Physiology. — "*Quantitative researches on phagocytosis. A contribution to the biology of phagocytes.*" By Prof. H. J. HAMBURGER and Dr. E. HEKMA.

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I. *Introduction and method of investigation.*

The investigations of which an abridged account is given in this paper [1]) are a continuation of those begun several years ago by one of us [2]), with the object of ascertaining the influence exercised by solutions of various concentration on the red corpuscles and other cells. These researches had been for the greatest part confined to the study of chemical and volumetrical alterations experienced by the cells through the modification of their media and of their significance with regard to the functions of the body. But until now, *the influence of these agents on the life of the cell itself,* had not been the object of a systematic investigation, although the plan had existed for some time and the expediency of the method had been proved [3]). The importance of such an investigation will be readily admitted. In the first place, because it enhances the value of the chemical and volumetrical researches mentioned above, and secondly, because the phenomena produced by the agency of solutions undangerous to life, are in fact nothing else but the effects of reaction, which finally will help us to penetrate farther into the chemical structure of the living cell. The red corpuscles, which were mostly used for the chemical and volumetrical researches, however, are no suitable objects for the study of the influence of reagents upon life, for they do not afford sure tests of vitality, nor is it possible to measure the value of their life functions.

We therefore looked elsewhere for our material and our choice fell on the phagocytes, for the twofold reason that they are simple, isolated cells in which it is possible to follow the effect of the chemical exchange with their natural medium, and to rate their very life by quantity; besides the phagocytosis is an essential factor in the functions of life. In support of this latter contention, we refer to the important place assigned by METCHNIKOFF to these cells in the struggle of the body against disease; a theory which he has

1) For detailed account, see "Biochemische Zeitschrift".

2) HAMBURGER, Zittingsverslag der Koninkl. Akad. v. Wetensch. 29 December 1883.

3) HAMBURGER, Het gedrag van witte bloedlichaampjes tegenover cyaankalium, Bijdrage tot de kennis der celpermeabiliteit. Feestbundel voor Rosenstein, 1902.

defended with such admirable acumen and unflagging energy. According to the same investigator, the part they play in the healthy body is no less important. The only thing therefore which remains to be done with regard to these cells, is to get a nearer insight into their conditions of life; as yet scarcely anything is known on this subject, a fact mentioned with regret by METCHNIKOFF, in the paper he read last year before the students of the University of Amsterdam, on: "Réactions phagocytaires" [1]).

The method of investigation employed by us was the following: White corpuscles from the blood of a horse, after having been transported into various media, were brought into contact with carbon and afterwards it was ascertained what percentage of the leucocytes had taken up particles of carbon. *This percentage was the measure for the degree of phagocytosis and gave the value of the influence of various agents on that function of life.*

These calculations were based on the principle that the phagacytorian power of the phagocytes present in a suspension is of unequal extent; i. e. the more detrimental the action of the agent is, the smaller must be the number of phagocytes able to take up carbon.

Our selecting a neutral indifferent substance of bacteria, had its ground in the fear that otherwise our work would have become too complicated. We here refer to the recently established fact that most kinds of bacteria, before they can be taken up by the phagocytes, must undergo a certain amount of preparation [2]). Hence it follows that not only will the intensity of phagocytosis be influenced by the agent as such, but also by the degree of preparation it has undergone. Another fact which had to be borne in mind, is that the bacteria sometimes secrete poisons which have an injurious effect on the phagocytosis.

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1) "Nous ne sommes qu'an début. Lorsqu'on connaîtra mieux *la physiologie des phagocytes* (the italics are ours) on cherchera des méthodes pour augmenter l'activité de ces éléments dans la lutte contre les microbes et on cherchera d'autres pour préserver contre l'attaque des phagocytes les cellules nobles de notre corps. En poursuivant ce but, il faudra tenir compte de ce que les phagocytes sont non seulement les destructeurs des microbes, mais qu'ils sont capables aussi de s'encorporer des poisons solubles et de les rendre inoffensifs. Leur rôle n'en devient que plus important."

2) WRIGHT and DOUGLAS, Proceed. of the Royal Society **72**, 1903, p. 357 and later studies prepared under WRIGHT. Further HEKTOEN and RÜDIGER, Journ. of Infect. diseases **2**, 1905, p. 128 and other studies prepared under HEKTOEN.

The selection of the indifferent substance carbon, instead of the usual grains of carmine was based on the greater facility and more accurate certainty with which the taking up of carbon can be ascertained. It was also for this reason that carbon had been the substance selected in former investigations on the action of carbonic acid [1]) and the action of cyanate of potassium [2]) on phagocytosis.

On the present, as well as on the former occasion, the leucocytes used in our investigations, were taken from the blood of a horse [3]). They were obtained by shaking blood with pieces of glass in a closed bottle and straining the defibrinated blood through a piece of muslin. The red corpuscles sink to the bottom, and the serum which covers them holds all the leucocytes. When this turbid fluid has been poured off we have a suspension of leucocytes in serum; this suspension can be made richer in leucocytes, by centrifugalizing it, removing part of the clear serum and mixing the leucocytes which have fallen to the bottom, with the remaining serum. A detailed description of this method, the process of preparing the carbon, the mode of bringing it into contact with the leucocytes, and the method of determining the percentage of the cells which have taken up carbon, will be found in our article in the Biochemische Zeitschrift [4]).

II. *The effects produced on phagocytosis by the addition of water.*

Our first experiments were directed to the solution of the question in how far phagocytosis is affected by the addition of water.

With this object in view, equal quantities of the suspension of leucocytes were mixed with serum, previously diluted with known quantities of water. The following table shows the results of one of the series of experiments. It will need no further explanation.

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1) HAMBURGER. VIRCHOW's Archiv, **156**, 1899. S. 329. Osmot. Druck u. Ionenlehre. 1. S. 416.

2) HAMBURGER Het gedrag van witte bloedlichaampjes tegenover Cyaankalium in ROSENSTEIN's Feestbundel. 1902.

3) At Groningen we experienced great difficulty in obtaining a regular supply of horses' blood. Mr. K. HOEFNAGEL, the Director of the abattoir at Utrecht, had the great kindness fully to meet our wants, for which we here beg to tender him our best thanks.

4) Compare also HAMBURGER, Osmot. Druck u. Ionenlehre, Bd. 1. S. 401.

TABLE I.

Effect of lessening the concentration of the serum.

Serum diluted with	Number of examined white corpuscles	Number of white corpuscles which have taken up coal	Percentage of number of white corpuscles containing coal	Decrease of the phagocytarian power
0% water	886	331	37	
20 „	754	246	32	13.5%
50 „	732	154	21	43 2 „
100 „	636	81	12½	66.2 „
140 „	530	0	0	
200 „	546	0	0	

From this table it appears that in serum, to which no water has been added, of the 886 examined leucocytes, 331 had taken up carbon, i.e. 37 %.

We must here point out that in the circulating, and also in the coagulated blood, the percentage of phagocytes is actually much smaller.

By a certain proceeding, however, we contrived that in our experiments, the leucocytes used for examination should contain a great number of phagocytes. This process is based on the fact that among the leucocytes, the phagocytes are the cells which soonest sink to the bottom. After this explanation, it will cause no surprise to find that, in normal serum, the percentage of the phagocytes which have taken up carbon, continually varies in different series of our experiments.

An addition of 20 % water, already lessens the phagocytarian power with $13^1/_2$ %. In calculating this loss on an addition of 5 % water, supposing the diminution to be proportionate, the decrease of the phagocytarian capacity would have amounted to: $\frac{5}{20} \times 13.5\,\% = 3.4\,\%$. In other words, *when the osmotic concentration of the blood plasma is lessened by 5%, a loss which in a healthy individual may be of daily occurrence* [1]*, the phagocytarian power falls about* 3.4 %.

By the side of this great sensitiveness of the phagocytes to the increase of their percentage of water, stands the fact, as shown from

[1]) Compare a o. Koeppe. Pflüger's Archiv. 62, 1896. S. 567. In his experim nts, Koeppe noticed a decline below the mean osmotic pressure of over 10 %.

the table, that on the other hand, there are a great number of phagocytes which can stand a dilution of their serum with 100 °/₀ of water. Former experiments have proved that this dilution causes an increase in the bulk of the cells, of considerably over 30 °/₀.[1])

We will now pass on to the following question: *Is this decrease in the phagocytosis of a permanent nature?*

In order to find an answer, we brought the white corpuscles which had been submitted to the action of diluted serum back into the normal, undiluted serum, and then tested again their power of taking up coal.

TABLE II.

After exposure to the action of diluted serum, the phagocytes are brought back into normal serum.

Serum diluted with	Number of white corpuscles examined	Number of white corpuscles which have taking up carbon	Percentage of white corpuscles containing carbon, *in normal serum*
0°/₀ water	500	105	21
20 „	500	99	19.8
50 „	500	107	21.4
70 „	500	96	19.2
100 „	500	78	15.6
200 „	500	61	12.2

This table shows that phagocytes, which had some time remained in serum, diluted with 20 °/₀ or 50 °/₀ water, dilutions which as the former series of experiments indicates, caused a reduction of the phagocytarian capacity of 13.5 °/₀ and 43.2 °/₀ respectively, after having been brought back into normal serum, *entirely recovered their original phagocytarian power.*

The addition to the serum of 100 °/₀ water, has on part of the phagocytes a lasting deleterious effect; the addition of 200°/₀ water is even more detrimental. Still, itis interesting to observe that, although in serum which had been diluted with 200 °/₀ water, all the phagocytes

[1]) HAMBURGER. Archiv. f. (Anat. u.) Physiol. 1898. S. 317.

had lost the power to take up carbon, after being replaced in the normal serum, over 50% of the phagocytes recovered their original capacity.

So, *the greater part of the phagocytes can support a considerable volume of water without permanent loss of their phagocytarian capacity.*

Here may be asked: On what does it depend, whether a phagocyte will regain its phagocytarian power? It is not impossible, nay, it is even probable, that here as well as in the case of the red corpuscles some lose their contents in serum diluted with 70% of water[1]. If the quantity of water added be raised to 100%, the number of destroyed erythrocytes will be found considerably larger. When the red corpuscles, which *have not lost their haemoglobin*, are removed from the serum diluted with 100% of water into undiluted serum, they entirely recover; they change from small globules into biconcave discs, which even arrange themselves like piles of coins.

However, this only applies to the cells which have not lost their colouring matter. These which have actually lost haemoglobin cannot recover. Now our microscopical investigations have revealed the fact, that in serum + 70% water, some of phagocytes lose a part of their contents: in that case we see a granular substance lying by their sides. In serum to which 100% water has been added, the effect is more apparent still. Then the number of leucocytes which have expelled granular matter is still larger. It is easy to understand that these cells, when again placed in normal serum, have lost the power of taking up carbon. The difficulty of ascertaining this with certainty however, is very great; in the transmission there is every chance of disturbing the granular substance by the side of the cells, and it is impossible to know whether one deals with a phagocyte which has lost part of its contents or not. Anyhow, taking into consideration the striking analogy existing between white and red corpuscles, both with regard to their permeability and to the osmotic pressure of their interior substance, and even to the percentage of the volume of their watery contents[2], we seem justified in our conjecture that the same fluid, which causes a loss of colouring matter in the least resisting of red corpuscles, also brings about the irremediate destruction of the phagocytarian power of the least resisting phagocytes.

It is a fact worthy of notice that the resisting power of the phagocytes reaches a higher maximum than that of the erythrocytes. In serum diluted with 200% water, all the erythrocytes of the horse are destroyed, and not quite half of the phagocytes.

III. *Effect on phagocytosis by the reduction of water.*

A similar method as had been used for studying the effect on phagocytosis by dilution of the serum, was now applied to ascertain the

[1]) HAMBURGER. Transactions of the Royal Academy of Sciences, 26th March 1885.

[2]) Osmot. Druck u. Jonenlehre I, S. 401—435.

influence of concentration. With this object in view, common salt was dissolved in the serum in quantities of 0.1, 0.2, 0.3, 0.4 % and more. The results of these experiments are shown in the following table:

TABLE III.

Effects on phagocytosis by increased concentration of the serum.

Serum in which is dissolved	The liquids in the preceding column are corresponding to:	Procentage of leucocytes which have taken up carbon	Decrease of the phagocytarian power
0 % Sod. Chl.	Sod. Chl. 0 9%	$\frac{208}{832} \times 100 = 26$ %	
0.1 "	" 1	$\frac{184}{874} \times 100 = 21.5$	17.3%
0.2 "	" 1.1	$\frac{184}{1005} \times 100 = 18.3$	29.6
0.3 "	" 1.2	$\frac{76}{941} \times 100 = 8$	69.2
0.4 "	" 1.3	$\frac{43}{793} \times 100 = 5.4$	79.2

Here we see that the injurious effect is very great, much greater than is the case when the osmotic concentration has been diminished. Then we observed that by diluting the serum with 20 % water, phagocytarian capacity fell 13.5 %; here we find that by raising the osmotic concentration by 10 %, the phagocytarian power is lowered by 17.3 %. This effect must already be perceptible within the physiological boundaries in which the osmotic pressure of the blood plasma usually varies in the normal body. For it may happen every day that in a normal individual the osmotic pressure of the liquor sanguinis, a few hours after dinner, is still raised by that of 0.1 % common salt [1]).

Here again, as we did before when studying the decrease of osmotic pressure, we ask whether the loss of phagocytarian power

1) KOPPPE, l. c.

D. SCHOUTE. Het physisch-chemisch onderzoek van menschelijk bloed in de kliniek. Diss. Groningen 1903.

See also Osmot. Druck u. Ionenlehre B. I S. 540 ff.; B. II S. 279 and 310 ff.

can be restored, by replacing the white corpuscles in the normal serum. The answer will be found in the following table.

TABLE IV.

After being exposed to the action of increased concentration, the leucocytes were replaced into normal serum.

Serum in which is dissolved	After replacement into normal serum, the phagocytarian power stands at
0 °/₀ Sod. Chl.	$\frac{273}{700} \times 100 = 39\%$
0.2 "	$\frac{246}{646} \times 100 = 38.4$
0.7 "	$\frac{226}{685} \times 100 = 33$
1.2 "	$\frac{170}{567} \times 100 = 30$
1.5 "	$\frac{149}{713} \times 100 = 21$
2 "	$\frac{87}{625} \times 100 = 14$
3 "	$\frac{57}{590} \times 100 = 9$

From this table it may be seen that after exposure to the action of serum in which 0.2 $\%$ common salt had been dissolved, a solution which had lowered the phagocytarian capacity by 29.6 $\%$ (see table III), replacement in normal serum brings it back to its original value. The action of serum in which 0.7 $\%$ of salt had been dissolved, however, causes a permanent loss of phagocytosis. Still, this loss is not so great considering that, in the serum with 0.7 $\%$, not a single cell has taken up carbon, — in other terms, the phagocytosis has been entirely paralysed.

Now the phagocytes had only been exposed for half an hour to the action of the concentrated media. This certainly may be considered long enough for the small cells to readjust themselves to their new medium. Still, it may be asked whether *after a more prolonged exposure the normal value of the phagocytarian power would be restored too.* This question is of great importance for the functions of normal life, in which the increase of osmotic concentration often lasts longer than half an hour. For this reason, the experiments in which the leucocytes were exposed to an action of much longer duration, were made with serum containing only 0.1 and 0.2 % NaCl; higher osmotic concentration does not occur in the body. The leucocytes were placed in the serum of increased osmotic concentration for 2, 24 and 48 hours, and then transferred into normal serum.

The experiment showed that after an exposure of 24 and 48 hours, the phagocytarian power had been diminished; but an *equal* decrease of vitality was also observed in phagocytes which had remained for 24 and 48 hours respectively in *normal* serum. This proved that the prolonged action of serum of increased osmotic concentration had had no *permanent* injurious effect on the phagocytarian capacity.

Thus we may conclude that, in the living body an increase in the osmotic concentration of the blood plasma, as well as a decrease of the same, has a deleterious effect on the phagocytarian power, but that the loss may be recovered; for as soon as the osmotic pressure has been restored to the normal, the phagocytes also entirely regain their inherent power.

If from these experiments we may conjecture, that what we have observed in the phagocytes, will also be applicable to other cells with semipermeable walls, it is reasonable to conclude from the results shown in tables II and III, *that the vital functions of the cell are in a large measure influenced by slight oscillations in the osmotic concentration of the environment and consequently of the cells themselves.*

IV. *Effect of simple solutions of Salt.*

1. Solutions of Sodium Chloride.

Now the question arises whether the loss of vitality described above, must be attributed to the variations of the quantity of water as such, or to the modification in the concentration of one or more of the substances.

In order to examine this question systematically, we might have alternately reduced the several elements in the diluted serum to their original concentration and then studied the extent of the improvement. But as in the mean time it had been clearly demonstrated to us that in a pure solution of Sodium Chloride of 0.9 % the phagocytes take up carbon in equal or almost equal quantities as in normal serum, we decided to abandon this mode of investigation.

Here we must incidentally remark that, after all that has been said by

Loeb [1]) and others, of the injurious action of a pure solution of a simple salt on the life of young moving larvae and the vital processes of higher animals, such as the beating of the heart and the movements of the intestines, we were at first rather astonished at the almost perfect innocuousness of similar solutions in regard to the phagocytes. However, we can easily find an explanation for this seeming inconsistency. Whenever a cell is surrounded by a simple isotonic solution of salt, two things are likely to happen: an exchange of ions may take place, thus causing a modification in the chemical structure of the cell, which interferes with, certain of its vital functions. This is the case with the larvae of fundulus with the muscle of the heart and that of the intestines. A supply of specified ions is then required to restore the chemical structure of the cell to its normal state. But — and this is the second possibility — if the permeability of the cell to ions is highly limited, a pure isotonic solution of salt will not cause any, or only a very slight alteration in the chemical structure of the cell. This is the case with the white corpuscles, the slight permeability of which to ions of salts has already been demonstrated in the most convincing manner.[2])

Bearing this fact in mind, it can cause no surprise that, contrary to the results of the observations on eggs and muscles, a pure solution of Sodium Chloride leaves the phagocytarian power entirely or almost entirely intact.

Under these circumstances, for determining the influence of the water as such, it was indicated to take solutions of Sodium Chloride of various strength.

Table V shows the action of diluted solutions of Sodium Chloride on phagocytosis.

TABLE V.

Effect of hyper-isotonic solutions of Sodium Chloride on phagocytosis.

Solutions of Salt.	Percentage of leucocytes which have taken up carbon.
NaCl-sol. 0.9%	$\frac{235}{756} \times 100 = 31$ %
NaCl 0.75% = NaCl 0.9% + 20% water	$\frac{208}{741} \times 100 = 28$
„ 0.6 = „ 0 9 + 50 „	$\frac{221}{1012} \times 100 = 21.8$
„ 0.45 = „ 0.9 + 100 „	$\frac{83}{745} \times 100 = 11\ 1$

1) J. Loeb, American Journal of Physiol. **3** 1900 p. 327 and 383; **5** 1901 p. 362 Pflüger's Archiv **80** 1900 S 229.
Lingle, Americ. of Journal of Physiol. **4** 1900 p. 265.
Miss Moore, Ibid. 1900 p. 386 etc.

2) Hamburger. Zeitschr. f. Biol. **35**. 1897 S. 252 and S. 280; Proceed. of the Royal Academy of Sciences 11 April 1897.
Archiv f. (Anat. u.) Physiol 1898 S. 31 and S. 317.
Virchow's Archiv **156** 1899 S. 329
Hamburger and van der Schroeff, Archiv f. (Anat. u.) Physiol. 1902. S. 251.

Here we see the marked effect of a diminution in the concentration of the salt solution.

TABLE VI.

Effect of hyper-isotonic solutions of salt.

Solutions.	Percentage of leucocytes containing carbon.	Decrease of the phagocytarian power.
NaCl 0.9 %	$\frac{250}{722} \times 100 = 34.6\%$	
" 0.95	$\frac{293}{875} \times 100 = 33.5$	3 %
" 1	$\frac{95}{802} \times 100 = 11.84$	60.6
" 1.1	$\frac{105}{981} \times 100 = 10.8$	69
" 1.2	$\frac{7}{990} \times 100 = 0.7$	98
" 1.3	$\frac{0}{200} \times 100 = 0$	
" 1.4	$\frac{0}{150} \times 100 = 0$	
" 1.5	$\frac{0}{150} \times 100 = 0$	

The surprisingly rapid decline of phagocytosis observed in serum of increased concentration (table III) is again clearly demonstrated in this instance.

Even the slight increase of 0.9 to 1 % lowers the phagocytarian power already 60.6 %. Another illustration of this rapid decline is afforded by the observation that in the 1 % concentration of Sodium Chloride, the amount of carbon present in the coal containing phagocytes is far less than in those that have stayed in the solution of 0.9 %.

Now, by comparing tables VI and III, we see at a glance that, when the experiments were made with a solution of Sodium Chloride of 0.9 %, to which afterwards salt had been added, *the decline in the phagocytarian power is more marked* than when they are made in serum supplemented with an equal quantity of salt. This proves that besides the osmotic pressure, which must principally be made accountable for the decline, there is still another factor at work, *and this factor can be no other than the modification — however slight — produced by a pure solution of NaCl in the chemical structure of the phagocytes.* Some time ago, one of us, in conjunction with Dr. VAN DER SCHROEFF [1]), already demonstrated that the leucocytes the same as the red corpuscles are in any case permeable to *an*ions. It is therefore evident that, owing to their chemical structure being interfered with, the cells most lose some of their vitality (phagocytarian power) under the action of pure salt solutions, — or rather, that they should lose *more* than in an isosmotic serum.

We have submitted this hypothesis to further experiments, starting from the following reasoning: If it is a fact that in a hyper-isotonic solution of salt, the phagocytes undergo a chemical variation through exchange of ions, it must be possible to restore this loss of phagocytarian capacity resulting from their modification in their structure, by replacing them in normal serum, and that this recovery will not be complete by immersion in a 0.9 °/₀ solution of salt. The following table proves that we were correct in our surmise.

TABLE VII.

Effect of solutions of salt on the chemical structure of the phagocytes.

White corpuscles immersed for $2^1/_2$ hours in the following solutions.	Phagocytarian power after being transferred into.	
	Normal Serum	Salt solutions of 0.9%
NaCl 0.9%	$\frac{319}{942} \times 100 = 33.9\%$	$\frac{284}{811} \times 100 = 35\%$
" 1	$\frac{258}{775} \times 100 = 33.3$	$\frac{251}{760} \times 100 = 33$
" 1.1	$\frac{233}{790} \times 100 = 29.5$	$\frac{209}{735} \times 100 = 28.6$
" 1.2	$\frac{202}{722} \times 100 = 28$	$\frac{175}{677} \times 100 = 26$

1) HAMBURGER and VAN DER SCHROEFF. l. c.

It is clearly demonstrated that the phagocytes, which have been exposed for two hours to the action of solutions of Sodium Chloride, exhibit a greater phagocytarian power when they are transferred into serum, than when they are placed into salt solution of 0.9 %.

No doubt the observation will strike the attentive reader as contradictory, that this is only the case with the phagocytes which had been exposed to the action of sodium chloride of 1 %, 1.1 % and 1.2 %, but not with those which for the same space of time had been immersed in a similar solution of 0.9 %; then the effect of this salt-solution and the serum is quite the reverse. This, however, is not actually the case; for in serum the phagocytes are likely to stick together and on this account do not offer as large a surface to the carbon as in the salt-solutions in which they remain more isolated. If then, as must be the case in an *isotonic* solution, the injurious effects of the Cl-ions of the pure salt solution are comparatively small, they may easily be exceeded by the unfavourable position of the cells caused by the serum.

When, however, by the use of *hyper-isotonic* solution of sodium chloride, the injurious action of the Cl-ions be increased, it may exceed the detractory influence of the agglomeration of the cells, and produce the results shown in the table.

With regard to these statements it may here be asked why, in *isotonic* solutions of sodium chloride, the injurious effect on the phagocytes cannot be determined, but is easily demonstrated when hyper-isotonic solutions are used, and the more readily in proportion as the concentration of the salt solutions are increased in strength. This question is very natural, because it concerns such a small increase in the considerable amount of ions of Cl or of Na already present. Here we are involuntarily reminded of the fact stated by HEDIN[1]) with regard to the red blood-corpuscles. The minute investigations of this scientist have brought to light the fact, that in isosmotic *isotonic* solutions of salt, the corpuscles possess an equal volume, but that in isosmotic anisotonic solutions their relative volume is no longer equal. HEDIN has not given an explanation of this important fact; but anyhow, it proves that simple solutions of salt, when anisotonic, exercise still another kind of action beyond that of their osmotic pressure. We propose to investigate this matter somewhat further: it is very probable that by a modification in the dissociation of the contents of the cell, an altered condition for the exchange of ions is produced.

[1]) HEDIN. Skandinavisches Archiv f. Physiol, 1895 S. 377.

2. Solutions of Chloric Potash.

In our description of the influence of sodium chloride, we attributed it to the ions of chlorine. This was based on the results of investigations in which we compared the action of sodium chloride and of potassium chloride, of which a few items here follow.

These investigations proved that isosmotic solutions of sodium chloride and potassium chloride have almost the same effects on phagocytosis.

TABLE VIII.

Comparison of isosmotic quantities of sodium chloride and potassium chloride.

	Percentage of leucocytes containing carbon.
Serum	$\frac{253}{722} \times 100 = 35\,\%$
NaCl-sol. 0.9%	$\frac{300}{836} \times 100 = 36$
KCl-sol. 1.15% (isot. m. NaCl-sol. 0.9%)	$\frac{258}{277} \times 100 = 34$
Serum + 0.1 % NaCl	$\frac{183}{672} \times 100 = 27$
„ + 0.127 „ KCl	$\frac{181}{715} \times 100 = 25$
„ + 0.3 „ NaCl	$\frac{45}{630} \times 100 = 7$
„ + 0.38 „ KCl	$\frac{54}{683} \times 100 = 8$
„ + 0.3 „ NaCl (afterwards placed into normal serum)	$\frac{184}{600} \times 100 = 30$
„ + 0.38 „ KCl (afterwards placed into normal serum)	$\frac{185}{621} \times 100 = 30$

Two other parallel-experiments in solutions of 0.9 % of sodium chloride produced the following results:

$$\frac{198}{863} \times 100 = 23\,\% \text{ of leucocytes containing carbon}$$

and $\frac{146}{677} \times 100 = 21.5\,\%$ „ „ „ „

in the isosmotic solution of KCl 1.15 %:

$$\frac{128}{615} \times 100 = 21\ \%\ \text{leucocytes containing carbon}$$

$$\text{and}\ \frac{165}{732} \times 100 = 22.5\ \%\quad ,, \quad ,, \quad ,,$$

Hence we may conclude, that there is no difference between the action of chloride of potassium and chloride of sodium.

3. Effect of chloride of calcium.

The great importance which, according to the most recent investigations must be ascribed to the ions of calcium,[1]) in the constitution of the fluid-matter of the tissues, induced us to test also the effect of this medium on phagocytes.

With this object in view, we dissolved various quantities of chloride of calcium in the serum of the blood of a horse and mixed the suspension of leucocytes thus obtained with carbon.

TABLE IX.

Effect of calcium chloride.

Serum +	Percentage of leucocytes containing carbon	Increase of the phagocytarian power
0% $CaCl_2$ 6 aq.	$\frac{132}{612} \times 100 = 21.2\%$	
0.01%	$\frac{225}{861} \times 100 = 26$	22.6%
0.1	$\frac{180}{652} \times 100 = 27.6$	30.2
0.5	$\frac{162}{598} \times 100 = 27$	27.3
1	$\frac{0}{724} \times 100 = 0$	

An addition of 0.01 % *of* $Ca\,Cl_2$ 6 *aq. to the serum already produces an increase of the phagocytarian capacity of* 22.6 %; by the addition of 0.1 % $Ca\,Cl_2$ 6 aq., the effect is somewhat

[1]) See especially the investigations of Loeb. Publications of the University of California and of Langendorff and Hueck. Pflüger's Archiv **96** 1903 S. 473; for the complete bibliography on the subject until 1904, see Osmotischer Druck und Ionenlehre B. III, S. 107 etc. Comp. also A. Netter, Importance biologique du Calcium. Paris. Masson et Cie. 1907.

greater, and by the addition of 0.5 % Ca Cl_2 6 aq., it again decreases.

The result registered in the first instance, which is produced by the addition of 0.01 % Ca Cl_2, must be considered the most valuable, for it denotes the nearest unalloyed effect of the calcium chloride. In the experiments where quantities of 0.1 %, 0.5 % and 1 % of Ca Cl_2 were added, the increase of phagocytosis is counteracted by the unfavourable influence of the raising of osmotic pressure.

This experience is in strict accordance with the observations made by LANGENDORFF, who found that the injection of very small quantities of calcium, causes the heart to beat with greater force. We ascribe this manifestation to the action of the ion of calcium on the contractile substance, and we may conclude that the muscular fibre and the phagocytes also, are permeable to this cation.

4. Effect of citras natricus.

The frequent use which, in consequence of the experiments of WRIGHT and DOUGLAS,[1]) is made of this medium at the present day by the bacteriologists, in order to prevent the coagulation of the blood, actuated us also to experiment with this substance for the sake of determining its action on the phagocytosis. The following table gives a survey of the results.

The customary solutions of 1 % and 2 % of citras natricus in 0.9 % solution of sod. chl. were used in these experiments.

TABLE X.

Effect of citras natricus.

	Percentage of leucocytes containing carbon
(*a*) 1 cc. suspension of leucocytes + 2 cc. solution of 1% citras natr. in 0.9% Sod.Chl.	0
(*b*) 1 cc. suspension of leucocytes + 2 cc. solution of 2% citras natr. in 0.9% Sod.Chl.	0
(*c*) leucocytes from (*a*) transferred in Sod.Chl. 0.9 %	$\frac{260}{686} \times 100 = 38\ \%$
(*d*) leucocytes from (*b*) transferred in Sod.Chl. 0.9 %	$\frac{255}{731} \times 100 = 35\ \%$
(*e*) 1 cc. suspension of leucocytes + 2 cc. solution of 0.9 % Sod.Chl. (Control test)	$\frac{369}{725} \times 100 = 50\ \%$

[1]) WRIGHT and DOUGLAS, Proceed. of the Roy. Soc. **72**, 1903, p. 357; **73**, 1904, p. 128.

From the above table it is shown: 1. That in 1—2 °/₀ solutions of citras natricus in 0.9 °/₀ of Sod. Chl. the phagocytarian power is nil.;

2. that the phagocytarian capacity again *partially reappears,* when the cells are transferred into 0.9 °/₀ solutions of Sod. Chl. *The permanent decline of the phagocytarian power still amounts to* 28 °/₀.

5. Effect of Fluornatrium.

Fluornatrium being also much used for preventing the coagulation of the blood, it seemed important to us also to study the effect of this medium on the phagocytosis.

TABLE XI.

Effect of Fluornatrium.

	Percentage of leucocytes containing carbon	
	Before being transferred into 0.9% Sod. Chl.	After being transferred into 0.9% Sod. Chl.
2cc suspension of leucocytes + 2cc NaFl 0.65% (isot. with NaCl 0.9)%	0%	$\frac{91}{677} \times 100 = 14\%$
" + 2cc NaFl 1%	0	$\frac{30}{511} \times 100 = 6$
+ 2cc NaFl 2%	0	0
+ 2cc NaCl 0.9%		$\frac{369}{725} \times 100 = 50$

Here we see that when the leucocytes have been exposed to a solution of Fluornatrium of 2 °/₀, 1 °/₀ or 0.65 °/₀ (isot. with 0.9 °/₀ NaCl) the phagocytarian power is entirely paralysed, yea, that even after transferring of the phagocytes in a solution of 0.9 °/₀ Sod. Chl. it shows to have been entirely destroyed for ever. *Hence we may conclude that NaFl is a powerful poison for the protoplasma of the phagocytes.*

V. *Effect of acid and alkali.*

1. Effect of acid.

The important part which the alkaline reaction of the blood-plasma seems to play, not only in connexion with the degree of oxydation taking place in the body, but also in infectious diseases, induced us to study its effects on the increase or decrease of the phagocytarian power.

The results of one of the experiment are shown in the following table.

TABLE XII.

Diminution of the alkaline reaction of the serum.

1 cc $^1/_2$ n. H_2SO_4 +	Amount of acid added	Percentage of white blood corpuscles containing carbon
9 cc serum	$^1/_{20}$ norm.	0
14 cc „	$^1/_{30}$ „	$\frac{13}{308} \times 100 = 4.3\%$
19 cc „	$^1/_{40}$ „	$\frac{35}{398} \times 100 = 9$
49 cc „	$^1/_{100}$ „	$\frac{165}{724} \times 100 = 21.4$
299 cc „	$^1/_{600}$ „	$\frac{255}{612} \times 100 = 41.7$
499 cc „	$^1/_{1000}$ „	$\frac{256}{530} \times 100 = 43.5$
normal serum		$\frac{227}{530} \times 100 = 43$

We observe that even the small addition of $^1/_{600}$ *n*-acid is injurious to the phagocytosis.

Now, we know that according to titration with lacmoïde, 100 c.c. horse serum in the mean is equivalent to 75.5 cc. $^1/_{25}$ *n*-acid [1]; consequently it is calculated that serum represents an alkaline fluid of $^1/_{30}$ normal.

The addition of $^1/_{600}$ *n*-acid, therefore lowers its alkaline reaction by 5 %.

Consequently, a diminution of the alkaline reaction of the serum by 5 % is already injurious to the phagocytes.

This result is in strict accordance with the injurious effect experienced

[1]) HAMBURGER, Verhandel. d. Koninkl. Akad. v. Wetensch. Second section, Vol. VI, N°. 1, 1897.

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by administering acid per os, and we are fully justified in ascribing the poisonous effects of the acid, to a diminution in the process of oxydation.

The results agree also with the observations recently published by J. LOEB, on the influence of the traces of NaOH (OH-ions) on the artificial fructification of the eggs of sea-urchins. The author has clearly demonstrated that the primary cause of this effect might be found in the acceleration of chemical reactions. [1]).

TABLE XIII.

Increase of the alkaline reaction of the serum.

1cc 1/2 n. NaOH +	Amount of alkali added	Percentage of white corpuscles containing carbon.
29cc Serum	1/60	$\frac{25}{622} \times 100 = 4$
37cc "	1/76	$\frac{57}{840} \times 100 = 6.8$
49cc "	1/100	$\frac{114}{707} \times 100 = 16$
99cc "	1/200	$\frac{179}{716} \times 100 = 25$
199cc "	1/400	$\frac{143}{531} \times 100 = 27$
399cc "	1/800	$\frac{149}{580} \times 100 = 25.7$
normal serum		$\frac{177}{664} \times 100 = 26.5$

It is seen from this table that, within a large margin, the addition of OH-ions to the serum does not exercise a perceptible influence on the phagocyterian power, it remains unaltered until the value is increased by $^{1}/_{200}$ normal: i.e., with 15 % of the original alkaline reaction. An additional supply of alkali causes a lessening of the phagocytarian power.

More pronounced still is the effect of acid and alkali on the phagocytes, when these substances, instead of being added to serum, are

[1]) J. LOEB, PFLUGER's Archiev **118**, 1907, H. 3/4, S. 181.

introduced in solutions of 0.9 % *sod. chl.* A more detailed account of the results of these investigations will follow later.

We also made a number of experiments to test the influence of other media on the phagocytarian power, e.g. with *ureum, chinine, argentum colloidale, heterogenous serum*, etc., the results of which will appear in a subsequent paper.

Summary.

The following are the principal conclusions derived from the above described experiments.

1. The action exercised by various media on the phagocyterian power of white corpuscles, can be accurately determined by counting the percentage of cells which have taken up particles of carbon.

2. *The addition of water to the inherent medium of the phagocytes i.e. to their own serum, acts injuriously on the phagocytarian power.*

Even a decrease in the osmotic concentration as may daily occur in a normal individual, causes a perceptible decline in the phagocytarian power.

So, it was shown in one of the experiments that, whilst in normal undiluted serum 37 % of the leucocytes had taken up carbon, in serum which had been diluted with 20 % of water the amount of cells containing carbon was only 32 %: this corresponds to a decline in the phagocytosis of $\frac{37-32}{37} \times 100 = \mathbf{13.5}$ %.

By the addition of 50 % water, the percentage of phagocytes containing carbon fell to 21 %, thus in this case a decrease of phagocytosis of $\frac{37-25}{37} \times 100 = \mathbf{43}$ %.

By addition of 140 and of 200 % water, the percentage of the carbon-containing leucocytes was lowered to nil, — in other words the phagocytarian power had been suspended, but only *temporarily, for*

3. *by replacing the cells damaged by the addition of water, into their own serum, the phagocytarian power is entirely or partially restored.*

So the recovery was complete, when the serum had been diluted with 20 % or 50 % of water, and only partial when 70 tot 100 % water had been added. Even when it had been diluted with 200 %,

a figure at which, it is shown under 2, the phagocytosis had been entirely suspended, — a recovery took place in the phagocytarian power to half of its original amount.

4. *The observations, here made with the phagocytes, correspond with those previously observed in the red corpuscles.*

1. The phagocytes, the same as the red corpuscles, can support a considerable quantity of water ($\pm$ 60 %) without a single cell being destroyed;

2. The modifications produced in the phagocytes by the addition of water, unless they have led to their entire destruction, may, judging from the phagocytarian capacity, be entirely obviated by replacing them in normal serum.

5. *A heightening of the osmotic concentration of the serum, as well as a lowering of the same,* (*comp. sub* 2) *has a very injurious effect on the phagocytosis. It was obvious that an increase of the osmotic concentration had even a more pronounced deleterious action than the decrease at the same ratio.* Already an addition of 0.1% NaCl to the serum caused the phagocytarian power to decline 17.3%.

By the addition of 0.4% NaCl this decrease amounted to 79.2% and by the addition of 0.5% Sod. Chl., the phagocytarian power was reduced to nil; but this considerable loss was but temporary, for

6. *when the cells which had been damaged by an addition of sodium chloride to the serum, were replaced in their original blood-serum, their phagocytarian capacity was again entirely or partially restored*; entirely when only from 0.1%—0.2% of the substance had been added; partially when a greater amount had been used.

7. If thus, as shown under 2 and 5, the phagocytarian power is specially impaired by modification of the normal osmotic concentration of the blood-serum, this capacity will be entirely restored as soon as the blood-plasma, principally owing to the activity of the kidneys, has recovered its normal osmotic concentration. The experiments have demonstrated that this recovery is still possible after the agency of the anisotonic serum for 24 hours and more.

8. *In solutions of* 0.9% *NaCl the phagacytarian power is almost equal to that of serum.* It considerably decreases under the action of weaker and stronger solutions of this salt, even more so than in serum which has been made isosmotic with these salt-solutions.

9. *This result leads to the conclusion that the decline of the phagocytarian capacity produced by anisotonic serum, has its cause principally in the alteration of the amount of water in the cells.*

10. *Besides the modification of the amount of water in the cells, another factor comes into play, namely the chemical change, which takes place consequently on the exchange of the contents of the cells with those of their environment and which, as a matter of course, is greater when the cell is surrounded by a simple solution of NaCl than when placed in an isosmotic serum.* This accounts for the fact, that phagocytes which have been submitted to the action of hyper-isotonic solutions of NaCl, when replaced into serum, exhibit a somewhat greater phagocytarian power, than when they are transferred to a 0.9 % solution of NaCl. In the latter case they have not the opportunity, given them in the former, of regaining the ions which they have lost in the anisotonic solutions of salt.

11. *It is very probable that the ions of Ca and of OH belong to this category.*

With regard to calcium, it has been proved that by the addition to the serum of the minute quantity of **0.01 % $CaCl_2$ 6 aq**, i.e. about **0.005 % $CaCl_2$**, the phagocytarian power was raised by about **22.6 %**. The inference is that ions of calcium must have penetrated into the phagocytes.

On the other hand it may be surmised that the phagocytes will lose ions of calcium when the amount of calcium in the medium is lower than that to which the phagocytes are accustomed. This loss of ions of calcium must cause a diminution of the phagocytarian power.

We observe a similar result in the case of the OH-ions; for our experiments have demonstrated that decrease of these ions causes a lowering of the phagocytarian power. *A 5 % diminution of the alkaline reaction of the serum,* which necessarily must lower the amount of alkali in the phagocytes, *produces a noticeable decline in the phagocytarian capacity.*

12. LOEB and after him other investigators have pointed out, that a pure solution of NaCl must be considered injurious to the larvae of lower sea animals, the muscles of the heart, and those of the intestines. *This opinion does not hold for the phagocytes.* The proof of this assertion is found in the fact that in a solution of NaCl isotonic with serum, the phagocytosis is almost as powerful as in the serum itself.

This seeming contradiction may be met by the explanation that the exchange of substance between the leucocytes and the solution of NaCl, especially when the latter is isotonic with the serum, is very small; whilst in the case of other cells (citiated cells, muscular fibre cells) the conditions of the exchange of ions are not so restricted, and consequently the chemical structure of these cells is more easily modified. And it is obvious that a modification of their chemical structure causes a disturbance in their inherent functions.

13. From the facts here recorded, it is evident that in studying the action of the phagocytes on bacteria *in vitro*, the degree of osmotic concentration and of the alkaline reaction of the medium, must be taken into account. This condition has been lost sight of in several of the experiments. They ought therefore to be repeated.

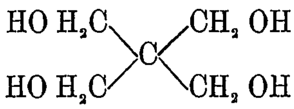
Groningen, June 1907.

Chemistry. — "*The decomposition of penta-erythritol tetraformate on heating.*" By Prof. P. VAN ROMBURGH.

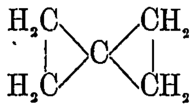
(Communicated in the meeting of June 29, 1907).

As the heating of the diformate of *s.* divinylglycol had led in such a simple manner to hexatriene 1.3.5, investigations have been set on foot in my laboratory for studying the decomposition of formic. esters of polyhydric alcohols, the results of which will be gradually communicated.

If for penta-erythritol we accept the formula:

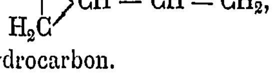


and if the reaction took place in a similar manner as with *s.* divinylglycol diformate, we might expect on heating the tetraformate[1]) the formation of a hydrocarbon of the formula:



in which occurs twice a 3-ring.[1])

[1]) GUSTAVSON C. R. **123** (1896) 242 obtained from the tetrabromide of penta-erythritol, by the action of zinc and alcohol, vinylcyclopropan:



instead of the above cited hydrocarbon.