Further Electro-biological Studies.

By

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1. Red Blood Corpuscles.

Concerning the electric conductivity and the alkalinity of the laked erythrocytes there are some differences among several animals. From the defibrinated blood of each animal a mass of the blood corpuscles was separated by means of a centrifuge and weighed. Having dissolved one gram of it in 10 cc. of distilled water (conductivity = $1{,}8 \times 10^{-6}$) the conductivity was measured by the KOHLRAUSCH method, and the pH electrometrically. The following table gives the result:

	Conductivity	pH
Rabbit	$9{,}31 \times 10^{-4}$	7,57
Cow	$8{,}14 \times 10^{-4}$	7,51
Pig	$9{,}14 \times 10^{-4}$	7,30
Goat	$8{,}76 \times 10^{-4}$	7,23
Dog	$7{,}89 \times 10^{-4}$	7,16
Cat	$7{,}94 \times 10^{-4}$	7,09

From this it will be seen that the conductivity and pH of the solution are the greatest in the rabbit and the least in the dog and cat. This result

coincides perfectly with our previous finding,[1] that the efficiency of the erythrocytes to neutralize an acid solution is the greatest in the rabbit and the least in the dog.

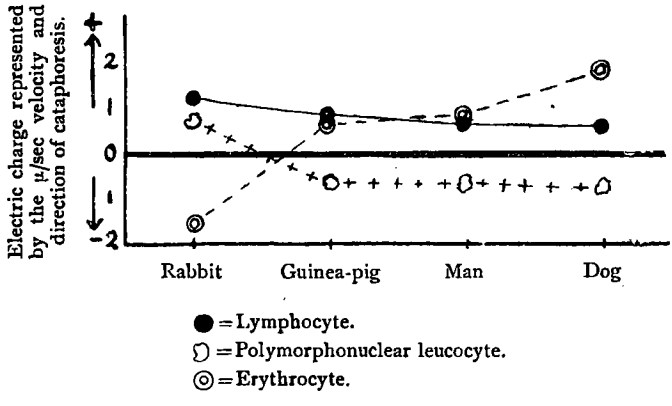
2. Leucocytes.

In our previous paper[2] it is stated that strongly negatively charged leucocytes extrude pseudopodia towards the cathode in a pronounced way, when a continuous current is sent through the fluid in which the leucocytes are still alive, while positively charged leucocytes protrude pseudopodia on the anodic side, and further, that the leucocytes of the rabbit and frog become remarkably negatively charged by the addition of alkali and throw out pseudopodia pronouncedly in the cathodic direction. Not only with regard to leucocytes of several animal species were these facts confirmed by further experiments, but just the same thing proved to be true in amoeba limax which reacted in an obvious manner. If an alkali solution is added to the amoeba culture while sending a continuous current through the culture the protrusion of pseudopodia on the cathodic side becomes soon strikingly evident. Even amoebae which are on the point of death because of the alkali react very significantly in this way. On the contrary, however, the addition of a little acid makes the direction of pseudopodia irregular. All these facts must be ascribed to a self-defensive action of the living cells. Hence, locomotive cells which are strongly negatively charged avoid generally the anode whence positive ions are coming and proceed or turn to the cathode, since the more negatively charged the cell body is, the more liable it is to be attacked by positive ions, a view which we expressed in detail in a previous essay[3] concerning the galvanotropism of ciliata.

1. Electric Charges of the red Blood Corpuscles. Okayama-Igakkwai Zasshi, No. 372, 1921.
2. Further Investigation on the Galvanotaxis and Cataphoresis (Japanese). Okayama-Igakkwai Zasshi, No. 357, 1919.
3. A Contribution to the Galvanotaxis (Japanese). Okayama-Igakkwai Zasshi, No. 345, 1918.

In order to examine the cataphoresis of leucocytes fresh blood was mixed with 1 or 1,5% sodium citrate solution made up in 0,9% NaCl solution and a mass of the blood corpuscles was separated by a centrifuge and washed with 0,9% NaCl solution. From this mass a white upper layer was taken and mixed with 0,9% NaCl solution in a quadrilateral cell upon a slide whose two opposite borders consisted of non-enameled porcelain pieces.[1] By putting a non-polarisable pencil electrode upon each of the non-enameled porcelain pieces a continuous current of 100 volts and 9 ma. was sent through the liquid in the cell, and the movement of the leucocytes was examined under the microscope which was always adjusted to a fluid layer 10 μ above the bottom.

Fig. 1.



● = Lymphocyte.
ℌ = Polymorphonuclear leucocyte.
◎ = Erythrocyte.

It was found that the polymorphonuclear leucocytes of the rabbit proceeded to the anode at a rate of 0,8 μ per second, while those of the man, guinea-pig and dog moved towards the cathode, the velocity of the former two being 0,7 μ and that of the dog leucocytes 0,8 μ per second. The lymphocytes of all the animals however went without exception to the anode, the distances which they passed per second being in the rabbit 1,2 μ, in the guinea-pig 1,0 μ, in the man 0,9 μ and in the dog 0,7 μ. Hence it will be seen that

1. Compare our previous paper "Electric Charges of the Red Blood Corpuscles."

in the rabbit the polymorphonuclear leucocytes as well as the lymphocytes are most negatively charged, while in the dog the former are most positively and the latter least negatively charged, so that in each animal there seems to exist some proportionality between both cells, as regards their electrical charges.

In Fig. 1 each curve shows the difference among the rabbit, guinea-pig, man and dog concerning the electric charges of the erythrocytes, lymphocytes or polymorphonuclear leucocytes respectively, the ordinates being the charges represented by the direction and speed of their cataphoresis.

3. Staphylococcus pyogenes aureus.

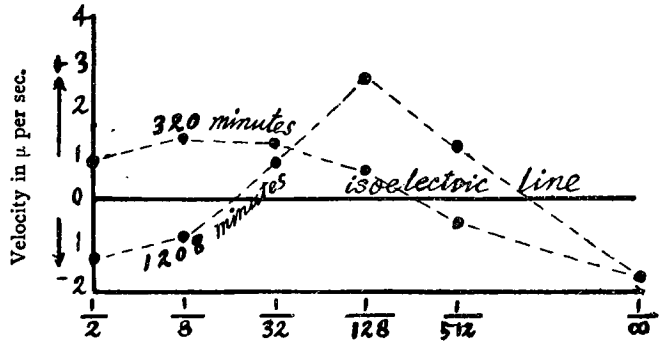
CENOVODEANU and HENRI[1] as well as E. PUTTER[2] report that the staphylococcus aureus is electronegatively charged, and in 5,5% grape sugar solution we too perceived, that it proceeded obviously to the anode without exception. In an electrolyte such as serum, 0,9% NaCl or RINGER solution we found however that the electric charge of the bacteria fluctuated according to the strain, and even the bacteria belonging to the same strain showed somewhat different electric charge according to the quality of cultur-medium and the number of the generation. But in majority of our cases the staphylococcus aureus had the same or a little more positive charge than the rabbit erythrocytes which we used always intermixed with bacteria in order to estimate the electric charge of the latter. Therefore we put forward the following statement, assuming that the staphylococcus aureus has a slight positive charge in an electrolyte solution, as was indeed the case in our experiments.

The staphylococcus aureus in the sugar solution loses its negative charge to a greater or a less degree by the addition of an electrolyte such as NaCl solution, peritoneal exudate or serum of the rabbit and gets finally a slight

1. Cit. after PUTTER.
2. Zeitschrift f. Immunitätsforsch. Org. Bd. 32, 1921, Heft 6.

positive charge, if the quantity of the added fluid is large enough. In this case it proceeds more swiftly to the cathode than the polymorphonuclear leucocytes of the dog, guinea-pig and man. The following treatment makes electric charge of the staphylococcus aureus change in a peculiar way. After the addition of the rabbit serum to the physiological salt solution containing the bacteria the mixture is left at a temperature of 20° to 37°C for 2—22 hours and then examined. As long as the concentration is not too

Fig. 2.



Effect of solutions of the rubbit serum upon the electric charge of the staphylococcus pyogenes aureus. Abscissae represent the concentration of serum added to the physiological salt solution. Ths fractional numbers are the reciprocals of serum dilutions, $\frac{1}{\infty}$ being the pure physiological salt solution containing no serum. Ordinates are the charges of the bacteria represented by the speed and direction of cataphoresis.

great the serum solution acts so as to diminish the positive charge of the bacteria, and the thicker it is the more it is so, until it gives them a maximal negative charge under certain concentration which must be considered as a turning point of the electric action of serum solution. Meanwhile there occurs a phenomenon of agglutination.* Beyond the concentration of the turning point, which is yet very different according to the duration of the serum

* This phenomenon occurs especially when the electric charge of the bacteria becomes neutral. This facts is in accordance with the reports of BECHHOLD (Colloids in Biology and Medicine. p. 205), NEISSER and FRIEDEMAN (Munchener Med. Wochenschr, 1904, No. 19), who state that the bacteria lose their charge on account of the agglutinin and precipitate between the electrodes.

action, the temperature and the strain of the bacteria, the serum solution acts to lessen the negative charge of the bacteria (Fig. 2), so that the staphylococcus aureus is in a mixture containing very much serum electrically neutral or positively charged. To give only an example, we added the rabbit serum to the 0,9% NaCl solution containing the staphylococcus aureus at the rate of 32 times dilution and allowed it to stand for 10 hours and 40 minutes at a temperature of 30°C, whereby the microorganism became negatively charged. Then it was mixed with an equal volume of a serum salt solution containing the serum in a proportion of one fourth and the mixture was allowed to stand further at a temperature of 30°C, in order to examine the electric charge of the bacteria at intervals of several minutes. As early as after 5 minutes there occurred a decrease of the negative charge and after 20 minutes the microorganism got a slight positive charge which became stronger after the lapse of further 60 minutes.

Within 2 hours the effect of the serum is not sufficiently manifested, so that only a concentrated serum mixture lessens the positive charge of the staphylococcus aureus or turns it to a negative one, the dilute mixture having no influence upon it (Chart I, Fig. 3). So in this case there is observed no turning point of the electric action of serum solution, and when the latter acts only for a very short time (say 5 minutes), the electric charge of the bacteria shows no alteration whatever.

The result of the above mentioned experiments may be modified, if a lower or higher temperature is used. The staphylococcus serum mixture preserved in an ice-box reveals little or no alteration concerning the electric charge of the bacteria, their positive charge being decreased only when the mixture is rich in serum and permitted to stand over circa 20 minutes. On the other hand the high temperature seems to act against the positive charge of the staphylococcus aureus. So the microorganisms preserved in the pure physiological salt solution without addition of serum at a temperature of 50°C lose their positive charge more and more in course of time and become finally negatively charged, which is however not the case at a temperature lower

than 37°C. Nevertheless there is no essential difference between a temperature of 50°C and that of 20° to 37°C concerning the effect of serum upon the electric charge of the staphylococcus aureus, only that at a temperature of 50°C the effect is generally more pronounced and appears quickly, if a strong serum solution is used. Comparing the effect of the normal rabbit serum solution with that of the heated one in which the complement is destroyed we find that the latter is a little stronger in reducing the positive charge of the staphylococcus aureus or giving it a negative charge, especially when the concentration of the serum solution is not too low (Chart I, Fig. 3). From this fact it may be inferred that the complement acts upon the staphylococcus as a contributor of positive electricity.

With regard to solutions of the immune rabbit serum it may be stated that they act upon the electric charge of the staphylococcus aureus generally in the same manner as those of the normal one. That is to say their effects do not develope completely in a short time (say within 80 minutes), and if they act long enough (say for 320 minutes) there is seen a turning point of their electric action under certain concentration which gives to the microorganisms the maximal negative charge. Beyond the concentration of the turning point of the electric action the solution of the immune serum acts upon the bacteria as a contributor of positive electricity, which action is far more powerful than that of the normal one, so that the microorganisms become markedly positively charged in a thick solution of the immune serum.

The action of the heated immune serum differs so far from that of the not heated one, in that the former is a little weaker than the latter in giving positive electricity to the microorganisms, and the concentrations of its solutions where the turning point of their electric action is met with are in general slightly higher than those of the not heated serum.

The serum of the guinea-pig which is far more insensible to the staphylococccus aureus than the rabbit has a very weak power of diminishing the positive charge of the organisms, when it is used as a weak solution. On the other hand its strong solution reveals a strong effect of promoting the

charge. Especially when the duration of its action is insufficient (say less than an hour) the positive charge of the staphylococcus aureus does not diminish at all however thin the concentration of the solution may be. On the contrary the electrical charge shows even in this case a slight increase by virtue of a thick solution (Chart I, Fig. 5). If the serum solution acts long enough there is seen a turning point of their electric action in certain concentration where the positive charge of the microorganisms reveals maximum diminution, being almost neutralized in the extreme case. Beyond the concentration of this point the serum solution acts upon the staphylococcus aureus as a giver of positive electricity which action becomes much pronounced with the ascending concentration of the solution, especially when the mixture is left for a long time, so that the long continued action of a thick serum solution is most powerful in promoting the positive charge of the staphylococcus aureus. As to the heated guinea-pig serum almost the same thing may be said of its solutions as in the case of the not heated one except that its thick solution does not raise the positive charge of the staphylococcus aureus so markedly as that of the latter, sometimes rather reducing it a little, if the duration of action is insufficient (Chart I, Fig. 5).

With regard to the sera of the dog and goat which were used to act upon the staphylococcus aureus always a half hour at a temperature of 30°C we found, that their thin solutions were electrically ineffectual, while concentrated raised positive charge of the microorganisms more or less, the serum of the dog being far more powerful than the goat serum in this respect. In the cases where the heated sera were used the concentrated solution of the goat serum lessened the charge of the staphylococcus slightly, whereas that of the dog raised it a little (Chart I, Fig. 4 & 6).

4. Staphylococcus pyogenes albus.

In opposition to the staphylococcus p. aureus the staphylococcus p. albus possesses a strong negative charge in 0,9% NaCl solution and moves very swiftly toward the anode, but the addition of a serum salt solution makes it less negatively or rather positively charged without causing any agglutination of the microorganisms, if the solution is allowed to act for 30 minutes* at a temperature of circa 24°C, and this change is the greater the more serum that has been added. So far the rabbit, goat, guinea-pig or dog serum behaves in the same manner, but each serum has a different power of giving the positive electricity to the microorganisms, so that the dog serum acts most effectively in this respect and next to it the guinea-pig serum, the rabbit serum being most feeble in efficacy (Chart I, Fig. 7—10). The same thing holds good for the heated serum too, but a comparison with the not heated serum shows, that the effect of the heated serum is generally a little weaker than that of the not heated (Chart I, Fig. 7—10). The latter fact furnishes again a proof, that the complement acts upon the microorganisms as a contributor of positive electricity. In opposition to the staphylococci the erythrocytes and leucocytes of the rabbit etc. preserve the same electric charge after addition of the serum taken from the same animal as in the pure physiological salt solution.

5. Immune serum as an effective contributor of positive electricity.

Concerning the rabbit serum we have already stated, that the immune serum solution acts upon the staphylococcus aureus more effectively than the normal serum solution as a bestower of positive electricity, if we use a thicker solution beyond the concentration of the turning point of the electric action.

* The action of a longer duration does not cause a further essential change.

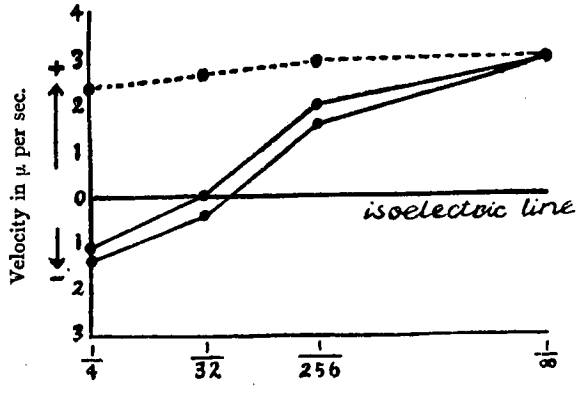
This change of serum shows its first step in a rabbit which has survived 48 hours after the first injection of the heated staphylococcus aureus and becomes more and more remarkable in the course of time until the animal is subjected to the second injection on the 7th day after the first treatment. Within 24 hours after the second injection however the action of the serum reveals electrically a slight decline in accordance with the negative phase, but later again a raising which corresponds to the positive phase. This second promotion of the serum action reaches somewhat higher than the first one. The same thing holds true after the third injection. We ascertained these facts not only in the staphylococcus aureus but in the staphylococcus albus which, having a strong negative charge in 0,9% NaCl solution, becomes less negatively charged even after the addition of a thin serum solution. In experiments concerning these matters the bacteria were allowed to stand under the serum action for 3 hours at a temperature of 20°C.

Besides the above-mentioned the strong electric action of immune serum was testified to, in a very obvious manner, of late by an examination of the bac. prodigiosis. This bacillus possesses a strong negative charge in the physiological salt solution and this charge is scarcely affected by the addition of the normal rabbit serum. On the other hand the serum of a rabbit subjected thrice to the injection of the bacteria at intervals of a week exerts a strong influence on the electric charge of the bacteria, its thin solution diminishing the negative charge greatly and a thick solution giving a positive charge to the microorganisms. In Fig. 11 a comparison of the normal and immune rabbit serum is given with regard to their influence over the electric charge of the bac. prodigiosis. This result is taken from an experiment in which every solution of both sera acted upon the microorganisms for 4 hours at a temperature of 20°C.

These facts as well as the fact that some haemolytic serum diminishes the negative charge of erythrocytes to a remarkable degree* lead us to the

* See our previous paper "Electric Charges of the Red Blood Corpuscles."

Fig. 11.



Effects of solutions of the normal and immune rabbit serum upon the electric charge of the bac. prodigiosis. The dotted line represents the effect of the normal serum solutions and the continuous lines that of the immune serum solutions.

following conclusion. Generally when an antibody is developed against an antigen and this antibody unites with another antigen of the same kind, there must occur a considerable change in the electric charge of the antigen, be it a bacterium or other particle. Perhaps this electric change, which can be observed by means of the microscopical examination of cataphoresis, will find its use in the diagnosis of several diseases.*

6. Phagocytosis.

The examination was made of the staphylococcus pyogenes aureus as well as the staphylococcus pyogenes albus with a special reference to the

* M. SEKI has found of late that this application is quite available in the diagnosis of syphilis. The serum of a luetic patient has a strong power in diminishing the negative charge of particles, which are found in the alcoholic extract emulsoid of the guinea-pig heart or in the lecithin emulsoid, while this is not the case with the normal human serum. By experimenting upon this fact he has obtained a result which coincides with that from the WASSERMANN reaction. This will be explained in detail in a later publication.

electric charge of the microorganisms. A serum salt solution was added to a salt solution containing the staphylococcus, and it was allowed to stand for 30 minutes at a temperature of 24°C (for the st. p. albus) or 30°C (for the st. p. aureus) and then was mixed with a definite quantity of a leucocyte emulsion prepared from the blood of the animal from which the serum was taken. The mixture was allowed to stand further for 15 minutes at a temperature of 30°C. Cover-glass films fixed with methyl alcohol were stained by GIEMSA's solution. The above-mentioned manipulation was many times repeated, using serum solution of different concentrations.

As to the staphylococcus albus, phagocytosis begins when the electric potential of the microorganisms becomes more or less equal to that of the leucocytes by the serum action, so that in the case of the rabbit, whose leucocytes are negatively charged, some few of the still negatively charged microorganisms may be seen adhering to the leucocytes, while in the case of the guinea-pig and dog, both of which have positive charged leucocytes the process appears only when the charge of the microorganisms becomes clearly positive. Generally speaking the average number of the microorganisms taken up by the leucocytes increases hand in hand with their positive charge, i. e. in proportion to the concentration of the serum.

In the case of the staphylococcus aureus too, the number of the microorganisms adhering to leucocytes increased with the concentration of serum solution, but here the bioelectrical relation was somewhat irregular. While in the case of the guinea-pig the degree of phagocytosis increased with the positive charge of the microorganisms, as in the case of the staphylococcus albus, the very reverse happened in the case of the rabbit, where the positive charge of the microorganisms decreased more and more with the ascending concentration of serum solution, on account of the insufficient duration of serum action. The latter fact is due to an unknown factor in serum action apart from the electric factor. The result of an experiment in which we used solutions of the heated rabbit serum instead of the active solutions demonstrated this plainly. The positive charge of the staphylococcus aureus showed

in this case generally a remarkable decline, so that the micro-organisms became negatively charged by virtue of a thick serum solution, yet there was seen no phagocytosis.

According to our conjecture it is very probable, that an electric change such as to diminish the negative charge of microorganisms or to increase their positive charge would prepare a favourable condition for the process of phagocytosis. Beside the abovementioned facts this idea is supported by results of the following two experiments.

Experiment A.

Sera taken from the rabbit, goat, guinea-pig and dog respectively were 16 times diluted by the physiological salt solution, and each solution was mixed with a quantity of the staphylococcus aureus all in the same ratio and was allowed to stand for 30 minutes at a temperature of 24°C. Then to each mixture half a volume of the emulsion of the guinea-pig leucocytes was added and it was allowed to stand further for 15 minutes at a temperature of 30°C. Cover-glass films fixed with methyl alcohol were stained by GIEMSA's solution. The average number of the microorganisms taken by a leucocyte was as follows.

In the case of the rabbit serum	0,15
„ „ „ „ „ goat „	0,25
„ „ „ „ „ guinea-pig „	1,15
„ „ „ „ „ dog „	0,45

The extraordinary number in the case of the guinea-pig serum is easily understood in consideration of the fact, that here the leucocytes and serum having been taken from the same animal the condition for phagocytosis was most favourable. Comparing the other 3 cases it is seen, that the dog serum acts most effectively and the rabbit serum least, in other words the most favourable condition is afforded to phagocytosis by the serum which acts most strongly against the negative charge of the microorganisms, and vice versa, if we exclude the case where the serum and leucocytes have been taken from the same animal.

Experiment B.

In this experiment the erythrocytes of different animals were used instead of the staphylococci in order to know if there exsists any relation between their electric charges and phagocytosis. The leucocytes taken from the abdominal cavity of the rabbit or guinea-pig to which 0,4% NaCl plus 2,5% grape sugar solution had been injected 4 hours previously were received in 0,9% NaCl + 1,0% sodium citrate solution and then washed twice with 0,9% NaCl solution. So treated leucocytes showed wanton movement of pseudopodia at a room temperature of circa 30°C.* Likewise each blood sample was received first of all in 0,9% NaCl + 1,0% sodium citrate solution and centrifuged in order to separate erythrocytes which were then washed twice with 0,9% NaCl solution to free them from serum. The leucocytes and erythrocytes emulsion were mixed together in a definite ratio which was exactly the same in all cases belonging to one trial. After 10, 20 and 40 minutes the mixture was poured on a slide and left for a further 15 minutes, meanwhile the blood corpuscles sank into the bottom. Then the supernatant liquid was drained away, and when the slide became dried a little it was exposed to vapour of osmium to be followed by GIEMSA's staining. From among 100 leucocytes in every case those holding erythrocytes were counted, which numbers permit a comparison of phagocytosis in cases belonging to the same trial. In the following two tables all these numbers are given.

The third trial shows a greater number of the leucocytes adhering to erythrocytes owing to the fact, that the blood corpuscles mixture was in this trial much more crowed with erythrocytes than in the other two. In cases where the goat erythrocytes were used the leucocytes holding erythrocytes are relatively numerous, since the goat erythrocytes having a small dimension may occupy the same space in greater number than other erythrocytes and offer a greater chance of collision with leucocytes. On the other hand the

* Here it may be noted that according to LEVADITI & MUTERMILCH (Compt. Rend. Soc. Biol. 1910), FRIEDMANN, ULRICH & SCHOENFED (Bioch. Zeitshr. Bd. 80, 1917) etc. the process of phagocytosis is independent from the vitality of leucocytes at least on its first step.

	First trial				Second trial			
	Rabbit leucocytes		Guinea-pig leucocytes		Rabbit leucocytes		Guinea-pig leucocytes	
Time in minutes that the mixture of the leucocytes and erythrocytes stand.	20	40	20	40	20	40	20	40
Rabbit erythrocytes	5	7	14	12	6	8	19	22
Cow „	17	18	8	14	23	24	20	24
Pig „	6	9	4	5	No examination			
Guinea-pig „	4	5	1	2	10	12	5	7
Human „	4	4	3	2	8	8	9	10
Goat „	No examination				10	9	12	14
Dog „	2	2	2	1	4	3	3	3

	Third trial					
	Rabbit leucocytes			Guinea-pig leucocytes		
Time in minutes that the mixture of the leucocytes and erythrocytes stand.	10	20	40	10	20	40
Rabbit erythrocytes	12	13	13	32	34	40
Guinea-pig „	15	17	18	13	14	11
Goat „	20	23	25	24	23	28

degree of phagocytosis is relatively insignificant in cases where the erythrocytes and leucocytes were taken from the same animal, evidently because the erythrocytes played here a part as physiological elements rather than as foreign corpuscles. Making allowance for these special cases we can say that the less negatively charged erythrocytes are generally more liable to be taken up by the leucocytes. In the above tables the species of erythrocytes is arrangedin order according to their electric potential,[1] beginning with the rabbit erythrocytes which have a slight positive charge unlike the other erythrocytes.

1. Compare our previous essay "Electric Charges of the Red Blood Corpuscles."

FENN[1] found that carbon was taken up by leucocytes more rapidly than quartz, but could not confirm the premise that quartz should carry a higher negative charge than carbon, although the results of WHITNEY and BLAKE, RONA and MICHAELIS as well as RONA and GYORGY speak in favour of this proposition. By using calomel and non-polarisable pencil electrodes* we examined carefully the cataphoresis of carbon, quartz, common glass and marble particles and ascertained that carbon had less negative charge than the others. This fact combined with the finding of FENN seems to afford another proof of the statement that the less negatively charged particles are generally the more easily taken up by leucocytes.

7. Influence of acid and alkali upon phagocytosis.

OKER-BLOM[2] states that the staphylococcus aureus which has previously been treated with $n/1000$ to $n/200$ H_2SO_4 solutions made up in 0,85% NaCl solution is more easily taken up by the guinea-pig leucocytes than that treated previously with the pure physiological salt solution, unless many bacteria escape phagocytosis on account of agglutination which takes place when the concentration of the acid solution is too high or its action continues too long. With regard to the action of NaOH he got various result, but generally speaking, it promotes phagocytosis in a less degree than the acid, sometimes even injuring the process. The neutralization of acid or alkali solution containing staphylococcus just before the mixing with the leucocytes fluid greatly increases phagocytosis, which is likewise accelerated remarkably in the case where the leucocytes have previously been treated with alkali and the bac-

1. Journal of General Physiology. Vol. III, No. 4 & 5.

* There is a slight difference in cataphoresis according to whether we use the non-polarisable pencil electrodes or the calomel electrodes. A curve concerning the μ/sec velocity of cataphoresis which we observed in the case of the non-polarisable pencil electrodes (100 volts, 9 ma.) shifts towards the anode by 0,6 μ, if the calomel electrodes (150 volts, 9 ma.) are used.

2. Zeitschr. f. Immunitätsforschung. I. Teil, Bd. 14, 1912.

teria with acid. OKER-BLOM tried to explain these facts by assuming changes in the electric charges of the leucocytes and especially of the bacteria, which should be caused by the action of acid or alkali, without however measuring the charges themselves. UCHIDA[1] reports in his researches of erythrophagocytosis that acid favours the process while alkali acts against it.

In order to test these results the staphylococcus aureus was thrown separately (1) into 0,9% NaCl + acid (HCl or oleïc acid) solutions, (2) into 0,9% NaCl + NaOH solutions and (3) into pure 0,9% NaCl solution (pH= 7,0),* the last being used as control, and each was allowed to stand 2 hours long. The bacteria which had been in the acid or alkali solutions were washed thrice with 0,9% NaCl solution before mixing with leucocytes. The latter were taken from the rabbit or guinea-pig blood and washed thrice with 0,9% NaCl solution. Then the bacteria and leucocytes were mixed, of course in all cases exactly in the same ratio. After 15 minutes the mixture was poured on the slide and when the blood corpuscles had sunk the supernatant fluid was drained off. The slide films were fixed with vapour of osmium and stained with GIEMSA's solution.

In each case 100 leucocytes were examined and the number of the bacteria adhering to them (phagocytosis number) and that of the leucocytes holding the bacteria were reckoned. However in cases where oleïc acid was used the bacteria became so agglutinated that it was impossible to count the phagocytosis number. The results are given in the following table.

1. Tokyo-Igakkwai Zasshi (Communication for the Tokyo Medical Society), Vol. 34, 1920.

* The salt solution in our laboratory is not exactly neutral but slightly acid in its reaction, its pH being often 5 or 6, perhaps on account of the fact that the room air contains much CO_2. To make the salt solution exactly neutral we added always a trace of NaOH until its pH became 7,0 in the colorimetric measurement.

	Cases where the rabbit leucocytes were used.		Cases where the guinea-pig leucocytes were used.	
	Phagocytosis number	Number of leucocytes holding bacteria	Phagocytosis number	Number of leucocytes holding bacteria
0,9% NaCl + $n/200$ HCl	38	26	61	31
„ + $n/800$ „	32	23	40	28
„ + $n/3200$ „	25	20	38	27
0,9% NaCl	13	9	29	21
0,9% NaCl + $n/3200$ NaOH	12	9	26	19
„ + $n/800$ „	12	8	28	21
„ + $n/200$ „	5	4	22	16
0,9% NaCl + Oleïc acid solution A*		30		34
„ + „ B		22		25

From this table it is evident that the acid promotes phagocytosis while the alkali represses the process a little. Now it is the question what changes in the electric charge of bacteria are caused really by the above mentioned treatment. Therefore we examined in every case the cataphoresis of bacteria before mixing them with leucocytes. The bacteria which had previously been treated with alkali proceeded to the anode without exception, their positive charge having turned to negative one, and this the more the more concentrated the alkali solution was. As to the action of acid we expected that it should promote the positive charge of the bacteria, but in reality we found that the bacteria subjected to the action of acid showed the same positive charge as those left in the pure physiological salt solution, or rather a slight decrease of the charge. Doubtless this fact is due to the far advanced

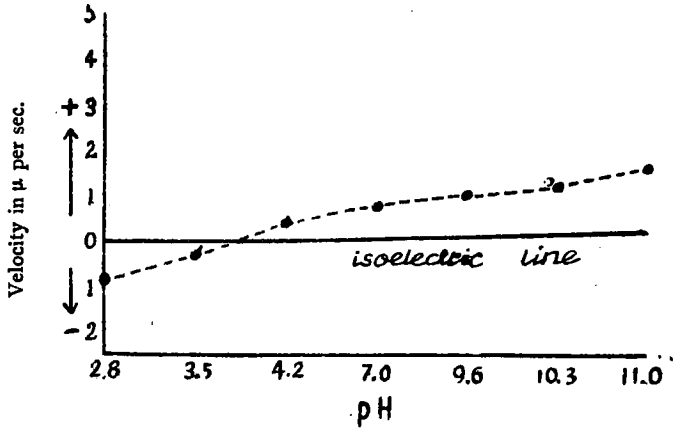
* Shake 20 cc. of 0,9% NaCl solution with 2 drops of oleïc acid. After filtration the filtrate is four times diluted with 0,9% NaCl solution. This is solution A. Solution B is prepared by diluting solution A further 4 times with 0,9% NaCl solution.

dissociation of the positive (hydrogen) ions from the bacterium body in consequence of the repeated washing by the pure physiological salt solution. The fact that the bacteria left in 0,9% NaCl solution showed somewhat less positive charge than those treated previously with acid, had they been washed thrice with the new salt solution, justifies this reasoning and proves that the acid must act upon the bacteria as a contributor of positive electricity. This action of the acid was more plainly testified by the following experiments concerning the staphylococcus albus and a strain of the staphylococcus aureus which had, as an exceptional one, a slight negative charge in 0,9% NaCl solution. Each was separately put in HCl or NaOH solutions of different concentration all made up in 0,9% NaCl solution and allowed to stand for 2 hours at ca. 20°C, the control having been preserved in the pure 0,9% NaCl solution under the same condition. The cataphoresis of every sample was examined in 0,9% NaCl solution without a previous washing of the bacteria, whereat a continuous current of 150 volts and 6 ma. was sent through the liquid by means of the calomel electrodes. The result are indicated with two curves in which the abscissae are the pH of the solutions previously used and the ordinates the charges of the bacteria represented by the speed and direction of the cataphoresis (Fig. 12).

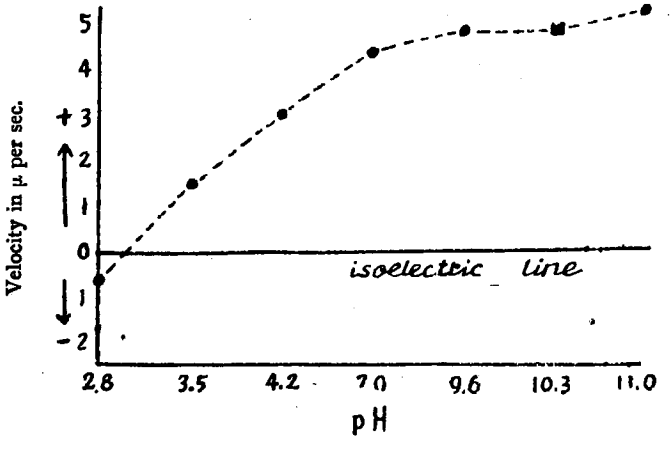
Therefore it is certain that the acid acts as a contributor of positive electricity upon the bacteria and especially when the negative charge of the bacteria is strong enough as in the case of the staphylococcus albus. And this action of the acid seems to be a preliminary condition to favour phagocytosis, the action of sera being taken into consideration. But a secondary elimination of the more or less increased positive charge which bacteria have obtained in the acid solution does not disturb the promoted phagocytosis, since a subsequent washing of the bacteria proves this fact, as well as the experiment of OKER-BLOM, who witnessed an increased phagocytosis even after neutralization of the acid solution. As the necessary condition for the promotion of phagocytosis we consider to be the fact that the electric charge of bacteria becomes once less negative or more positive shortly before they meet leucocytes.

Fig. 12.

Staphylococcus aureus.



Staphylococcus albus.



ADDENDUM.

After completion of this essay we had an opportunity to examine the blood of a patient suffering from paroxysmal haemoglobinurine. From this blood, which according to the dominant opinion contains autolysin, we separated partly serum, partly erythrocytes, the former after coagulation of blood, the latter by centrifugating the blood received in 0,9% NaCl + 1% natrium citrate. The erythrocytes were washed thrice with 0,9% NaCl solution. Likewise we prepared serum and erythrocytes from the normal human blood. Then the following 4 mixtures of serum (in 4 and 8 times dilution with 0,9% NaCl solution) and erythrocytes were made: (1) both serum and erythrocytes from the normal blood, (2) both serum and erythrocytes from the patient's blood, (3) the erythrocytes of the patient and the serum of the normal blood, (4) the erythrocytes of the normal blood and the serum of the patient. Each mixture was allowed to stand for 5 hours at a room temperature of circa 15°C, before any examination was made. The erythrocytes in the first mixture proceeded to the anode with a velocity of 2,4—2,5 μ per second, when they were examined in 0,9% NaCl solution. Almost the same charge was shown by the erythrocytes in the second and third mixture, although strictly speaking they moved a little slower than those of the first mixture. On the other hand the erythrocytes in the fourth mixture had much less negative charge, the velocity of their cataphoresis in 0,9% NaCl solution being only 1,4 μ (when the serum solution of 4 times dilution was employed) and 1,8 μ (when the serum solution of 8 times dilution was used) per second. From this fact it may be concluded that the patient's serum contained something which united with the normal erythrocytes at a temperature of 15°C and diminished their negative charge strongly, but we can not say whether this something is autolysin or isolysin, or both. On the supposition that the erythrocytes in the fourth mixture would be taken up by leucocytes readily we tried to examine phagocytosis by using the rabbit

leucocytes, but in vain, since there occurred a strong agglutination of the erythrocytes.

Chart I. Curves concerning the change in the electric charges of the staphylococci caused by the action of serum solutions.

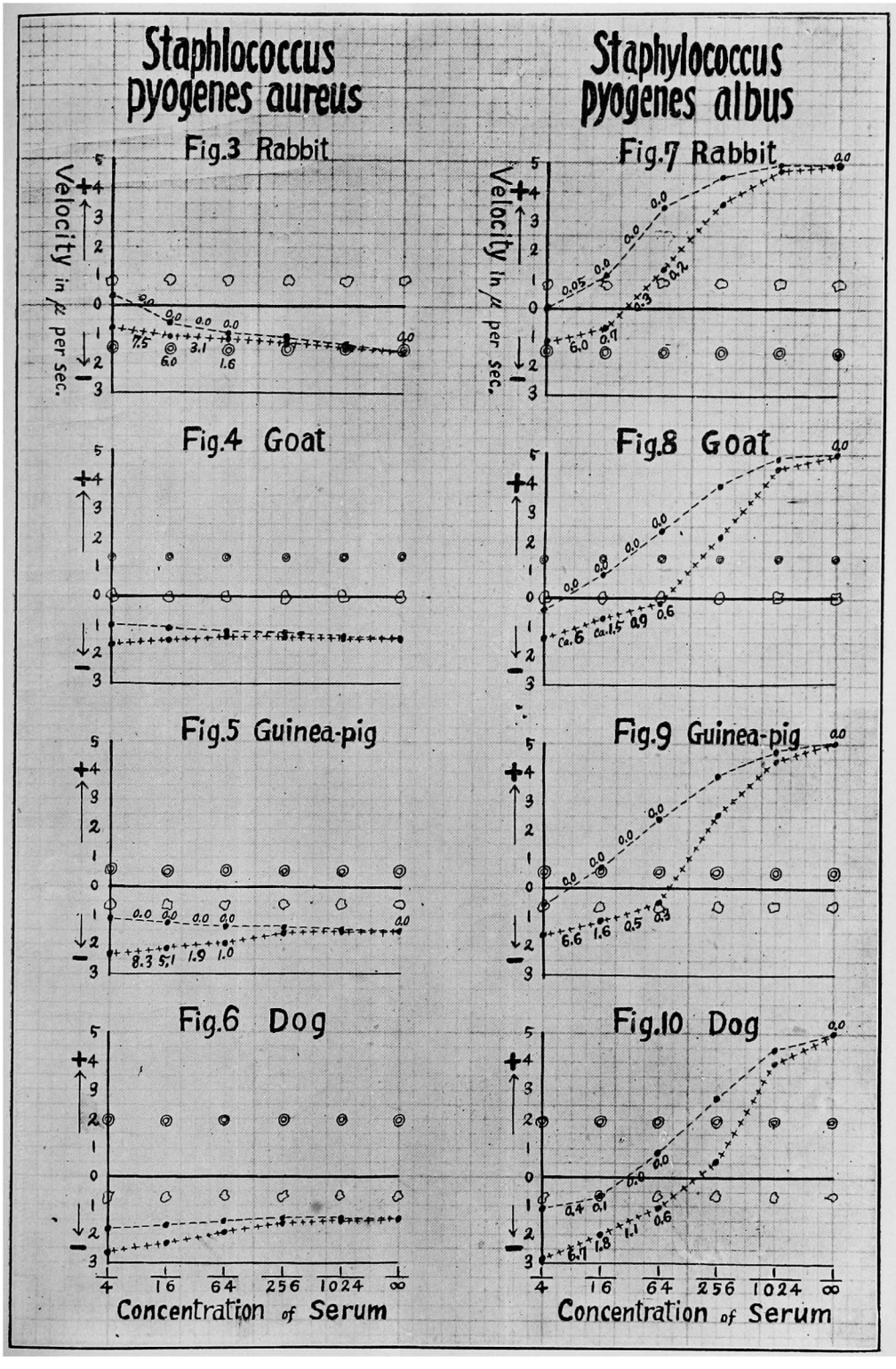
Abscissae represent the concentrations of serum added to the physiological salt solution. The fractional numbers are the reciprocals of serum dilutions, $\frac{1}{00}$ being the pure physiological salt solution containing no serum.

Ordinates are the electropotentials of the bacteria and blood corpuscles shown by the direction and speed of their cataphoresis.

– – – – – – – – lines are curves referring to the heated sera and + + + + + + + + lines those of the normal sera. Everywhere is seen the fact that the complement has a power to lessen the negative charge of microorganisms or to promote their positive charge.

◎ is the charge of erythrocytes and ♀ that of leucocytes, both referring to the animal from which the serum was taken.

The numbers which accompany curves are average numbers of the microorganisms taken up by each leucocyte, when the charge of the staphylococcus becomes so changed as shown by the curves. In cases where curves have no such affix numbers no examination of phagocytosis was made.


Staphlococcus pyogenes aureus
Fig.3 Rabbit
Velocity in μ per sec.
Fig.4 Goat
Fig.5 Guinea-pig
Fig.6 Dog
Staphylococcus pyogenes albus
Fig.7 Rabbit
Velocity in μ per sec.
Fig.8 Goat
Fig.9 Guinea-pig
Fig.10 Dog
4 16 64 256 1024 ∞
Concentration of Serum
4 16 64 256 1024 ∞
Concentration of Serum