance. With the stronger solutions this change takes place in 10-15 minutes, but with the weaker solution it/

required a longer time, and may not become absolute for 90 minutes. A dilute solution of 1:2500 causes the cells to become less actively mobile, but does not alter their appearance; whereas still weaker solutions of 1:3500 and upwards seem to have no action at all. A statement of R. Sand is quoted by Korentschewsky (14) to the effect "that quinine in very weak solution 1 to 10,000 stimulates the activity of unicellular organisms by causing a more rapid reproduction".

Some information can also be obtained from the numerous investigations upon the chemotactic influence of quinine salts. Leber (15) used the following method. He filled sterile glass capillary tubes with drugs of various kinds and introduced the open tubes into the anterior chamber of the eye of rabbits. If the drug attracted leucocytes these drugs found their way to the tube and accumulated inside it. He was able to show that under these conditions quinine attracted leucocytes. This hardly bears out Binz's statement that quinine even in weak solution checks protoplasmic movements. It accords, however, with the experience of Massart and Bordet, who found it impossible to prevent the chemotatic influence of a staphylococcic culture by subcutaneous injection of quinine salts. Gabritchewsky (16) found that in the strength of 0.5% quinine exerted a negative chemotatic influence. Sterile glass capillary tubes sealed at one end were filled with the substance to be tested, and inserted into the peritoneal cavity of frogs. With substances which exerted a positive chemotatic influence, the tubes became filled with leucocytes. Thus a culture of bacillus pyocyaneus attracted great numbers of leucocytes, but the effect of mixing this culture with a 5% solution of quinine was to reduce very considerably the number of leucocytes

that/

that accumulated. Leber found that mercury attracted leucocytes. He filled two capillary tubes, one with mercury, the other with a mixture of solid quinine sulphate and a saturated solution of the same drug, and introduced them into the anterior chamber of the eye. The result was that the tube with mercury attracted leucocytes, while the other tube remained for several days almost free and then gradually became full of cells, though it still contained undissolved quinine. The question whether a similar effect is produced upon the white cells by the internal administration of quinine is not so clear. (18) Wood discusses this point and comes to the conclusion that in therapeutic doses the leucocytes are not affected, but poisonous doses have a demonstrable action upon the white cells.

In the following experiments the action of quinine was studied in three ways;-

(1) A few experiments were made to determine the phagocytic power of blood before and after the administration of large doses of quinine.

(2) The effect of washing the corpuscles in a saline solution of quinine was tested.

(3) The process of phagocytosis in the presence of definite quantities of quinine salts was investigated.

Details of the Experiments.

First series to determine the effect of previous administration of quinine salts.

In these experiments the quinine was given by the mouth and the blood was tested six hours thereafter, since at that period the maximum quantity of quinine is circulating in the blood.

Experiment A.

12 grs of quinine sulphate taken by myself.

Effect tested on staphylococcus pyogenes albus.

Blood separated into serum and corpuscles.

(1) Before quinine my blood acted as follows;-

S + C + staphyl. emulsion = 6 cocci for each phagocytic cell.

(2) Six hours after quinine.

S + C + staphyl. emulsion = 6 cocci for each phagocytic cell.

Result - no effect.

Experiment B.

30 grs. of quinine sulphate were taken by myself. Effect tested on staphylococcus pyogenes albus.

The blood was not separated into serum and corpuscles, but was used as "citrated blood" i.e. an equal quantity of citrate/

citrate of soda solution was added to prevent clotting and one volume of this mixture mixed with one volume of the emulsion of cocci was incubated and films were made.

(1) Before quinine.

Cit. blood + emulsion of cocci = 13 cocci per cell.

(2) 3 hours after the quinine was taken, I felt distinct buzzing in the ears, and a test of the blood showed this result;-

Cit.blood + emulsion of cocci = 10 cocci per cell.

(3) 6 hours after the quinine was taken, the effect was;-

Cit. blood + emulsion of cocci = 2.7 cocci per cell.

The same emulsion of cocci was used in the three experiments.

Experiment C.

Quinine sulphate 30 grs. taken by me at 11-30 a.m. Effect tested on staph. pyog. aur. emulsified in .71% Na Cl. Equal volumes of blood and sodium citrate solution were thoroughly mixed. Of this mixture one volume was taken, and mixed with an equal volume of the emulsion, incubated 20 minutes, and examined. At the commencement, before the quinine was taken, the average phagocytosis was 13 cocci per cell.

At 2-30 p.m. (3 hours after quinine) it was 10 cocci per cell

At 4 p.m. 4½ " " " " " 4.5 " " "

At 5-30 p.m. 6 " " " " " 4.4 " " "

Experiment D.

A rabbit received 20 grs. of quinine salicylate by stomach tube. The blood was separated into serum, the corpuscles were washed, and the effect of the drug was tested on staph. pyog. albus.

(1) Before quinine.

Ser.+ Corp.+ emulsion = 4 cocci per cell.

(2) 6 hours after dose

Ser.+ Corp. + emulsion = .9 cocci per cell.

Experiment E.

Similiar experiment with same dose.

(1) Before quinine.

Ser. + Corp. + emulsion = 6.6 cocci per cell.

(2) 6 hours after quinine.

Ser. + Corp. + emulsion = 2.6 " " "

These results are fairly uniform but are too few in number to permit the drawing of any conclusions, yet they indicate that in these cases the administration of large doses of quinine had a marked effect upon the phagocytic power of the blood. While the dose of 12 grs. proved inactive, upon two occasions 30 grs. markedly reduced the phagoytic power of my blood, and a similar result followed the administration of 20 grs. of quinine salicylate to rabbits.

III (b)

Effect of washing the corpuscles in a dilute solution of quinine hydrochloride (1 of quin. hcl. in 2000 parts of normal saline) Organism staph. pyog. alb. The effect in each cases was tested against a control experiment in which the corpuscles were washed in normal saline solution which contained no quinine.

Experiment A.

Serum + saline washed corp. + emulsion gave an average of 20 cocci per cell.

Serum + quinine washed corp. + emulsion gave an average of 1.9 cocci per cell.

Experiment B.

Similar to A.

Ser. + saline washed corp. + emulsion gave an average of 13.4 cocci per cell.

Ser. + quinine washed corp. + emulsion gave an average of 0.2 cocci per cell.

Experiment C.

Similar to A.

Ser.+ saline washed corp.+ emulsion gave an average of 9.4 cocci per cell,

Ser. + quinine washed corp.+emulsion gave an average of 0.1 cocci per cell,

These experiments show that the effect of washing the corpuscles in quinine is very marked and renders them incapable of taking up cocci. As the quinine-washed corpuscles were certain to retain some traces of the quinine after the fluid was pipetted off, these experiments practically come under the next category of experiments, on phagocytosis in the presence of dilute quinine solution.

III (c)

Phagocytosis in definite presence of quinine salts.

Experiment A.

Solution of one part of quinine hydrochloride in 500 parts of normal saline. Organism staph. pyog. alb. One blood used. The effect of previous action of quinine upon corpuscles and serum was tested by using a mixture of equal volumes of serum and quinine solution which had been allowed to act upon each other for 30 minutes before being used = Q S

Similarly the washed corpuscles.were mixed with an equal volume of the quinine solution, which was allowed to act for 30 minutes = Q C.

S = pure serum. C = washed corpuscles. E = emulsion of cocci.

Control (1) S + C + E - 7.1 cocci per cell.

(2) S + QC+E - 1.1 " " "

(3) QS+ C+E - 1.2 " " "

(4) QS+QC+E - 0.4 " " "

Experiment B.

Similar experiment but in addition the effect of allowing the quinine solution to act upon the emulsion of cocci for 12 minutes was observed = QE.

Quinized serum = QS. Quinized corpuscles = QC.

Quinized cocci = QE.

Control (1) C + S + E = 2.6 cocci per cell.

(2) S + QC + E = 0.2 " " "

(3) QS + C + E = 0.3 " " "

(4) QE + C + S = 0.16 " " "

These two experiments show that the presence of a strong solution of quinine had a distinct action and that it was immaterial whether the quinine was previously mixed with the serum, corpuscles, or emulsion of cocci.

111 (d)

In this series of experiments, quinine was used in various proportions dissolved in normal saline solution. As in previous experiments equal volumes of washed corpuscles (C), Serum (S), Emulsion of cocci (E), and the quinine solution were used. The dilution of the quinine solution is represented by the fraction preceding the capital Q. Thus 1 2000 means that one volume was used of a solution of one part of quinine in 2000 of normal saline solution and so on.

Experiment A.

Control (1) C + S + Normal saline + E = 9 cocci per cell.

(2) C + S + 1 1000 quin. + E = 0.9 " " "

(3) C + S + 1 2000 " + E = 2.6 " " "

(4) C + S + 1 4000 " + E = 4.2 " " "

(5) C + S + 1 8000 " + E = 4 " " "

Experiment B.

In this and the following experiment the blood was not separated into serum and corpuscles, but was mixed with an equal volume of sodium citrate to prevent clotting. Equal volumes of this citrated blood, emulsion of cocci and solution of quinine were used.

Control/

Control (1)	Citrated blood	+	Normal saline	+	E =	11	cocci p cell
(2)	"	"	+ 1 : 1000 quin.	+	E =	2	" "
(3)	"	"	+ 1 : 2000 "	+	E =	5	" "
(4)	"	"	+ 1 : 4000 "	+	E =	5.7	" "
(5)	"	"	+ 1 : 8000 "	+	E =	10	" "

Experiment C.

Similar to B.

Control (1)	Citrated blood	+	Normal sal.	+	E =	11.7	cocci p cell
(2)	"	"	+ 1:1000 quin.		E =	1.5	" "
(3)	"	"	+ 1:2000 "	+	E =	3.8	" "
(4)	"	"	+ 1:4000 "	+	E =	6	" "
(5)	"	"	+ 1:8000 "	+	E =	6	" "

These experiments show very definitely that the precence of quinine exerts a very marked influence upon the process of phagocytosis in vitro. Strong solutions practically cause the phagocytosis to cease while the presence of relatively very small per centages of quinine still exerts an influence. It should be noted that in an experiment such as A (2) where one volume of a solution containing 1:1000 part of quinine is mixed with equal volumes of serum, washed corpuscles, and bacterial emulsion, the proportion of quinine to the whole mixture is 1:4000. Experiments A (2), B (2), C (2), show that in this proportion phagocytosis is almost inhibited. With the next strength, i.e. where one volume of 1:2000 of quinine is used (the proportion of quinine to the whole mixture being 1:8000) the phagocytosis is only about half that which takes place in the control experiment. Cp. experiments A (3), B (3), E (3).

In the proportion of 1:16000 the effect is less marked, but is still visible when the quinine is present in so dilute a form as 1:32000. Cp. A (5), B (5), C (5).

The molecular weight of quinine hydrochloride is 393.76 therefore the osmotic tention of a solution containing 0.1% of/

of quin. hydr. is equal to $\frac{58.5}{393.75} \times 0.1 = 0.015\%$ Na Cl.

This figure is so small that we cannot ascribe the marked difference to any alteration in osmotic tension.

In a note to an article by Bordet (19) I find that he was aware of the fact that a solution of hydrochloride of quinine prevents phagocytosis. He states that even dilute solutions render the leucocytes immobile. An exudate containing leucocytes is incapable of phagocyting germs if it is mixed with an equal volume of .1% solution of quinine hydrochloride. This is in complete agreement with my own observations.

IV

Action of sodium salicylate.

Salicylic acid is stated to possess the property of checking the movements of leucocytes. Prudden found that a solution of 1:4000 completely cheqkes the emigration of leucocytes out of the mesenteric vessels of the frog and that this action remains after the acid has been removed by washing the part. According to Dixon (12) the salicylate resembles the acid in this effect but is less active. Leber (15) investigated the chemotactic influence of salicylic acid and found that it did not attract leucocytes. Gifford (18) found that to check the growth of pus cocci required a strength of 1:500 - 1:2000 of sodium salicylate. According to Buchholz (18) the sodium salicylate is about equal to the pure acid in preventing the development of bacteria, 0.4% destroying the germs.

Experiment A.

Organism staph. pyog. alb. My own blood was used mixed with an equal volume of 1½% sodium citrate = C.B.

The sodium salicylate was dissolved in normal saline solution

Control (1) C.B.+ Normal saline +E = 9.2 cocci per cell.

(2) " + 1:100 sod.sal.+E = 6.3 " " "

(3) " + 1:900 " " +E = 10 " " "

(4) " + 1:3000 " " + E = 13 " " "

Experiment B.

Same procedure.

Control (1) C.B. + Normal Saline + E = 9 cocci per cell.

(2) " + 1% sod. sal. + E = 0.7 " " "

(3) " + 0.1% " " + E = 10 " " "

(4) " + 0.01% " " + E = 9 " " "

Experiment C.

Same procedure.

Control/

Action of Sodium salicylate.

Control (1) C.B.+Normal saline +E = 5.99 cocci per cell.
(2) " +1% sod. sal. +E = 2.9 " " "
(3) " +0.1% " " +E = 5 " " "
(4) "+0.01 " " +E = 5.1 " " "

Experiment D.

Blood of patient suffering from Phthisis Pulmonalis tested on emulsion of strepococcus

Control (1) Serum+corp.+Normal saline +E = 9.3 cocci p cell
(2) " + " +1% sod. sal. +E = 6 " "
(3) " + "+0.1% " " +E -14 " "
(4) " + "+0.01% " +E -15.3 " "

Experiment E.

Similar to D. but my own serum was tested on patient's corp.

Control (1) Serum+Corp.+Normal sal.+E = 8.9 cocci p cell
(2) " + " +1% sod. sal.+E - 5.8 " "
(3) " + " +0.1% " " +E -10.6 " "
(4) " + "+0.01% " " +E = 7.9 " "

Experiment F.

Patient with chronic rheumatism gave the serum which was tested with my own washed corpuscles on a streptococcic emulsion.

Control (1) Serum+corp.+Normal Saline+E = 5 cocci p cell
(2) " + " +1% sod. sal. +E - 0.3 " "
(3) " + " +0.1% " " +E = 3.6 " "

Experiment G.

Similar to F. but my own serum used.

Control (1) Serum+Corp.+Normal sal.+E = 2.6 cocci p cell
(2) " + " +1% sod. sal.+E = 1.6 " "
(3) " + " +0.1% " " +E = 3.8 " "
(4) " + "+0.01% " " +E = 2.6 " "

Experiment H.

Serum of patient with chronic arthritis tested on my corpuscles with emulsion of staphylococcuc pyogenes albus.

Control/

Action of Sodium salicylate.

Control (1) Serum+Corp.+Normal sal.+E = 10.4 cocci p cell
(2) " + " +1% sod. sal.+E = 1.6 " "
(3) " + " +0.1% " " +E = 7 " "

Experiment I.

Similar to H. but my own serum used.

Control (1) Serum+Corp.+Normal sal.+E = 9 cocci p cell.
(2) " + " +1% sod. sal.+E = 4.25 " "
(3) " + " +0.1% " " +E = 4.5 " "

Experiment J.

Similar to H. but serum obtained from patient suffering from chronic lead poisoning.

Control (1) Serum+Corp.+Normal sal.+E = 10 cocci p cell.
(2) " + " +1% sod. sal.+E = 5.6 " "
(3) " + " +0.1% " " +E = 14.2 " "
(4) " + " +0.01% " " +E = 12.3 " "

Experiment K.

Similar to J. but my own serum tested.

Control (1) Serum+Corp.+Normal sal.+E = 8.9 cocci p cell.
(2) " + " +1% sod. sal. +E = 4.9 " "
(3) " + " +0.1% " " +E = 8.8 " "
(4) " + " +0.01% " " +E =11.4 " "

These experiments show that the action of sodium salicylate is very much less marked than that of quinine. In the strength of 1:400 i.e. (addition of one volume of 1% solution) it invariably reduces the degree of phagocytosis, but as a rule this reduction amounts only to about 50% of the control experiment in which normal saline is used. In three experiments B.F. and H. the reduction was more marked. The effect of smaller proportions of sodium salicylate is interesting. With the addition of 1:1000 in 8 out of 11 experiments the number of germs phagocyted was greater than in the control experiment. In the other three experiments viz;- F.H. and I the number of germs taken up was greater than with the stronger solution of a salisylate but still less than the control/

control experiment. The effect of using the salicylate in still more dilute form is variable, in some cases the average per cell rose, in others it fell.

The molecular weight of sodium salicylate is 158, therefore a 1% solution corresponds in osmotic tention to $\frac{58.5}{158}$ = that of a solution of 0.37%. Na Cl.

V

ACTION OF SODIUM FORMATE

The next drug tested was sodium formate which has recently been recommended by various writers as a general stimulant to nervous and muscular tissue. On the supposition that it might possibly have some effect upon the process of phagocytosis it was examined in a series of tests though I was unable to find any support of this view.in the literature of phagocytosis. Duclaux (26) investigated the antiseptic action of formic acid and found that it was a strong antiseptic. In the strength of 8 mgr. to the litre it prevented the growth of yeast, and in stronger solution it checked the growth of various pathogenic organisms. Behring found that 0.267% (27) checked the growth of the anthrax germ. In Germany the use of formic acid as a preservative for canned fruits and meat has recently been given up as it was found that the administration of formic acid produced methhaemoglobin. In my experiments I did not observe any formation of methhaemoglobin. As will be seen from the subjoined experiments the drug has no marked action on phagocytosis. It somewhat closely resembles the sodium salicylate but the stimulating effect is less marked.

Experiment A.

My own blood tested on a emulsion of staph. pyog. alb. The sodium formate was dissolved in normal saline solution. Control/

Control (1) Serum + Corp. + Normal sal. + E = 7.9 cocci p cell.

(2) " + " + 1% sod. form. + E = 2.4 " "

(3) " + " + .1% " " + E = 8 " "

Experiment B.

Similar experiment but serum obtained from a patient suffering from Phthisis was tested on my corpuscles.

Control (1) Serum + Corp. + Normal sal. + E = 7.7 cocci p cell

(2) " + " + 1% sod. form. + E = 3 " "

(3) " + " + 0.1% " " + E = 6.2 " "

Experiment C.

My own serum and corpuscles were used.

Control (1) Serum + Corp. + Normal sal. + E = 6.3 cocci p cell

(2) " + " + 1% sod. form. + E = 2.4 " "

(3) " + " + 0.1% " " + E = 5.5 " "

(4) " + " + 0.01% " " + E = 6.4 " "

Experiment D.

Similar to C.

Control (1) Serum + Corp. + Normal sal. + E = 7 cocci p cell.

(2) " + " + 1% sod. form. + E = 3 " "

(3) " + " + 0.1% " " + E = 6 " "

(4) " + " + 0.01% " " + E = 6.6 " "

Experiment E.

Similar to C.

Control (1) Serum + Corp. + Normal sal. + E = 10.8 cocci p cell

(2) " + " + 1% sod. form. + E = 5.4 " "

(3) " + " + 0.1% " " + E = 11.3 " "

(4) " + " + 0.01% " " + E = 8.7 " "

Experiment F.

Similar to D.

Control (1) Serum + Corp. + Normal sal. + E = 16.7 cocci p cell

(2) " + " + 1% sod. form. + E = 7.2 " "

(3) " + " + 0.1% " " + E = 9 " "

(4) " + " + 0.01% " " + E = 8.4 " "

These experiments are uniform in their results and show that the presence of 1:400 sodium formate reduces the phagocytic power by one half, while in the proportion of 1:4000 it is as active as when normal saline is used. There is no evidence that reducing the proportion of formate still further increases the phagocytic power.

VI

ACTION OF SODIUM BENZOATE.

Benzoic acid is a weaker antiseptic than salicylic acid. Lubbert found that the development of staph. pyog. aur. was completely checked by a solution of 1:400 of benzoic acid, while Koch states that a solution of 1:200 of sodium benzoic caused inhibition of growth of anthrax cultures. I did not find that benzoate of sodium has any marked effect upon the process of phagocytosis, so that only two experiments are recorded here.

The Benzoate was dissolved in water and my own blood was tested on an emulsion of staph. pyog. aur.

Experiment A.

Control (1) Corp.+ Serum +.77% Na Cl.+E = 7.4 cocci p cell
(2) " + " + 1% sod. Ben. - 7.1 " "

Experiment B.

Similar to A.

Control (1) Corp.+ Serum.+.71% Na Cl.+E = 2.24 cocci p cell
(2) " + " + 1% sod. Ben+E = 2 " "

VII

ACTION OF SODIUM CINNAMATE.

This drug is not a strong antiseptic, Koch found that cinnamic acid in the strength of 1:1000 is without any effect upon the growth of the bacillus of anthrax. It has no curative action in rheumatic fever. The cinnamate of soda has been largely used in the treatment of phthisis. It is supposed to cause an increase in the number of leucocytes circulating in the blood, but the oral administration of 15 grs. every four hours for 10 days did not markedly increase the/

the leucocytosis in a case of rheumatic fever. An investigation by Cathcart and myself (20) showed that it had very little effect upon the blood and bone marrow of rabbits. The subjoined experiments show that in respect of phagocytosis it is also an inactive drug even in the strength of 1:400.

Experiment A.

Sodium Cinnamate dissolved in water. My own blood tested on an emulsion of staph. pyog. aur.

Control (1) Serum.+Corp. + .71% Na Cl.+E = 7.4 cocci p cell

(2) " + " + 1% Sod. Cinn.+E= 3.8 " "

Experiment B.

Similar to A.

Control (1) Serum+Corp.+.71% Na Cl.+E = 2.24 cocci p cell

(2) " + " + 1% Sod Cinn.+E = 3.2 " "

VIII

ACTION OF ALCOHOL.

Diluted with water alcohol is largely used as an antiseptic. Salzwedel and Elsner (21) found that a 7% solution checkes the growth of staph. pyog. aur., while Harrington and Walker (18) found that 40% of alcohol is effective against non-sporulating pathogenic bacteria, within five minutes. Gabritschewsky (16) states that alcohol exerts a negative chemotactic influence.

Experiment A.

In a rough way the effect of alcohol was tested by adding large and small quantities to my blood and an emulsion of staph. pyog. aur. made with sterile ascitic fluid, which possessed no opsonic properties.

Control (1) Corp.+Serum +E = 6.4 cocci per cell.

(2) " + " + E +Trace alcohol = 5 cocci p cell

(3) " + " + E +50% " = 0 " "

Experiment B.

Similar to A.

Control (1) Corp.+ Serum + E = 13 cocci per cell.

(2) " + " + E + Trace alc. = 4.7 cocci p.c.

(3) " + " + E + 50% " = 0.1 "

More careful experiments with definite quantities of alcohol dissolved in saline solution were then carried out. The emulsion of staphylococci was made with normal saline solution.

Experiment C.

Control (1) Serum + Corp. + Normal sal. + E - 18.6 cocci p cell
(2) " + " + 0.25% alcohol + E = 10.5 " "
(3) " + " + 0.025% " + E = 13 " "

Experiment D.

Similar to C. but serum obtained from a patient who had had pneumonia.

Control (1) Serum + Corp. + Normal sal. + E = 5 cocci p cell
(2) " + " + 0.25% alcohol + E = 5.8 " "
(3) " + " + 0.025% " + E = 7 " "

Experiment E.

Similar to C.

Control (1) Serum + Corp. + Normal sal. + E - 10.8 cocci p cell
(2) " + " + 0.25% alcohol + E = 8.1 " "
(3) " + " + 0.025% " + E = 12 " "

Experiment F.

Similar to C.

Control (1) Serum + Corp. + Normal sal. + E - 10.4 cocci p cell
(2) " + " + 0.25% alcohol + E = 7.2 " "
(3) " + " + 0.025% " + E = 9.6 " "

The effect of alcohol is evidently not very marked and not dissimilar to that obtained on increasing the saline content as in the experiments with Na Cl. The molecular weight of alcohol is 45.7 consequently its osmotic tention is rather greater than that of Na Cl. 0.25% of alcohol corresponds to 0.32% of Na Cl. in osmotic tention. This alteration alone, irrespective of specific action by the alcohol, would cause a considerable effect upon the phagocytosis. In weaker solution alcohol apparently tends to increase the activity of phagocytosis.

IX.

ACTION OF CARBOLIC ACID.

Phenol in aqueous solution is a general protoplasmic poison. In weak solution it arrests movements of ciliated cells and of white corpuscles (Prudden, Labbe) but very minute quantities increase the activity of undifferentiated protoplasm (Dixon).(12) The effect of phenol is much less marked than that of per-chloride of mercury upon phagocytosis in vitro, as the following experiments show. My own blood was used and the germs tested were staphylococcus pyogenes aureus, emulsified with 1% Na cl. Phenol was dissolved in water.

Experiment A.

Control (1) Corp. + Serum + E + .77% Na Cl = 3.5 cocci p cell

(2) " + " + E + 0.5% Phenol = 2 " "

(3) " + " + E + .025% " = 3 " "

Experiment B.

Similar to A.

Control (1) Corp. + Serum + E + .77% Na Cl. = 10.4 cocci p cell.

(2) " + " + E + 0.5% Phenol = 7 " "

X

ACTION OF PERCHLORIDE OF MERCURY.

This drug is so largely used as an antiseptic that it seemed advisable to investigate its effect upon the process of phagocytosis. It is one of the most active germicides which we possess, and it is therefore extremely likely that the presence of traces of per-chloride of mercury would affect the white corpuscles. Koch originally stated that a solution of 1:1,000,000 of Hg Cl checked the growth of the anthrax germ, while a solution of 1:5000 killed the spores. But Behring showed that the presence of albumen markedly reduced the power of per-chloride of mercury, and stated that even a solution of 1:1000 in the presence of albumen could not be depended upon with certainty to kill anthrax spores within 20 minutes. Geppert still further reduced its value/

value by showing that the complete chemical neutralisation of $HgCl_2$ by ammonium sulphide proved that even this strength did not kill after acting for several hours. Notwithstanding these criticisms perchloride of mercury is one of our most reliable antiseptics. Heinz and Seitz (22) show that 0.1%, 0.075% and 0.05% solutions prevent the growth of staphylococcus pyogenes aureus for three days. Upon the movements of white corpuscles it has a paralysing influence. Thus the application of a solution of 1:10,000 to the inflammed mesentery of the frog will prevent the exudation of leucocytes and arrest the amoeboid movements of those already outside the vessel wall. Dougal (13) found that a solution of 1:6500 kills spermatozoa. Kowalevsky (23) states that a 1:1000 solution exerts a marked chemotactic influence in rabbits. On the other hand Bloch (24) found that it had no such effect.

Experiment A.

Blood separated into serum, and corpuscles washed. Emulsion of staphylococci made in ascitic fluid which contained no opsonins.

$HgCl_2$ mixed with ascitic fluid 1 1000.

Control (1) S + C + E + ascitic fluid = 5.4 cocci per cell.

(2) S + C + E + 1 1000 $HgCl_2$ = 0.3 " " "

Experiment B.

Same type. Blood tested of man suffering from malignant tumor.

Control (1) S+C+E + ascitic fluid = 6 cocci per cell.

(2) S+C+E + 1 1000 $HgCl_2$ . . =0.5 " "

Experiment C.

Own blood tested on staphylococcus pyogenes aureus in normal saline. Perchloride in watery solution.

Control (1) S + C + E + Normal sal. = 10 cocci per cell.

(2) S + C + E + 1 1000 $HgCl_2$ = 0.1 " " "

Action of Perchloride of Mercury.
Experiments with Antibacterial Sera.

Experiment D.

Own blood citrated; Staphylococci emulsified with normal saline.

$HgCl_2$ 1:1000 water diluted with Na Cl.

.77 1:2000 1:8000.

Control (1) Cit. blood + E + Na Cl. = 10 cocci per cell.

(2) " " + E + 1:2000 $HgCl_2$. = 0.1 " "

(3) " " + E + 1:8000 " = 0.1 " "

These experiments show that even very dilute solutions of perchloride of mercury are able to check the process of phagocytosis. Thus in experiment D(3) the proportion of $HgCl_2$ was only 1:24,000 but this almost completely checked phagocytosis. The presence of albumen rather lowers this power. Thus when mixed with the ascitic fluid as in experiments A and B the effect was less marked than in the later experiments where it was used as a watery solution.

XI.

EXPERIMENTS WITH ANTIBACTERIAL SERA.

The last series of drugs investigated consisted of commercial sera. Crofton (25) has investigated a few of these and found that manufactured anti-toxic sera had very little action as opsonins. He states that they may even contain certain substances which act as antibodies inimical to opsonins of normal serum.

The serums I examined were Burroughs, Welcome & Co's antistreptococcic serum, Menzer's antistreptococcic serum, and Roemer's antipneumococcic serum. None of these were found to possess any opsonic value. Illustrative experiments of each are given.

Experiment A.

B.W. & Co's Antistreptococci serum. In this experiment the streptococci were emulsified with normal saline. My own

serum/

serum and washed corpuscles were used as tests.

Control (1) Corp.+Serum + E = 13.3 cocci per cell.

(2) " + " +Antistrep. serum +E = 1.7 " "

Experiment B.

Roemer's serum. This is a polyvalent serum made with various strains of pneumococci and is intended for use in cases of local and general pneumococcic infection. The pneumococcic emulsion was prepared with normal saline and the blood used was my own.

Control (1) Corp.+ Serum + E = 6 cocci per cell.

(2) " +Roemer's Serum +E = 0.2 " "

Experiment C.

Menzer's serum is a polyvalent antistreptococcic serum, which has proved of use in puerperal sepsis and in selected cases of sub-acute rheumatism. The serum which was used in these tests was very active therapeutically.

The action was tested upon an emulsion of streptococci in normal saline solution. My own serum and corpuscles again were used.

Control (1) Corpuscles + Serum + E = 14 cocci per cell.

(2) " +Menzer's Serum +E = 4.7 " "

The corpuscles in this case were not very thoroughly washed free from opsonin, since in the control test the corpuscles still took up 4.8 cocci.

The experiment was continued on the following lines It is possible, as Crofton has suggested, that the serum may not only not contain opsonin but may have an antagonising influence, either, as he suggests, from the presence of an antibody, or possibly from the presence of a trace of antiseptic substance added to preserve the serum. To test this the Menzer's serum was tested against corresponding quantities/

quantities of normal serum and normal saline.

(1) C + S + Normal saline + E = 13.5

(2) C + S + Menzer Serum + E = 6.5

(3) C + Normal sal. + Menz. Ser. + E = 4.3

(4) C + Normal sal. + E = 4.4

This seems to indicate that the addition of Menzer's serum did diminish the activity of normal serum.

XII.

EFFECT OF STARVATION.

I have also investigated the effect of starvation upon the phagocytic power of the blood. The observations were made upon a professional faster, V.B. who fasted under continuous observation for 14 days. Careful examinations of the excretions showed that the fast was absolute. During the fasting period the blood was examined repeatedly and its phagocytic power tested, my own blood being used as a control.

The results are briefly as follows;-

On the day before fast began the phagocytic index for the fasting man was 7.5 for staph. pyog. alb; for F.J.C. 8.7

2nd day of fast	V.B.5	" "	6
5th " " "	V.B.3	" "	3
7th " " "	V.B.1.9	" "	1.8
8th " " "	V.B.2.8	" "	1.6
12th " " "	V.B.0.8	" "	0.8
15th " " "	V.B.0.6	" "	0.7
After 3 days of food.	V.B.2	" "	1
After six days of food.	V.B.11	" "	12

The opsonic index for Bacillus Typhoses was

before fast for	V.B. 1.5	for F.J.C.	1.9.
5th day of fast.	V.B. 6	" "	6
8th " " "	V.B. 2	" "	1.8
12th " " "	V.B. 2	" "	0.7.

In these observations the fasting man's blood and my own blood were tested at the same time of the day and under similar conditions; the two serums being tested upon the same washed corpuscles following Wright's technique. The results show that in this experiment the fasting had no effect, B's blood remaining as actively phagocytic as my own. Canalis & Morpurgo (28) have shown that pigeons after starvation for 6-7 days loose their natural immunity against anthrax, while hens starved for 3-7 days succumbed more readily to anthrax than normal hens. On the other hand rats remained immune despite starvation, and hens previously rendered immune by inoculations retained this immunity during starvation. Roger & Josue (29) found that absolute starvation for 5-7 days increased the resisting power of rabbits against inoculations of bacterium coli compared with normal unstarved animals, which fact they ascribed to the increased proliferation which takes place in the bone marrow during starvation.

CONCLUSIONS.

1. The effect of increasing the proportion of sodium chloride affects the phagocytic power of the blood.

2. Quinine in solution exerts a very marked retarding influence. Strong solutions practically check all phagocytosis. In the proportion of 1:8000 the phagocytosis is reduced to one half that of the normal amount. While even in extreme dilution when only 1:16000 or 1:32000 of quinine is present the retarding influence is still visible. The administration of quinine salts in large doses by the mouth seems to produce a similar effect to what it does in experiments in vitro.

3. Of the other drugs examined, the salicylate of sodium, the formate of sodium, the benzoate of sodium, and the cinnamate of sodium, have little effect upon phagocytosis in vitro.

4. The effect of adding the perchloride of mercury is very marked. Even in so small a proportion as 1:24000 of the mixture it checks phagocytosis almost completely.

5. Alcohol has little influence in checking phagocytosis unless it is present in large quantities. The addition of 1 to 8 of alcohol checks all phagocytosis, but the addition of a solution of less than 0.25% has little action.

6. The action of Phenol is not marked.

7. The antitoxic sera examined did not produce any phagocytosis.

8. The effect of prolonged fasting did not reduce the phagocytic power of the blood of a fasting man.

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