Physiology. — *"The influence of small amounts of Calcium on the motion of Phagocytes.* By Prof. H. J. HAMBURGER.

Former investigations have shown that small amounts of calcium are able to promote phagocytosis considerably [1]). An addition for instance of 0.005 % $CaCl_2$ to the serum caused an increase of about 22 % in the phagocytarian power. This favourable effect of chloride of calcium becomes even more strongly manifest when, instead of being added to the serum, it is added to NaCl-solutions.

These investigations have been continued now in two directions.

In the first place we have asked ourselves whether the influence of Ca would also manifest itself *in the living body.* All experiments had hitherto been made outside the body. If — we argued — the phagocytarian power, heightened by Ca-Ions, is based upon an acceleration of the amoeboid motion then it may be expected that by Ca chemotaxis will be promoted likewise. And therefore we determined *the chemotaxis with and without the addition of chloride of calcium* in the manner described below.

At the same time this investigation would furnish an answer to a question raised in another quarter. In the Zeitschrift für Balneologie of August 15, 1909 we read that the Prussian Ministry of Public Worship, Education and Medical Affairs has addressed the following question to the Kaiserliche Gesundheitsamt: "Ist ein Mineralwasser, das eine isotonische Kochsalzlösung darstellt, durch einen Gehalt von 0.1 % Chlorcalcium gemäss den Untersuchungen des Prof. HAMBURGER in Groningen geeignet, dem Körper Stoffe zuzuführen, die in dem Serum die Aufgabe haben, den Verdauungsprocess der Bakterien vorzubereiten, die Phagocytose erheblich zu steigern? Sind einschlägige Untersuchungen in staatlichen Instituten mit einem Mineralwasser, das jene chemischen Vorbedingungen erfüllt, zu empfehlen?"

Prof. Dr. H. KIONKA (Jena) sent in a report on the matter, setting forth the great desirability of these investigations.

With a view to the interest from balneological quarters I have therefore at the same time made some experiments with a mineral-water containing much Ca; I took for it the water of the Virchow-Quelle at Kiedrich near Eltville (Wiesbaden).

Still in another direction we have carried on our investigations. We have namely made attempts *to penetrate further into the nature of the remarkable influence exercised by calcium.*

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[1]) HAMBURGER and HEKMA. These Proceedings Vol. X p. 144.
Biochemische Zeitschrift 9, 275, 1908.

A. Influence of calcium on the chemotaxis.

To test the influence of calcium upon the chemotaxis two methods were applied.

The first method consisted in placing under the skin of a rabbit small capillary tubes, closed at one end, and filled with a suspension of B. coli commune in NaCl solutions, containing or not containing $CaCl_2$. After some time the lengths of the leucocyte columns which had entered were measured.

The second method consisted in $CaCl_2$ being introduced into the intestinal canal of some rabbits and not in that of others; and investigating after some time to what extent the capillary tubes filled with the coli-suspension had attracted in the first rabbits a longer phagocyte-column than in the second.

Besides cultures as such, we also put into the capillary tubes instead of them, the liquid without bacteria, that is to say the products of the bacteria.

For the technical details we refer to the Biochemische Zeitschrift. Here we may mention that the capillary tubes were fastened into small flat pieces of cork, in which holes had been pricked beforehand, and further that for the experiments rabbits were used viz. the inside of the thigh. It is easy to make a pocket in the skin there, in which the piece of cork with the capillary tubes can find a place. This having been put in, the wound was closed.

We will now mention the result of some of the experiments.

First Method.

Experiments with bacteria suspensions with and without calcium.

For the following experiment three pieces of cork were each provided with two capillary tubes. In the two tubes of the first cork we put a suspension of B. Coli in NaCl 0.9 %. In the two tubes of the second cork the fluid was NaCl 0.9 pCt. + 0.01 % $CaCl_2$ and of the third cork NaCl 0.9 % + 0.05 % $CaCl_2$. The first and the third cork were placed under the skin of the right leg, the second (the one with 0.01 % $CaCl_2$), under that of the left leg. They remained there for 24 hours. Then the lengths of the leucocyte columns which had entered, were measured.

The following table gives the results of one of the experiments.

It is seen that by the addition of $CaCl_2$ to the bacteria suspension, chemotaxis has increased.

TABLE I.

Influence of calcium upon the chemotaxis.

Suspension of B. Coli commune in a solution of NaCl 0.9% in which has been dissolved:	Lengths of the phagocyte columns in two capillary tubes:
0 % $CaCl_2$	$\frac{1}{2} + 2 = 3\frac{1}{2}$ m.M.
0.01 „ „	2 + 2 = 4 „
0 05 „ „	$2\frac{1}{2} + 2\frac{1}{2} = 5$ „

The same result was obtained when instead of the suspension as a whole we took the suspension freed from bacteria. We subjoin as an example:

TABLE II.

Influence of Calcium upon Chemotaxis.

	Right Leg	Left Leg
	Extract of Colibacteria in NaCl 0 9%	Extract of Colibacteria in NaCl 0 9% + $CaCl_2$ 0 01%
Total of 3 leucocyte-columns after 24 hours	4.25 mM.	4.75 mM.

Of the many other experiments made in the same way as those on which Table I and II are based, a detailed account will be found in the Biochemische Zeitschrift. The results were in all cases the same.

Second method.

Introduction into the intestinal canal of fluids containing calcium.

The difference between this method and the first lay in the fact that we added Ca not to the contents of the capillary tubes, but to the tissue fluid. We accomplished this by rectal injection of fluids containing Ca. As such we used in the first place NaCl sol 0.9 % in which $CaCl_2$ had been dissolved, and secondly a mineral water containing calcium.

a. NaCl-solutions containing Ca.

After the faeces in the rectum had been removed by soft pressure on the belly 60 cc.fluid were brought into the rectum of each of 4 rabbits, 15 cc. four times a day. The first rabbit got 60 cc. NaCl

0.9 %; the second 60 cc. NaCl 0.9 % in which 0.1 Gr. $CaCl_2$ had been dissolved; the third received in the same way 0.2 Gr. $CaCl_2$ and the fourth 0.5 Gr. $CaCl_2$. In each case a cork-slice with three capillary tubes containing a filtrated culture of B. coli commune had been placed under the skin. The result is found in the following table.

TABLE III.

Influence of calcium upon chemotaxis.

	Fluids introduced into the rectum:	Total length of the 3 leucocyte columns:
Rabbit 1	60 cc NaCl sol of 0.9 %	5 mM.
„ 2	60 „ „ „ +1 Gr. $CaCl_2$	9.5 „
„ 3	60 „ „ „ +0.2 „	9 „
„ 4	60 „ „ „ +0.5 „	9 30 „

It follows from these experiments:

1. that all solutions containing $CaCl_2$, have effected a more extensive chemotaxis than the pure NaCl-solution.

2. that an introduction of more than 0.1 Gr. $CaCl_2$ has caused no further increase of chemotaxis.

Further we wished to know whether the phenomenon would repeat itself the next day, if the experiment was continued, in other words if new capillary tubes were put in, and fresh injections were added. The following table may serve as an answer.

TABLE IV.

Influence of Calcium upon Chemotaxis.

	Fluid injected into the rectum:	Total length of the 3 lococyte columns
Rabbit 1	60cc NaCl sol of 0 9%	4.5 mM.
„ 2	60 „ „ „ +0.1 Gr. $CaCl_2$	8.7 „
„ 3	60 „ „ „ +0.2	10 5 „
„ 4	60 „ „ „ +0.5	8 „

It appears that when the experiment is continued a second day Ca has promoted chemotaxis likewise.

We may add that the same results were obtained when *unfiltrated cultures* were used. Comparative experiments, however, with filtrated and unfiltrated cultures upon one and the same animal showed that

the leucocyte columns were longer in the capillary tubes with the filtrated culture. It is not difficult to explain this, seeing that the white blood corpuscles which have entered, are partly filled up with a considerable amount of coli-bacteria and are destroyed in consequence.

b. Mineral water rich in calcium (Virchow-Quelle).

The experiments were identical with those made on the influence of NaCl sol. containing $CaCl_2$. First, however, it had to be calculated, how much mineral water had to be injected into the rectum. According to the analysis of H. FRESENIUS it contains about 0.1% $CaCl_2$. To administer therefore 0.1 Gr. $CaCl_2$, as in the above experiments, it would be necessary to take 100 cc. of the water per day. In doing this there was no reason to expect that part of the water would be thrown out, but yet the volume could not be called small. Guided by the amount which is given to man, we come to a smaller volume for a rabbit than 100 cc. For in the case of men an average quantity of 1 L. of the water is prescribed per day. Calculating the weight of a man at 65 K.G., that of the rabbits at 3.5 KG., the rabbits would have to receive in proportion $\frac{3.5}{65} \times 100 = 54$ cc. Therefore we have given to the animals 60 cc. per day, distributed over four times, that is to say 15 cc.every time. *So they got* 0.06 *Gr.* $CaCl_2$ *per day.*

Let us now communicate some of the results. It need hardly be said that to control the experiments rabbits were also injected with pure NaCl-sol. 0.9%.

Results: The total length of the 6 leucocyte-columns (2 legs) amounts to:

7.25 m.M. in the NaCl rabbit.
9 m.M. in the Virchow-Quelle rabbit.

Similar results are given by the following experiment:

Result: The total length of the 6 leucocyte columns (2 legs) amounts to:

8.4 m.M. in the NaCl rabbit.
12 m.M. in the Virchow-Quelle rabbit.

These experiments show that an introduction of only 0.06 *Gr.* $CaCl_2$ *promotes chemotaxis considerably.* Even without measuring the lengths of the phagocyte columns, one may convince oneself that this conclusion

is the correct one. When opening the skin wound, it is immediately seen that *in the Virchow-rabbit a much thicker mass of phagocytes has gathered round the tubes than in the NaCl-rabbit.* The same thing we observed invariably in all experiments where NaCl-solutions containing $CaCl_2$ were injected.

After some reflection one is surprised at the great influence of this exceedingly small amount of Ca. The increase of Ca-percentage in the lymph must be very slight indeed. Let us assume for a moment that the 0.06 Gr. $CaCl_2$ have been distributed equally over the blood- and tissue-fluids of the animal, then the increase in Ca-percentage can only be very small. A rabbit of 3500 Gr. contains $\frac{3500}{100} \times 8$ Gr. $= 280$ Gr. of blood i. e about $280 \times \frac{2}{3} = 185$ cc. of serum. If further we assume that the animal contains 100 cc. tissue-fluid, then the Ca percentage of the tissue-fluid will have been raised by $\frac{100}{285} \times 0.06\% = 0.02\%$. As has been said above we take for granted, that the Ca has been distributed entirely and exclusively over the 285 cc. of fluid, in other words that nothing has penetrated into the tissue-cells or into the blood corpuscles [1]) or has left the kidneys. This calculation is very arbitrary, but still it gives some idea of the slight increase of calcium concentration, necessary to raise the chemotaxis from 7.25 to 9 or from 8.4 to 12 i. e by $\frac{12-8.4}{8.4} \times 100 = \pm 40\%$.

And this increase represents only a minimum value. For when a column of leucocytes has entered the capillary tube it impedes a further entrance of the movable cells, chiefly by the fact that now the liquid contents of the capillary tubes cannot diffuse freely into the surroundings. This furnishes an explanation why the influence of calcium is not so manifest when the capillary tubes are left under the skin for a longer time, for instance for 48 hours instead of 24. From this point of view it would have been advisable to leave the capillary tubes for a shorter time than 24 hours. Then the difference in percentage between the leucocyte-columns in normal and in calcium animals would undoubtedly have been greater. A technical drawback would have been, however, that the absolute lengths of all columns would have been smaller, and not so easy to measure accurately.

1) Which is indeed the case with the red blood corpuscles. Compare our treatise on the permeability of blood cells to ions of Ca. These Proc. XI p. 718.

It is obvious that this remark also holds good as regards the results with Na Cl-solutions, containing or not containing calcium.

All these experiments show plainly that chemotaxis is considerably promoted even by slight quantities of calcium.

We now come to the second question: how to account for this promotion of chemotaxis and for the increased phagocytosis observed before?

B. Why are phagocytarian power and chemotaxis heightened by calcium?

As regards chemotaxis the answer is pretty obvious. The entering of a large number of phagocytes into the capillary tubes can scarcely be explained in any other way than by a greater mobility of the cells. Is the increase of phagocytosis to be explained in the same way? Or are we to think of a greater development of force, manifesting itself by the presence of calcium in the phagocytes, and enabling cells which under normal circumstances would be too weak to take up particles, to do so now. There are grounds for thinking of this possibility, if we remember the way in which the phagocytarian capacity was determined by us. To a suspension of leucocytes, carbon-particles were added, and now it was investigated which percentage of leucocytes, both with and without calcium, had absorbed carbon.

It seemed not difficult to establish which of the two factors must be held responsible for the favourable effect of calcium: the acceleration of the amoeboid motion or the increase in force of this motion.

All we had to do to investigate this, was to take two equal suspensions of leucocytes, add calcium to one and not to the other, add carbon particles to both and to examine if the suspension without calcium after sufficient lapse of time would develop as great a phagocytarian capacity as the one with calcium. If this was really the case, then the favourable action of calcium had only to be attributed to an increased velocity of the amoeboid motion.

The following experiment may serve as an answer to the question. The method we adopted is shown in the following table.

To form a correct idea of it, it must be observed that before the addition of carbon the suspension had been heated to 37° and further that when the time of action was finished, the leucocyte-carbon suspensions were immediately placed in cold water to cut short the phagocytarian process as soon as possible.

TABLE V.

Influence of the Time on the extent of Phagocytosis [1]).

Time during which the phagocytes could take up carbon:	Percentage of Leucocytes having absorbed carbon:		Increase of phagocytosis by Calcium in %
	The leucocytes are in: NaCl 0.9 %	The leucocytes are in: NaCl 0 9% + $CaCl_2$ 0.05%	
10 minutes	$\frac{156}{500} \times 100 = 31.2\%$	$\frac{155}{506} \times 100 = 30.6\%$	2 %
20 „	$\frac{146}{460} \times 100 = 31\ 7$ „	$\frac{171}{428} \times 100 = 39.9$ „	26 „
30 „	$\frac{219}{529} \times 100 = 41\ 5$ „	$\frac{246}{420} \times 100 = 58.5$ „	40 1 „
1 hour	$\frac{270}{560} \times 100 = 48.2$ „	$\frac{339}{514} \times 100 = 65\ 9$ „	37 „
1½ „	$\frac{322}{503} \times 100 = 64$ „	$\frac{359}{602} \times 100 = 59\ 6$ „(?)	
2 „	$\frac{302}{478} \times 100 = 65.1$ „	$\frac{309}{467} \times 100 = 66.1$ „	1 5 „

This table shows, that already after 10 minutes a considerable number of phagocytes have taken up coal. The influence of calcium cannot yet be observed here. This is the case, however, where the leucocytes have been in contact with carbon particles for 20 minutes, still more when the time was 30 minutés, whilst the greatest difference is to be observed after they have been together for one hour. It is seen that in the suspension without calcium 48.2% of the leucocytes have taken up carbon, whilst in the suspension containing calcium this figure was already 65.9%. This is evidently the maximum. This same maximum, however, is very nearly reached in the fluid without Ca, but about *half an hour after.*

This experiment, made with blood-corpuscles of another animal gives similar results as the preceding one: after one hour the phagocytosis in the suspension containing Ca is still considerably greater than in the suspension without Ca. After two hours they are about equal (59.8 and 59.4).

[1]) The experiments on phagocytosis mentioned in this treatise have been made in collaboration with Mr. J. DE HAAN, Med. Cand., assistant at the Physiologica Laboratory.

Repetition of the Experiment.

TABLE VI.

Effect of the time on the extent of Phagocytosis.

Time during which the phagocytes could take up carbon-particles:	Percentage of Leucocytes having taken up carbon:		Increase of Phagocytosis by Ca in %
	The leucocytes are in: NaCl 0 9%	The leucocytes are in: NaCl 0.9%+$CaCl_2$0.05%	
10 minutes	$\frac{104}{284} \times 100 = 36.6\%$	$\frac{214}{574} \times 100 = 37.2\%$	1 7%
30 „	$\frac{127}{327} \times 100 = 38.8$ „	$\frac{146}{330} \times 100 = 44.2$ „	14 „
1 hour	$\frac{171}{404} \times 100 = 42\ 3$ „	$\frac{235}{458} \times 100 = 51\ 3$ „	91 3 „
2 „	$\frac{304}{520} \times 100 = 58.4$ „	$\frac{200}{334} \times 100 = 59.8$ „	2 4 „
4 „	$\frac{165}{302} \times 100 = 57\ 9$ „	$\frac{153}{253} \times 100 = 60.4$ „	4.3 „

The fact of its remaining a little greater in the solution containing Ca than in the liquid without Ca must probably be attributed to the circumstance that with the first the phagocyte-contents are better balanced, in other words sustain less change than in the NaCl-solution without Ca.

Finally a third experiment may be mentioned. It gave the same results as the 2 first.

TABLE VII.

Influence of the time on the extent of phagocytosis.

Time during which the phagocytes could take up carbon-particles:	Percentage of leucocytes, having taken up carbon:		Increase of phagocytosis by Ca in %
	The leucocytes are in: NaCl 0 9%	The leucocytes are in NaCl 0.9%+$CaCl_2$0.05%	
10 minutes	$\frac{183}{493} \times 100 = 37.1\%$	$\frac{185}{405} \times 100 = 45.9\%$	23 7%
1 hour	$\frac{142}{294} \times 100 = 48\ 2$ „	$\frac{241}{369} \times 100 = 65.3$ „	35.4 „
2 „	$\frac{214}{333} \times 100 = 64.2$ „	$\frac{243}{384} \times 100 = 64.2$ „	0 „

The results obtained in the *experiments on chemotaxis* and in *those on phagocytosis show uniformly that the influence of Ca is based upon an acceleration of the amoeboid motion.*

It may further be asked *why* calcium accelerates the amoeboid motion of the phagocytes. We might suppose a modification in the agglomeration of the colloid protoplasm-particles. This might be occasioned by a modification in the electric charge owing to the entering of the bi-valent kation. If this were the case then it would not be improbable that the other bi-valent metal-ions such as barium, strontium or magnesium would promote phagocytosis likewise. The experiment has taught, however, that this is by no means the case. I shall mention here only one of the many experiments made on the subject.

To three solutions, viz. NaCl 0.9%, NaCl 0.9% + 0.11% $BaCl_2$ + 2 Aq and NaCl 0.9% + 0.05% $CaCl_2$ equal quantities of a leucocyte suspension were added. After the fluids had acted upon it for 2 hours, carbon was added and half an hour after, it was investigated what percentage of the leucocytes had taken up carbon. The following table gives the results of the experiments.

TABLE VIII.
Influence of Barium and Calcium.

Solution:	Percentage of leucocytes having taken up carbon
NaCl 0.9%	$\frac{58}{234} \times 100 = 24.8\%$
	$\frac{54}{229} \times 100 = 23.4$ „
	$\frac{68}{270} \times 100 = 25.1$ „
NaCl 0.9% + 0.11% $BaCl_2$ 2 Aq	$\frac{60}{279} \times 100 = 21.7$ „
NaCl 0.9% + 0.05% $CaCl_2$	$\frac{192}{377} \times 100 = 50.9$ „

This experiment shows that barium has exercised no determinable influence upon phagocytosis, calcium on the other hand in a very high degree.

This result is confirmed in the case of the same leucocytes, after

they have been left to themselves *for 24 hours in a 0.9% NaCl-solution.* After that time a fixed amount of the leucocytes is added to a fresh solution of NaCl 0.9,%, of NaCl 0.9% + 0.11% $BaCl_2$ and of NaCl 0.9% + 0.05% $CaCl_2$.

TABLE IX.
Influence of Barium and Calcium.

Solution:	Percentage of leucocytes having taken up carbon:
NaCl 0 9%	$\frac{15}{473} \times 100 = 3.2\%$
" + 0.11% $BaCl_2$	$\frac{16}{453} \times 100 = 3.5$ "
" + 0.05% $CaCl_2$	$\frac{113}{216} \times 100 = 52.3$ "

These experiments show that when the phagocytes, by being exposed a long time to NaCl 0.9%, have almost entirely lost their power, *they cannot be revived by barium.* An isosmotic quantity of *calcium however, produces this effect in a very marked degree.*

The action of strontium was identical with that of barium.

Finally we may add an experiment with magnesium.

TABLE X.

Solution:	Percentage of leucocytes containing carbon:
NaCl 0 9%	$\frac{1}{529} \times 100 = 0.2\%$
" + 0 05% MgCl	$\frac{8}{807} \times 100 = 2.2$ "
" + 0.05% CaCl	$\frac{261}{532} \times 100 = 49$ "

Here it appears again that the disabled phagocytes are somewhat revived by the addition of some magnesium, but that the effect is incomparably much greater, an isosmotic amount of $CaCl_2$ being added.

From these experiments a greater number of which, with more detailed descriptions will be found in the Biochemische Zeitschrift, it may be concluded that the considerable increase of phagocytarian capacity effected by calcium, cannot be explained by the electric charge inherent in Ca as a bi-valent ion, but that we have to deal here with *a specific, biochemical property of this element.*

To throw more light upon the special significance of calcium we may incidentally mention that it is especially this element which represents the favourable effect of RINGER's fluid on phagocytosis. The following experiment may serve as an illustration.

Equal amounts (0.15 cc.) of the same leucocyte-suspension were mixed with equal amounts (2 cc.) of a sol. of NaCl 0.9 %, of RINGER's fluid without Ca (viz NaCl 8, $NaHCO_3$, KCl 0.075, 1000 aq.) and of RINGER's fluid containing different quantities of $CaCl_2$.

The leucocyte-suspension having been exposed to carbon for 30 minutes it was determined in the usual way what was the percentage of leucocytes containing carbon. The following table gives the results of an experiment.

TABLE XI.

Importance of Calcium in RINGER's fluid.

Solutions:	Percentage of leucocytes containing carbon:	
		Average:
NaCl 0.9%	38.6%—42 % [1])	40.3%
RINGER's fluid without $CaCl_2$	37.1 „ —41 „	39 „
„ „ with 0.005% $CaCl_2$	41.5 „ —43.1 „	42.3 „
„ „ „ 0.01 „ „	42.8 „ —45.5 „	44.1 „
„ „ „ 0.05 „ „	49.3 „ – 51.7 „	50.5 „

It is seen that RINGER's fluid without calcium is not more favourable to phagocytosis than NaCl 0.9 % alone; it would rather seem to be a little impeded by it. *Addition of calcium, however, even of mere traces, promotes phagocytosis considerably.*

What influence this element exercises here can only be guessed

[1]) The two values are in all cases given by two observers. It is remarkable that the one always gets a higher figure for phagocytosis than the other, although the preparations were taken from the same suspension. Evidently the one sees coal in a cell sometimes, where the other does not.

at the present moment. About the real cause of the amoeboid motion of living protoplasm we know at present nothing with any amount of certainty. We have only suppositions; so we have thought for instance of the possibility that calcium would bring about a decrease in the surface tension of the phagocytes. Now the surface tension or rather the molecular constant of two contiguous layers is, as we know, expressed by the formula $K_{1\cdot 2} = K_1 + K_2 - A_{1\cdot 2}$, K_1 representing here the molecular constant of the extreme layer of phagocytes, K_2 the molecular constant of the surrounding fluid, and $A_{1\cdot 2}$ representing the energy resulting from the contact of the two surfaces. It would be of importance to be able to demonstrate that under the influence of calcium $K_{1\cdot 2}$ decreases. Hitherto, however we have failed to determine this value, even approximately. The only thing we could do was to establish whether the surface tension of the surrounding medium (fluid) viz. K_2 underwent any change under the influence of calcium. But we have not been able to discover any such change, neither in a positive nor in a negative sense.

We are still occupied with a further investigation concerning the nature of the effect produced by calcium. Perhaps in its turn it lays open a road to penetrate into a more general and more important problem viz. the cause of the motion of living protoplasm.

SUMMARY.

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

1. *Chemotaxis is considerably promoted by slight amounts of calcium.*

This was demonstrated in two ways:

a. by placing under the skin capillary tubes containing bacteria cultures (B. Coli) with and without calcium and comparing the lengths of the columns of leucocytes which had entered into the tubes.

b. by injecting NaCl solutions with and without Ca into the intestinal canal and measuring subsequently in both cases the lengths of the columns of leucocytes which had been attracted into the capillary tubes filled with the bacteria suspension.

The experiments sub *a*, and sub *b* were carried out with bacteria suspensions as such, and with filtrates obtained by means of CHAMBERLAND's filters.

The results were similar in both cases.

The fluids containing calcium which were brought into the intestinal canal were:

1. NaCl-solution containing $CaCl_2$.

2. The water of the Virchow-spring (Kiedrich near Eltville, Wiesbaden) which contains a great amount of Ca. The influence of both fluids turned out to be very considerable:

If only 60 cc. of the above mineral water was injected daily into the intestinal canal of rabbits, a quantity corresponding with 0.06 Gr. $CaCl_2$, the chemotaxis increased by about 40 %. It must be observed that this increase represents only a minimum value.

2. *These chemotactic investigations have proved that calcium increases the activity of phagocytes to a very considerable extent, not only in vitro but also in the living organism.*

During 48 hours this influence remained undiminished. Very probably it extends over a much longer period. The way in which the experiments were conducted, however, did not admit their being continued for a longer period, with the same animal.

3. *This increased activity of the phagocytes cannot be accounted for by an increased intensity of the cell contractions, but finds its cause in an acceleration of the amoeboid motion.*

As regards chemotaxis this needs no further proof; as regards phagocytosis this could be demonstrated by the following experiment: when suspensions of leucocytes without calcium are left only sufficient time to take up carbon particles, the percentage of leucocytes having taken up carbon becomes equal to that which is observed, in a shorter time indeed, in suspensions with Ca.

4. If we ask ourselves what may be *the cause* of calcium accelerating the amoeboid motion of phagocytes, we might be inclined to think of a modification in the agglomeration of the colloid protoplasm-particles as a consequence of the electric charge, caused by the entering of a number of bi-valent calcium ions. This explanation however can hardly be the correct one. For the experiment teaches that other bi-valent kations namely barium, strontium, magnesium do not cause an acceleration of the amoeboid motion.

It must be assumed then, that the action of calcium in this case, is based upon a specific, hitherto unknown, biochemical property. As another example of the great influence of calcium we may also mention the fact that the favourable effect of RINGER's fluid, on phagocytosis, must be exclusively attributed to this metal.

(June 23, 1910).