STUDIES IN SPONTANEOUS PHAGOCYTOSIS.

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Part I.

Phagocytosis as an adsorptive process.

Both the sensitation of typhoid bacilli by serum and their ingestion by leucocytes when sensitised, have been shown by Ledingham (1912) to take place in accordance with the laws regulating an adsorption process, the removal of opsonin from a serum by bacteria, and of sensitised bacteria from an emulsion by leucocytes following very satisfactorily the adsorption equation $y = kx^n$, where $y =$ the amount adsorbed, $x =$ the amount left free and k and n are constants.

It remained to be seen whether spontaneous phagocytosis, that is, phagocytosis occurring in the absence of serum, either active or inactive, also follows a similar law.

For this purpose a *Staphylococcus aureus* was employed, thick emulsions of an overnight agar slope culture being made in physiological saline and a series of dilutions prepared therefrom. The leucocytes used were those present in an emulsion of human blood corpuscles, taken up in citrate solution and washed three times with physiological saline.

Equal volumes of staphylococcal emulsion, leucocyte emulsion and saline were incubated in a shaker at 37° C. for half an hour, at the end of which time the mixtures were removed, films drawn, fixed and stained. In each film never less than 100 leucocytes were counted. Besides the phagocytic index thus obtained, the ratio of cocci to

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leucocytes was calculated, the coccal emulsion having been counted in one of its dilutions in a Thoma-Zeiss counting chamber and the leucocytes taken as being roughly 8000 per c.mm. This approximation is allowable, seeing that for each series the leucocytic emulsion is the same throughout. Reckoning from the number of cocci that were available for each leucocyte and the number which each, on the average, had actually taken up, as given by the phagocytic index, one obtained the percentage ingested.

In the following experiments, I–VI, one notices first of all that the results obtainable in a spontaneous phagocytosis experiment are not so reliable as when serum is present. There is, for instance, only a rough consistency with regard to the index one may expect from a coccal emulsion of a certain strength, although it must at the same time be remembered that the leucocyte emulsion varied in strength from experiment to experiment to a certain extent and would introduce a variable factor.

Experiment I.

Cocci per c.mm.	$\frac{\text{Cocci}}{\text{Leucocytes}}$	Phagocytic index	% of cocci ingested
$22{,}000 \times 10^6$	2,750	30·58	1·11
$11{,}000 \times 10^6$	1,375	—	—
$5{,}500 \times 10^6$	687·5	9·53	1·39
$2{,}750 \times 10^6$	343·75	1·62	0·47
$1{,}375 \times 10^6$	171·875	4·53	2·64
688×10^6	85·9375	4·35	5·06

Experiment II.

$11{,}448 \times 10^6$	1,431	15·55	1·09
$5{,}774 \times 10^6$	715·5	20·12	2·81
$2{,}862 \times 10^6$	357·75	7·68	2·15
$1{,}431 \times 10^6$	178·875	7·25	4·04
715×10^6	89·4375	4·45	4·97
358×10^6	44·7187	3·26	7·29

Experiment III.

$3{,}120 \times 10^6$	390	9·3	2·4
$1{,}560 \times 10^6$	195	4·1	2·1
780×10^6	97·5	3·1	3·2
390×10^6	48·75	1·26	2·6
195×10^6	24·375	1·94	7·9
$97{\cdot}5 \times 10^6$	12·1875	0·92	7·5
$48{\cdot}75 \times 10^6$	6·0937	0·29	4·8
$24{\cdot}375 \times 10^6$	3·0468	0·32	10·5

Experiment IV.

$7{,}696 \times 10^6$	962	12·2	1·3
$3{,}848 \times 10^6$	481	5·2	1·1
$1{,}924 \times 10^6$	240·5	7·2	3·0
962×10^6	120·25	2·2	1·8
481×10^6	60·125	1·4	2·3
$240{\cdot}5 \times 10^6$	30·0625	1·2	4·0
$120{\cdot}25 \times 10^6$	15·0312	1·2	8·0
$60{\cdot}125 \times 10^6$	7·5156	1·05	14·0
$30{\cdot}0625 \times 10^6$	3·7578	0·34	9·1

In Exps. V and VI the actual strength of the coccal emulsion was not estimated. Consequently the percentage of cocci ingested could not be determined, but the ratio of the relative strength of coccal emulsion to the phagocytic index gave a similar indication as to the adsorptive nature of the process.

	Experiment V.		*Experiment VI.*	
Strength of coccal emulsion	Phagocytic index	Phagocytic index / Relative no. of cocci	Phagocytic index	Phagocytic index / Relative no. of cocci
$32x$	49·05	1·53	10·78	0·34
$16x$	17·60	1·10	17·61	1·10
$8x$	14·52	1·81	13·18	1·65
$4x$	9·64	2·41	12·97	3·24
$2x$	4·84	2·42	11·02	5·51
x	4·86	4·86	9·20	9·20

Turning to the figures representing the percentage of cocci ingested, it is to be noted that while they suggest that the process of ingestion is of an adsorptive nature, there being with a decrease in the number of cocci available, a marked rise in the percentage ingested, these figures are too variable to be used mathematically, the experimental error apparently preventing more accurate proof as had been possible in the case of phagocytosis in the presence of serum.

PART II.

The influence of hydrogen-ions on phagocytosis.

The influence of acid and alkali on spontaneous phagocytosis has been considered by Hamburger and Hekma (1908). They used *horse leucocytes* and as object for phagocytosis *carbon particles*, taking the percentage of leucocytes that had ingested any carbon at all as index. They came to the conclusion that any addition either of acid or alkali

to the phagocytic system, resulted in a lowering of the index, this being particularly the case with acid. It must be noted, however, that they worked with comparatively strong solutions of H_2SO_4 and NaOH, the strength in the mixture of leucocytes and carbon particles being from $n/20$ to $n/200$ in the case of the acid and for the alkali from $n/100$ to $n/500$.

In an endeavour to find proof for electrochemical influence on the phenomenon of phagocytosis, a series of experiments was carried out in which phagocytosis took place under the influence of concentrations of acid and alkali weaker than those used by Hamburger and Hekma and in the absence of serum either active or inactive, control experiments being performed without either of these additions and simply in the presence of physiological saline solution.

In these experiments the organism used throughout was again a *staphylococcus aureus*, emulsions of it and the leucocytes being prepared as described above.

Acetic acid and ammonia were the acid and alkali employed, both in dilutions of $n/50$ and $n/330$, physiological saline being used to prepare the dilutions in place of distilled water. One volume of the staphylococcal emulsion was added to an equal volume of either acid, alkali or saline as the case might be, the two digested at room temperature for varying periods, then a third and equal volume of leucocyte emulsion added, and the whole incubated at 37° C. for half an hour, a shaker being employed to maintain a homogeneous distribution of the leucocytes throughout the mixture. At the end of the half hour's incubation films were made, fixed and stained in the usual way. The volumes used were capillary ones, the same capillary pipette serving for the measurement of all three volumes. For each count 100 leucocytes were taken. All slides were examined under cipher, as only in this way can subjective influences on one's counting be avoided; and by recounting slides and comparing separate counts from the same slide it was seen that the error occurring in this part of the experiment was very small. Yet it was soon apparent, as had been the case in the earlier adsorption experiments, that, in a phagocytic system not containing serum, the experimental error was greater than when phagocytosis took place in the presence of serum; a series of control experiments demonstrated that an average error of about 10 % above or below the average was to be expected, though individual counts might fall still further out of line. Therefore it could only be on larger differences in the indices that one could lay any stress, or by grouping a number of

experiments together, that it was possible to arrive at reliable conclusions.

I have been unable to explain the reason of this rather wide reach of error. The greatest care was always taken that the conditions, under which the various experiments were carried out, should be as similar as possible. It was thought that the irregularity might be due to the use of imperfectly cleansed glass ware, traces of serum still adhering to the small tubes from previous experiments, or that glass tubing had been used the walls of which gave off alkali on being drawn out into pipettes. This idea was tested by having all glass specially cleaned and by the use of Jena glass, but no improvement in the result took place.

Notwithstanding these irregular indices it was quite obvious that the tendency was for digestion of the cocci with acid to give a raised count, while digestion with alkali apparently brought about a fall, though this latter did not seem to be so pronounced.

TABLE I.

	Phagocytic index			Saline coun brought to 100		
Digestion with	1′	30′	1¾ hrs.	1′	30′	1¾ hrs.
Acetic acid *n*/50	22·55	34·81	25·36	116	202	137
Acetic acid *n*/330	18·54	18·61	37·59	96	108	203
Physiological saline	19·37	17·20	18·52	100	100	100

In Table I is given a typical experiment in which the cocci were digested for various periods with the two different strengths of acetic acid before the addition of the leucocytes to the system. A well-marked increase in phagocytosis is noticeable in those cases where the stronger acid or a longer period of digestion was given.

TABLE II.

	Phagocytic index				Saline count brought to 100			
Digestion with	1′	30′	2½ hrs.	6½ hrs.	1′	30′	2½ hrs.	6½ hrs
Ammonia *n*/50	10·90	9·12	11·61	15·32	82	60	64	84
Ammonia *n*/330	12·63	12·87	14·52	29·47	94	85	80	161
Physiological saline	13·37	15·19	18·04	18·29	100	100	100	100

In Table II is shown a corresponding experiment in which digestion with ammonia took place. As will be seen, the general effect is to lower the indices, but this lowering is not as marked as is the increase in the case of digestion with acid.

In both tables are appended columns in which the acid and alkali indices are stated relative to saline indices of 100; this with the purpose of giving a more comprehensive view of the numbers. One point has to be decided before reading the results from such experiments, especially in view of the fact that sometimes, as is seen in Table II, with an increase in the time of digestion with saline itself, a rise in the phagocytic index is observable. The point is, might not this rise possibly be due to a multiplication of the cocci during the digestion period, and the greater rise, in the case of digestion with acid, to an accelerated multiplication? However, by counting the number of cocci in the emulsions on mixing with saline, acid and alkali, and after these mixtures had stood for varying periods at room temperature up to several hours, it was seen that no appreciable increase in the numbers of the cocci had taken place and that consequently the elevation of the phagocytic index after digestion could not be due to the simple fact of there being more cocci available at a later period of digestion than at an earlier.

A striking feature of these experiments is, that an alteration in the length of digestion, or in the strength of acid or alkali used, does not result in any regular corresponding alteration in the phagocytic index. If there should be, as there well might be, differences due to these factors, they are apparently not sufficiently marked to escape masking by the irregularities to which the counts are liable. One must have recourse to the average and to judge from this, as shown in Table IV, one would conclude that the weaker acid produces the greater increase in phagocytosis and the stronger alkali the greater decrease.

TABLE III.

Digestion with	Digestion period																			
	1′	1′	1′	15′	15′	15′	30′	30′	30′	30′	30′	30′	30′	30′	1¾ h.	1¾ h.	2½ h.	5½ h.	6½ h.	6½ h.
Acetic acid *n*/50	116	—	—	271	113	272	119	202	135	89	202	44	163	261	137	30	—	351	—	—
Acetic acid *n*/330	96	—	—	240	106	818	—	108	—	153	210	34	135	179	203	29	—	195	—	—
Physiological saline	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Ammonia *n*/330	94	47	106	—	106	286	85	—	150	34	—	—	58	—	—	—	80	121	161	37
Ammonia *n*/50	82	30	136	—	147	90	60	58	59	77	—	—	59	—	—	—	64	75	84	39

Table III is a summary of all experiments, with the indices given so as to correspond in each experiment with a saline index of 100. Arranged as the indices are, in order of length of digestion, it is clearly

seen how little the amount of rise or drop appears to depend on this factor, but that notwithstanding the aberrant indices, that fall right out of line (four of the 28 indices obtained under the influence of acid and six of the 27 indices obtained under the influence of alkali give in the former case markedly lowered, in the latter case markedly raised counts), the general tendency is for counts to be raised by the addition of acid and if anything to be lowered by the addition of alkali. This is more clearly observed by taking the average of all counts and comparing them as has been done in Table IV, where it is seen that digestion with acid produces an index considerably above the saline one, while digestion with alkali one on the whole slightly below it.

TABLE IV.

Digestion with			Average phagocytic index	
Acetic acid *n*/50	...	...	167	179
Acetic acid *n*/330	...	...	193	
Physiological saline	...	...	100	
Ammonia *n*/330	...	...	105	90
Ammonia *n*/50	...	...	76	

This lowering of the phagocytic index in alkali digestion however, taking the average of all experiments, does not fall beyond the limit of experimental error; it is only when the series of indices obtained under the influence of the stronger ammonia solution (*n*/50) is grouped together that a definite retarding effect on phagocytosis is observed.

The results of these phagocytosis experiments seem clearly to point to an acceleration of the spontaneous ingestion of cocci by leucocytes when under the influence of acid, while by the addition of alkali to a spontaneous phagocytic system very little difference is occasioned unless the stronger alkali be employed, in which case phagocytosis is retarded to a certain extent.

Oker-Blom (1912) working also with a *staphyloccocus*, but employing ionic solutions somewhat stronger (H_2SO_4 and NaOH in strengths of *n*/200 to *n*/1000), at least so far as those experiments are concerned which could approximately be compared with mine, has apparently obtained similar results, although he interprets them differently.

Table V gives a summary of some 33 indices obtained by Oker-Blom under the influence of H_2SO_4 and 36 indices obtained under the influence of NaOH, which were the result of experiments carried out much on the same lines as my own and therefore capable of comparison. The other experiments of Oker-Blom were carried out under methods so

different from my own as to preclude them from all comparison, *e.g.* the use of H_2SO_4 and NaOH in strengths of $n/40$ and $n/20$; the neutralization of the acid or alkali used for digestion before the addition of the leucocytes; digestion of leucocytes or both leucocytes and cocci with acid and alkali.

TABLE V. (*From Oker-Blom.*)

Digestion with	Average phagocytic index	
H_2SO_4 $n/200$	186	
H_2SO_4 $n/400$	183	185
H_2SO_4 $n/1000$	186	
Physiological saline	100	
NaOH $n/1000$	114	
NaOH $n/400$	115	112
NaOH $n/200$	107	

There was one difference, and that in counting, between Oker-Blom's methods and my own. Oker-Blom counted what he called the "verankerte" cocci, which, as he explained in a private communication, were all cocci found in contact with the leucocytes, *i.e.* not only the ingested ones, but also those which were merely attached to the periphery of the leucocytes. Such "attached" cocci were not frequent in my slides, and to me it seems a matter of considerable difficulty to decide which cocci are attached and which are merely in juxtaposition to the leucocytes. It is very interesting that with two such dissimilar methods of counting, results comparable to the degree that they are should have been obtained.

Although Oker-Blom decides that the indices obtained under the influence of NaOH may be looked upon as showing an accelerating effect of the alkali on phagocytosis, my experience would lead me to believe that the average alkali count as given in Table V still lay within the zone of experimental error, and that the inference to be drawn was, that while acid had the effect of markedly increasing phagocytosis, alkali on the other hand had very little or no influence.

To account for an accelerating effect on the part of both acid and alkali Oker-Blom argued that as bacteria were notoriously negatively charged and as leucocytes judging by the acidophilic staining reaction of their protoplasm was obviously positively charged, there existed from the beginning a mutual attraction and one would only have to explain how by digestion with either acid or alkali this attraction was heightened. This he attempted to do as follows, supposing bacteria and leucocytes to be affected by the acid and alkali in three phases.

(1) On mixing bacteria and acid, bacteria enter *First Phase* and have a lessened negative charge.

(2) At end of digestion of bacteria and acid bacteria are in the *Second Phase*, there being a state of balance between the two; the bacteria have a neutral charge.

(3) On adding leucocytes suspended in physiological saline and thus lowering the acidity of the mixture, the bacteria enter the *Third Phase* and show a negative charge greater than the initial one, while the leucocytes suddenly finding themselves in an acid environment take on a stronger positive charge than they possessed before (*First Phase* for the leucocytes).

In the same way, apparently, when digesting bacteria with alkali, the bacteria end up with a positive charge and the leucocytes with a negative one. In both cases it is assumed that the mutual attraction between the bacteria and the leucocytes has been increased.

To test this theory of Oker-Blom, it was necessary to determine experimentally the charge possessed by both bacteria and leucocytes in neutral solutions and under the influence of acid and alkali. The attempt to demonstrate the convection of leucocytes in an electric field by the U-tube method had to be abandoned; the leucocytes proved too heavy, and sedimented before any cataphoresis could be observed. Subsequently, the glass slide method was employed for both bacteria and leucocytes, and proved very satisfactory. An ordinary glass slide has cemented to it at either end a broad, flat platinum electrode; a few drops of the suspension to be tested are placed between the electrodes and covered by a cover slip which rests on the two electrodes, thus forming a shallow cell some ·2 to ·3 mm. in depth, in which by the aid of a dark-ground illumination microscope cataphoresis may be observed. The electrodes were connected with the poles of a battery possessing a charge of about seven volts, a switch being interposed to allow of the current being quickly and conveniently closed, opened or reversed. As the formation of any gas at the electrodes would be fatal to a correct observation, it is impossible to carry out the experiments in physiological saline solution. For the suspension of bacteria, distilled water can of course be used, but for leucocytes, at any rate for longer observations, it is necessary to suspend in an isotonic solution. In these experiments 8·8 % cane sugar in distilled water was taken. Acid and alkali solutions similar in strength to those used in the phagocytosis experiments were prepared, in the one case distilled water, in the other isotonic sugar solution being the diluent.

Suspended in a neutral solution, the staphylococci showed a marked negative charge and this charge was not altered in the presence of either $n/1000$ or $n/100$ acetic acid, even after they had been in contact as long as 24 hours. Indeed in no way could the cocci be induced to take on a positive charge under the influence of the acid, unless there was present in the solution an amphoteric electrolyte, *e.g.* the haemoglobin that had laked out of red corpuscles—when, apparently adsorption of protein on the cocci took place. As the protein coat took on a positive charge in response to the acid, the whole coccus moved towards the negative pole.

Leucocytes, obtained either in the form of pus cells from urine or from the peritoneal cavity of the guinea-pig, in response to an injection of broth, or from citrated blood, and washed and suspended in isotonic sugar solution, show a decided negative charge, moving, on the current being closed, smartly towards the anode.

Leucocytes in the presence of red cells assume under the influence of even $n/1000$ acetic acid a positive charge, but this occurs for the same reason as that advanced for the giving of a positive charge to cocci —viz. that they are coated with adsorbed protein.

Michaelis and Takahashi (1910) have shown that by increasing the hydrogen-ions in a red corpuscle suspension beyond 1×10^{-5}, the red cells are laked. Acetic acid $n/1000$ represents $[H^+] = 0{\cdot}13 \times 10^{-3}$. When blood corpuscles are suspended in $n/1000$ acetic acid, the red cells lose haemoglobin which, by coating the leucocytes, gives them a positive charge. How the leucocytes would behave without red cells present to yield haemoglobin does not concern one here, as red corpuscles are always present in phagocytic systems such as those under consideration. As was to be expected, both cocci and leucocytes retain their negative charge in the presence of alkali, ammonia up to $n/100$ being used. On bringing a mixture of cocci and blood corpuscles, all previously showing a negative charge by moving in an electric field towards the anode, to $n/1000$ with acetic acid, the charge of both the cocci and the blood corpuscles becomes a positive one, migration taking place towards the kathode. The addition of ammonia in the place of the acid leaves the bodies negative as they were before.

The facts gained from these observations would seem to point to the following explanation of the results obtained in the experiments on spontaneous phagocytosis.

If the fact of bacteria and leucocytes possessing an electric charge of their own is going to play a part in phagocytosis, and explain or help

to explain why it is that on the spontaneous phagocytosis of staphylococci acid exerts an accelerating influence and alkali either none at all or a retarding one, it is obviously because both cocci and leucocytes possess, in the neutral control experiments, a negative charge which is either unaltered or somewhat increased by alkali, and decreased or altered to positive by acid.

With alkali present one would thus suppose phagocytosis, *i.e.* the coming together of coccus and leucocyte, to be confronted with difficulties similar to, if not greater than, those met with in the control experiments. The coccus and the leucocyte charged with electricity of the same sign, to the same or perhaps a higher degree than in a neutral solution, will tend to keep apart to the same or even greater extent, *i.e.* phagocytosis will be the same as in the control experiments or will be retarded.

With acid present, unless the electric charge of the cocci and the leucocytes is carried as far on the positive side as it is on the negative side in a neutral solution, the forces tending to keep the two apart must be less, *i.e.* phagocytosis will be accelerated.

Before applying this hypothesis to the phagocytic experiments recorded earlier in this paper, it is necessary to examine the leucocytes and cocci in the actual phagocytic system, *i.e.* using the same thick emulsions as are used in spontaneous phagocytosis. It is not to be expected that such marked changes of charge will be observable, seeing that the absorption of acid and alkali by the large number of corpuscles and cocci present, will have lowered the hydrogen-ion concentration of the mixture very considerably.

The usual thick staphylococcus emulsion and washed corpuscles and acid and alkali solutions were then taken, the only difference being that in every case isotonic sugar solution was used instead of physiological saline in order to avoid disturbance in the electric cell. Equal volumes of staphylococcal emulsion and acetic acid $n/50$ and $n/300$, and ammonia $n/50$ and $n/300$, and isotonic sugar solution (this in place of the usual physiological saline control) were digested for half an hour at room temperature, then third and equal volumes of corpuscles added and the whole incubated in the shaker at 37° C. for half an hour. At the end of this time the various mixtures were examined for cataphoresis in the electric cell, and as they were much too thick for direct examination, a preliminary centrifuging to sediment the corpuscles and most of the cocci was necessary.

Taking the supernatant and a few of the corpuscles it was possible to observe the charge possessed by the leucocytes and by the cocci.

In all cases however, whether phagocytosis had taken place under the influence of acid or alkali or neither, the cocci and corpuscles moved towards the positive pole, nor did the rate at which they moved appear to differ to any extent. There was therefore no proof here that the electric condition of the cocci and leucocytes had been greatly altered by the addition of acid or alkali to the system. The hydrogen-ion content probably differs so little in these final mixtures that a difference in charge cannot be demonstrated by cataphoresis in the electric cell. Yet from this one may not conclude that no alteration at all in the amount of negative charge possessed by leucocytes and cocci has taken place. Any change in charge brought about by either acid or alkali would necessarily be a very much smaller one and so very likely to escape detection in the rather rough estimation possible in the electric cell. What the charge of a particle may be is easily decided, but whether it is a little more or a little less negatively charged is a difficult question to decide.

To determine exactly what the hydrogen-ion concentration of these phagocytic systems is, thick emulsions of blood corpuscles and cocci were prepared and acid, alkali or saline added as was done in the experiments detailed earlier in the paper. In these mixtures estimations were carried out by the gas-chain method and it was apparent to what a very considerable extent both acid and alkali had been absorbed. In every case the mixture was on the alkaline side of the neutral point. The addition of acid, even in the strength of $n/50$, did not suffice to give an acid reaction. With a lowering of the amount of alkali added or an increase in the amount of acid a rise in the hydrogen-ion concentration took place but the range of alteration was very small, the hydrogen-ion concentration being in the phagocytic system to which $n/50$ ammonia had been added $10^{-7\cdot72}$ normal and in that to which $n/50$ acetic acid had been added $10^{-7\cdot21}$ normal.

In thick emulsions of corpuscles and cocci as used in spontaneous phagocytosis there is always a certain amount of protein present in solution and, judging by those cataphoresis experiments where the hydrogen-ion content was greater, it is to be concluded that the negative charge of the cocci and leucocytes has sunk with the rise in the hydrogen-ion concentration of the mixture, although not to a sufficient extent to be noticeable in a cataphoresis experiment, and indeed the small extent to which the hydrogen-ions are increased or decreased by the addition of acid or alkali, would not lead one to expect a greatly altered charge.

It is to be supposed that in a phagocytic system alterations of charge differing in degree, though similar in character to those demonstrable when the proportion of acid to cocci and leucocytes in the emulsion is greater, have occurred, and that the lowering of the negative charge of cocci and leucocytes by acid may help to explain the acceleration of phagocytosis observed to have been caused by acetic acid, while the addition of alkali to a phagocytic system may retard phagocytosis by increasing the negative charge of the cocci and leucocytes.

CONCLUSIONS.

(1) Spontaneous phagocytosis appears, like normal phagocytosis occurring in the presence of serum, to be a process of an adsorptive nature, but owing to the wide range of experimental error this is not capable of exact proof as is the case with phagocytosis in the presence of serum.

(2) The spontaneous phagocytosis of staphylococci taking place on the addition of acid (acetic acid $n/50$ to $n/330$) undergoes marked acceleration, the average count for all experiments thus carried out being about 80 % higher than when no acid was present.

(3) Alkali (ammonia $n/50$ to $n/330$) affects spontaneous phagocytosis in weaker solutions scarcely at all; in stronger dilutions ($n/50$) it retards the ingestion of the cocci, the experiments performed giving an average count about 25 % lower when the stronger alkali was used than when alkali was absent.

(4) In neutral and alkaline suspensions both cocci and leucocytes possess a negative electric charge.

(5) In acid suspensions with haemoglobin or other amphoteric electrolyte present in solution cocci and leucocytes take on a positive charge, this being apparently due not to a direct effect of hydrogen-ions on the cocci and leucocytes, but to their action on the coat of protein these bodies had obtained by adsorption of the haemoglobin in solution.

(6) In a phagocytic system a certain amount of protein is free in solution, and it is suggested that, on the addition of acid, the cocci and leucocytes, owing to their possessing adsorbed protein coats, lose a certain amount of their negative charge, while on the addition of alkali the charge is somewhat raised, the extent to which this occurs however being small, as the addition of even $n/50$ acetic acid or ammonia to a phagocytic system results in only a small alteration in the $[H^+]$ concentration (the major portion of the acid and alkali being absorbed by

the protein of the corpuscles and cocci). This alteration of charge would explain why acid, by causing a decrease in the forces tending to keep the cocci and leucocytes apart, raises the phagocytic index, while alkali, by bringing about an increase in these forces, lowers the phagocytic index.

(7) Experiments carried out with the actual emulsions of cocci and leucocytes similar to those used in spontaneous phagocytosis have failed to show a decrease of negative charge in the cocci and leucocytes on addition of acetic acid up to $n/50$ or an increase under the influence of ammonia. It is possible that the alterations in charge in these cases are too small to be observable in a cataphoresis experiment and yet sufficiently large to influence phagocytosis.

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