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violett" nor in any other dye experimented upon in this respect. Only „Säure-fuchsin", a dye which is no chloride and deviates considerably in composition from fuchsin showed something like it. It does not make any difference whether Säurefuchsin or Rubin S of GRÜBLER or Saurefuchsin S.M.P. of the Actien-Gesellschaft für Anilinfabrikation of Berlin is used for this purpose. I have never been able to state with certainty whether in a $^1/_{50}$ % Säure-fuchsin-solution being almost entirely decolored the color partly returns after the filtration of the carbon; But I have experienced, that in the almost decolored solution after the filtration and even after the lapse of some weeks the color can suddenly and very intensely be reproduced by acetic acid. It must be taken in consideration in this case, that acetic acid stains likewise a diluted Säure-fuchsin-solution which has never been in contact with carbon, somewhat more deeply, but by far not so much as the solution almost decolored by carbon.

I desist from suggesting an hypothesis for the explanation of the last mentioned phenomenon, and only hope, that the nature of what I have communicated here may, at some time or other, be explained and increase our knowledge of the theory of histological staining methods.

Physiology. — *"Phagocytes and respiratory centre."*

Their behaviour when acted upon by oxygen, carbonic acid, and fat-dissolving substances. Explanation of the excitement-stage in narcosis." [1]) By Prof. H. J. HAMBURGER.

(Communicated in the meeting of March 27, 1915).

Introduction.

In a former paper it was shown that Iodoform, even in extremely slight quantities can accelerate phagocytosis, to a considerable extent [2]). We explained this action by assuming that this substance, after being dissolved in the lipoid surface, softens the cells, thus facilitating the amoeboid motion.

If this view were correct, it might be expected that other substances which are soluble in lipoids, would act in the same way. This was indeed the case, without a single exception, with all the substances investigated, only not, as we found afterwards, with carbon sulphide. But in chloroform, chloralhydrate, ethylalcohol [3]), butyric acid, propionic acid [3]), benzole, turpentine, camphor, Peruvian balsam [3]) (cinnamic

[1]) A detailed account will appear in the Internationale Zeitschrift für physikalisch-chemische Biologie. (ENGELMANN, Leipzig).

[2]) H. J. HAMBURGER, J. DE HAAN and F. BUBANOVIC, These Proceedings, March 25, 1911.

[3]) H. J. HAMBURGER and J. DE HAAN, Ibid, October 28, 1911.

acid) the same property became manifest, even when they were taken in very weak concentrations e.g. propionic acid 1 : 10.000.000, chloroform 1 : 5.000.000, chloralhydrate 1 : 20.000, alcohol 1 : 10.000, concentrations answering to the division-coefficients of these substances between oil and water.

In order to penetrate more deeply into the nature of this phenomenon, we asked ourselves if the entrance of these substances into the phagocytes resulted in a decreased viscosity or in a decreased surface-tension. But experiments in this direction made by BUBANOVIC in our laboratory [1]), and after another and better method in that of Prof. ARRHENIUS [2]) at Stockholm, gave negative results; so did other experiments taken by myself later on. The object of these experiments was to investigate if the surface-tension of oil decreased under the influence of small quantities of chloroform and similar substances.

It must, however, be remembered that the lipoids of the cell-surface may not be considered identical with oil, so that it is not impossible that after all we have to deal with a decreased surface-tension. In order to ascertain if this is really the case the experiments of BUBANOVIC would have to be repeated with the lipoids of the white blood-corpuscles, but it is very difficult to obtain these substances in sufficient quantities. Perhaps in the future, methods may be available enabling us to determine these values with slighter quantities than are required at present.

But however this may be, as yet the experiments which aimed at establishing a modification in the viscosity or surface-tension under the influence of traces of fat-dissolving substances, have led to negative results. [3])

Whilst looking forward to these researches with the lipoids of the blood-corpuscles or, better still, with naked protoplasm, we asked ourselves whether perhaps the acceleration of phagocytosis would not be accompanied by an increased oxygen-consumption, would perhaps even be caused by it.

This possibility had already been suggested by us before [4]), and

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[1]) F. BUBANOVIC, Zeitschr. f. Chemie und Industrie der Kolloide. **10** (1912), 178.

[2]) F. BUBANOVIC, Middelanden f. K. Vetenskaps-Akademiens, Nobelinstitut N°. 17 (1911).

[3]) Later experiments however have shown, that small amounts of chloroform diminish the viscosity of YOLK. [Note added to the translation].

[4]) H. J. HAMBURGER: Physikalisch-chemische Untersuchungen über Phagozyten. Ihre Bedeutung von allgemein biologischem und pathologischem Gesichtspunkt. Wiesbaden, J. F. BERGMANN, 1912, S. 167.

had led HEGER and BARUCH to investigate the absorptive power of red blood-corpuscles for chloroform in chloroform-narcosis. These investigators found indeed that during the chloroform-narcosis the oxygen-percentage of the red blood-corpuscles is modified [1]). It was found to have increased. Because less oxygen is used?

We began now by investigating, to what extent in an ordinary leucocyte-suspension i.e. without chloroform the phagocytosis depended on the oxygen-percentage of the medium.

For these investigations no carbon was used, because, as we know, this substance possesses the property of absorbing gases to a considerable extent. Instead of it we made use of amylum of rice-flour. The technical part had been worked out by Dr. J. DE HAAN, who, in consequence of the European war was prevented from completing his investigation. A detailed description of the technical part will, therefore, be published later on.

The principle for determining the degree of phagocytosis was the same as that for the taking up of carbon. It was namely determined which percentage of the leucocytes counted, had taken up amylum after a certain time.

I. *Comparison of the extent of the phagocytosis in a NaCl-solution which had been treated with nitrogen, with atmospheric air and with oxygen.*

As regards the way in which the experiments were carried out the following may be observed.

A thick suspension of horse-leucocytes in NaCl 0.9 % is prepared in the manner we described before. [2])

Further a considerable volume of NaCl-sol. 0.9 % is boiled out, an increase in the concentration being obviated.

a. part is treated with nitrogen.
b. „ „ „ „ atmospheric air.
c. „ „ „ „ oxygen.

Thus NaCl-solutions with increasing oxygen-percentages were obtained. We satisfied ourselves of this by oxygen-determinations according to the method of WINKLER with manganous chloride, natrium-thiosulphate, hydrochloric acid and I in KI. 1 cubic centimetre of the thiosulphate-solution corresponds with 0.0782 mmg. of oxygen.

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[1]) HEGER et BARUCH. Instit. Solvay 13 Fasc. 1; Bulletin de l'Acad. Royale de Médecine de Belgique Séance du 26 Juillet 1913.

[2]) Cf. inter alia. Physik. chem. Untersuchungen über Phagozyten. Wiesbaden, J. F. BERGMANN 1912.

A description of the method is given a.o. by HANS FILLIÉ, Zeitschrift f. allgem. Physiol. **8**, (1908) 496.

To 4 cc. of the solutions *a*, *b* and *c* 0,1 cc. of serum is added and to these mixtures 0.3 cc. of the thick leucocyte-suspension.

After they have been exposed to room-temperature for half an hour, during which time they were repeatedly stirred gently, 0.3 cc. of an amylum-suspension in NaCl 0.9 % is added to the suspensions, after which they are kept at 37° in an incubator. After 20 or 30 minutes they are simultaneously taken out and the phagocytosis is stopped by placing them in icewater and adding formol. Then preparations are made which are examined after.

The reader will have noticed that in these experiments serum is added. Unlike carbon, amylum is only taken up if the fluid contains some serum. The most desirable quantity amounts according to DE HAAN's researches to $2^1/_2$ vol. percent. This was confirmed by OUWELEEN, who will soon publish further particulars in a dissertation.

Further particulars relating to the technicalities of the amylum-phagocytosis are omitted here. We can now proceed to summarize the results of one series of experiments in a table.

TABLE I.

Comparison of the extent of the phagocytosis in NaCl-solution, which had been treated with nitrogen, with oxygen and with atmospheric air. Phagocytes and amylum had been in contact for 20 minutes [1]).

The leucocytes are in	Number of leucocytes counted	Number of leucocytes having taken up amylum	Percentage of leucocytes containing amylum
NaCl-solution *treated with nitrogen*	577	159	28.5 %
NaCl-solution *treated with air*	672	130	19.3 %
NaCl-solution *treated with oxygen*	835	110	13.1 %

[1]) If the leucocyte-suspension remains at 37° in contact with amylum for a *longer time*, the values denoting the extent of phagocytosis will be greater. But the *differences* in the degree of phagocytosis become smaller and smaller. At length a time will come when in all three fluids the phagocytosis is the same. This is the case mostly after about $1^1/_2$ hour. The reason is that we have to do with a difference in velocity. Evidently the phagocytosis went slowest in the solution treated with oxygen, fastest in the one treated with nitrogen. If the phagocytes in the oxygen solution are left sufficient time, they will finally have taken up amylum in as ample a degree as the phagocytes in the nitrogen-medium in a shorter time.

This table brings the unexpected result, the phagocytosis is greatest where the slightest amount of oxygen was present.

We see namely that in the NaCl-solution treated with nitrogen the phagocytosis is about $\frac{28-19}{19} \times 100 = 47\%$ greater than in the one treated with air; and in the latter again $\frac{19-13}{13} \times 100 = 46\%$ greater than in the one treated with oxygen.

A repetition of the experiment when only the NaCl solutions were compared which had been treated with nitrogen and with oxygen gave a similar result.

TABLE II.

Effect of nitrogen and of oxygen on phagocytosis.

In the fluid treated with *nitrogen*	22.2 %
" " " " " *oxygen*	17.6 "
" " " " " *nitrogen*	29.4 "
" " " " " *oxygen*	23.4 "

In the following series of experiments NaCl-solutions which had not been boiled out have been compared; some had been treated with nitrogen, others had not. This treatment consisted in N-gas (from a metal cylinder) being led for $^1/_2$ hour into the bottle with NaCl-solution of 0.9% whilst the fluid was shaken every 5 minutes with the gas on the top of it.

It goes without saying that just as in the experiments of Tables I and II a complete expulsion of oxygen could not be expected, but this was not desired. If this had been aimed at, the suspension which was added afterwards, should also have been treated with N.

TABLE III.

Effect of nitrogen on phagocytosis.

The fluid is not treated	It is treated with *nitrogen*
20.7 %	27.9 %
17.7 "	22.3 "
19.6 "	24 "
16.6 "	28.8 "

Here again the phagocytosis is increased everywhere by nitrogen.

A new confirmation is supplied by the following series of experiments.

TABLE IV.

Effect of a treatment with nitrogen on phagocytosis.
Degree of phagocytosis.

The fluid is not treated	It is treated with *nitrogen*
22.9 %	26.1 %
23.8 „	33 „
23.2 „	27.8 „
20.5 „	31.2 „

Here again a higher degree of phagocytosis showed itself unmistakably, where only a slighter amount of oxygen was met with.

It must be noted that in two instances the results were different. It appeared namely that in one of the experiments the result was as follows:

in the NaCl-solution treated with *air* . . . . . phagocytosis 34.7%
„ „ „ „ „ *oxygen* . . . . „ 36 „

and in the other case:

NaCl-solution treated with *air* . . . . . phagocytosis 40.6%
„ „ „ *oxygen* . . . . „ 41.7 „.

It is obvious, that an increased O-percentage has caused no decrease of the phagocytosis here, rather a slight increase. But these two results will have to be attributed either to mistakes in the experiment, or to individual differences, often met with in the phagocytes of different horses. The considerable amount of material which we have experimented with for many years, leaves no doubt about such differences. It has even occurred that the same horse which had been used 6 times at long intervals, and which had always supplied leucocytes that gave satisfactory results, gave cells the 7th time with which hardly any phagocytosis could be obtained.

This could not be attributed to the nature of the fluids, for with the same fluids another horse gave irreproachable results.

Yet in the results obtained with nitrogen, the possibility remained that this gas contained substances which had accelerated the phagocytosis. This was not very probable since the N, supplied by the

Company "Oxygenium" at Schiedam had been prepared by fractionated distillation of liquid air. It still contains about 1% of oxygen, further gases of the helium group, and a bit of oil-products due to the pumps. At any rate it seemed desirable to carry out experiments with hydrogen likewise.

II. *Effect of hydrogen phagocytosis.*

These experiments were carried out like those with nitrogen. Here too compressed gas was used which had been purified in the usual way. The results, however, were different from what we had expected, the phagocytosis was found to have decreased instead of increased.

The phagocytosis was compared in fluids of which the NaCl-solution had not been treated, and which contained therefore comparatively much oxygen, with the phagocytosis in fluids of which the NaCl-solution had lost the greater part of its oxygen by being treated with H.

TABLE V.

Effect of hydrogen on phagocytosis.

Degree of phagocytosis.

The fluid is not treated	It is treated with hydrogen
24.5 %	14.7 %
20.9 „	14.9 „
18.9 „	20.7 „
21.9 „	18.1 „

The average of the first column comes to 21.5%, that of the second to 17.1%. There can be no doubt, therefore, but the hydrogen has impaired the phagocytosis.

The most obvious explanation was, that some noxious impurity had not been removed altogether. Therefore we used in the following experiments hydrogen which we had prepared ourselves from chemically pure zinc, which had been provided with a thin layer of copper by means of a copper-sulphate-solution of 5%.

Now the results were entirely different; *invariably the phagocytosis was promoted by the treatment with hydrogen.*

TABLE VI.

Effect of Hydrogen on the phagocytosis
Degree of Phagocytosis.

The fluid is not treated	It is treated with hydrogen
20.1 %	25.7 %
20.5 „	22.8 „
18.9 „	26.3 „
18.3 „	24.9 „

This table shows that if the salt-solution is not treated with hydrogen, the phagocytosis averages **19.4**%, if it is treated with hydrogen **24.9**%.

Besides these experiments several others were carried out, which *all resulted invariably in phagocytosis being promoted by the action of hydrogen.*

Only a few series of experiments must be more particularly drawn attention to. Their purpose was to investigate to what extent an intense hydrogen-treatment would produce another degree of phagocytosis than a less intense one.

It appeared then that a less intense treatment raised the phagocytosis from 41.2 % to 47.1 %, whilst an intense treatment only raised it to 45.4 %.

It seemed to us that this must be due to the fact that an *extensive removal* of oxygen causes incipient paralysis, which will make itself the more felt as the oxygen is more completely removed.

If this view was correct, then it must be possible to lower the phagocytosis still more by a still more energetic removal of oxygen, nay to make it fall below that observed in the fluids not treated with hydrogen. It was indeed found possible to do so. We shall give an account of a few experiments taken with nitrogen.

III. *Effect of an extensive removal of oxygen.*

A NaCl-solution of 0,9 % is thoroughly treated with nitrogen; this is also done with the bloodserum, which we did not do as yet; of this serum $2^1/_2$ vol. perc. is added to the NaCl-solution. Of this we take 4 ccm., add 0,3 ccm. of a thick leucocyte-suspension (in NaCl 0,9 %) and leave the mixture exposed to roomtemperature for half an hour. Thus the leucocytes lose oxygen. Now 0,3 ccm. of a

suspension of amylum in NaCl-solution are added, which had likewise been treated with N, and the mixture thus obtained is exposed to the effect of body-temperature for 25 minutes.

If, however, the same experiment was carried out in exactly the same manner, but only with this difference that the fluid treated with nitrogen could act at room-temperature *for 5 hours instead of half an hour* on the phagocytes, then the phagocytosis was found to be much less than in the original fluid, which had not been treated with nitrogen. Hence after a longer exposure of the phagocytes to a medium which contains little oxygen, paralysis will set in, the available amount of oxygen being consumed to a great extent.

This may appear from the following experiments.

TABLE VII

Effect of an extensive withdrawal of O on phagocytosis by a long exposure of the phagocytes to the normal medium and to the medium treated with N.

	Phagocytosis
After a 5 hours' exposure of the phagocytes to the serous NaCl-sol. which had *not* been treated with N.	$\frac{681}{1341} \times 100 = 50.71\%$
After a 5 hours' exposure of the phagocytes to the serous NaCl-sol. which *had* been treated with N.	$\frac{521}{1174} \times 100 = 44.38\%$

Whilst formerly after an exposure of one hour an increased phagocytosis was invariably observed, this increase has changed into a decrease after a 5 hours' exposure.

We shall add another experiment, showing the effect on the *same* leucocytes of an exposure of $^1/_2$ hour and $4^1/_2$ hours.

TABLE VIII.

Effect of a short and of a long exposure of the phagocytes to a solution containing only traces of oxygen.

Exposure of $^1/_2$ hour	Exposure of $4^1/_2$ hours
In the normal serous NaCl-sol.: $\frac{244}{941} \times 100 = 25.9\%$	In the normal serous NaCl-sol.: $\frac{443}{1125} \times 100 = 39.4\%$
In the serous NaCl-sol contain. a trace of O: $\frac{321}{930} \times 100 = 34.5\%$	In the serous NaCl-sol. contain. a trace of O: $\frac{372}{1145} \times 100 = 32.5\%$

Hence we see that the same phagocytes which, after being exposed to nitrogen for half an hour, give a considerable increase viz. $\frac{34\,5-25.9}{25.9} \times 100 = 33.2\,\%$, show a *decreased* phagocytosis of $\frac{39.4-32\,5}{39.4} \times 100 = \mathbf{17.5}\,\%$ after an action of $4^1/_2$ hours, the loss of O having become greater in that time. A longer exposure to the medium containing little O would probably have lowered the phagocytosis still more. The phagocytes will consume more and more their own oxygen.

IV. *Respiratory centre and phagocytosis. Effect of carbonic acid and of potassium cyanide. Discussion of the results obtained.*

If we submit the results obtained to a close examination, we are struck by the agreement between the effect which a withdrawal of oxygen has on the respiratory centre on the one hand, and on the phagocytes on the other.

After the many researches on the respiratory centre we may take it for granted that, besides by the action of carbonic acid, the respiratory centre is also stimulated by a withdrawal of oxygen.

If in an animal the O-percentage of the blood is increased by frequent deep respiration, then this respiration may be stopped for some time without the animal showing any need of it (apnoea). Under these circumstances the stimulus passing from the respiratory centre on to the nerve centres of the respiratory muscles is evidently too weak to act upon it successfully. Likewise with the phagocytes we observe that a considerable increase of the O-supply leads to a decreased activity, a decreased phagocytosis. *If the O-percentage decreases, the phagocytes are stimulated into a higher activity, the phagocytosis increases, while it decreases more and more, subsequently, as more O is lost, in accordance with the fact that all cells of the animal organism need oxygen, if they are to continue their functions.*

The respiratory centre too increases its activity when O is very scarce (dyspnoea), and is paralyzed when O continues to be withdrawn.

Hitherto we have made no quantitative comparisons between the O-percentage of the fluid in which the phagocytes are paralyzed, and that in which the nervous centre refuses to act. These comparisons, however, can only relate to the medium, hardly to the cells themselves. In view of these considerations and also owing to the fact that a quantitative determination of phagocytosis is very tiresome, no experiments have been made in this direction. It may be expected that the respiratory centre will be more sensitive to a withdrawal of oxygen than the phagocytes. The higher nervous centres are certainly still more sensitive than the respiratory centre.

In view of this agreement between phagocytes and respiratory centre the question suggests itself whether other substances have likewise the same effect on both.

Therefore we have in the first place investigated the effect of *carbonic acid* on phagocytosis. Some years ago already we published investigations on the effect of CO_2 on phagocytes, and arrived at the conclusion that the use of somewhat large amounts of CO_2 had an injurious effect on phagocytosis [1]). The effect of slight quantities was not investigated then.

Now that we had a more accurate methode at our disposal, it became desirable to repeat the experiments with slighter quantities of CO_2.

NaCl-solutions were made with different CO_2-percentages by mixing different quantities of a boiled out NaCl-solution with the same NaCl-solution which had been saturated with CO_2.

We prepared the following mixtures: containing

4	Vol.	NaCl-sol.	+	1	Vol.	of the	NaCl-sol.	saturated	with	CO_2...	35	Vol. pct CO_2
9	„	„	+	1	Vol.	„	„	„	„	„	17.5	„ „ „
19	„	„	+	1	Vol.	„	„	„	„	„	8.75	„ „ „
49	„	„	+	1	Vol.	„	„	„	„	„	3.5	„ „ „
99	„	„	+	1	Vol.	„	„	„	„	„	1.75	„ „ „

TABLE IX.

Effect of CO_2 on phagocytosis.

Boiled out NaCl-sol. containing:	Phagocytosis
no CO_2	46.3 %
35 Vol. Perc. CO_2	0
17.5 „ „ „	0.7 %
8.75 „ „ „	4 2 „
3.5 „ „ „	41.9 „
1.75 „ „ „	42 „

This table shows that carbonic acid has effected an entire or entire paralysis of the phagocytosis, except in the concentrations 3.5 % and 1.75 %.

[1]) Hamburger. Virschow's Archiv **156** (1899), 329.

Now the question was whether perhaps below, or in the neighbourhood of the concentration of 1,75 %, there would not be one, in which the phagocytosis was increased. Therefore the experiment was repeated also with weaker concentrations.

TABLE X.

Effect of CO_2 on phagocytosis.

Boiled out NaCl-sol. containing	Phagocytosis
no CO_2	$\frac{145}{519} \times 100 = 27.9\%$
17.5 Vol. percent CO_2	0
3.5 „ „ „	$\frac{150}{565} \times 100 = 26.7\%$
1.75 „ „ „	$\frac{159}{497} \times 100 = 31.9\%$
0.35 „ „ „	$\frac{148}{492} \times 100 = 30\%$
0.175 „ „ „	$\frac{140}{506} \times 100 = 27.6\%$

From this series of experiments it appears, just as from the preceding table, that in the NaCl-solution containing 17.5 % CO_2 the phagocytosis is 0, in that containing 3.5 % about the same as if there had been no CO_2 in it. At 1.75 vol. perc. *the phagocytosis has risen* 14.2 % and at 0.35 vol. perc. CO_2, 7 %. At 3.5 vol. perc. the promotive action is therefore compensated by the noxious effect peculiar to CO_2.

Consequently this series of experiments plainly demonstrates that in weak concentrations carbonic acid increases the phagocytosis, and that in higher concentrations it has a paralyzing effect.

We shall adduce no more experiments in this short article. Let the statement suffice that the result was repeatedly and invariably confirmed.

It should, however, be pointed out that the amount of CO_2, required to effect an increase (or also a paralysis) will have to be greater when the phagocytes are surrounded by serum, than in our experiments where the medium was a NaCl-solution containing only $2\frac{1}{2}$ vol. perc. of serum. On another occasion we shall, for a different

purpose, (the effect of artificial venous congestion on the phagocytosis of bacteria) determine the amount of CO_2 which accelerates phagocytosis when only serum is used.

At any rate it may now be looked upon as an established fact that, as regards carbonic acid, the phagocytes behave exactly like the respiratory centre. For the respiratory centre is also stimulated by slight quantities and paralyzed by greater ones.

As we know, *potassiumcyanide* has a highly stimulating effect on the respiratory centre before paralysis sets in. A violent dyspnoea manifests itself.

It is all but certain that this symptom must be connected with the property this substance has of obstructing the oxygen-consumption of the cells. This becomes manifest, for instance, when we note the effect of KCN on muscular contraction. Even if to the blood with which the muscle is supplied, oxygen is added in an ample degree, traces of potassiumcyanide lower the oxygen-consumption considerably.

What may be the effect of potassiumcyanide on phagocytosis?

The following tables will supply an answer.

TABLE XI.

Effect of KCN on phagocytosis.

Serous NaCl-solution + KCN	Phagocytosis
0	12 1 %
1 : 1000	0
1 : 2000	0
1 : 5000	6.6 %
1 : 10.000	9.7 „
1 : 50.000	23.9 „
1 : 100.000	19.2 „

From this table it appears that in a concentration of 1 to 1000 and also of 1 : 10000, KCN has had a noxious, but on the other hand in weaker concentrations, a favourable effect on phagocytosis.

The following table contains experiments also with weaker concentrations.

TABLE XII.

Effect of KCN on phagocytosis.

Serous NaCl-sol. + KCN	Phagocytosis
0	$\frac{187}{622} \times 100 = 30$ %
1 : 10.000	$\frac{204}{564} \times 100 = 36.1$ „
1 : 50.000	$\frac{289}{675} \times 100 = 42.8$ „
1 : 100 000	$\frac{231}{624} \times 100 = 36.8$ „
1 : 1000.000	$\frac{185}{617} \times 100 = 29.9$ „

Hence we see that in slight quantities potassiumcyanide has a highly stimulating effect on phagocytosis, which is checked by greater quantities. Here again a perfect agreement in the behaviour of respiratory centre and phagocytes.

V. *Explanation of the stimulating effect of traces of chloroform on phagocytosis, and of the excitement-stage in narcosis.*

Let us now return to our startingpoint, viz. to the question what may be the reason why traces of chloroform and similar substances cause an acceleration of phagocytosis.

By VERWORN and his school it has been demonstrated that in the chloroform-narcosis the cells have lost the power of using the oxygen offered to them, for oxydation purposes. There is asphyxia. The supposition suggests itself that *the application of small amounts of chloroform brings about this blockade of oxygen imperfectly,* and that the phagocytes are thus reduced to a condition similar to that which is met with when a short treatment with nitrogen and hydrogen has caused them to lose part of their oxygen, which loss has brought them into a state of increased sensitiveness.

The action of greater amounts of chloroform will cause the potential oxygen percentage, if we may call it thus, to fall still lower, the phagocytosis will begin to decrease: a decrease which likewise sets in at a long action of a medium containing little oxygen, as we obtained it by treatment with nitrogen or hydrogen. (Comp. § III).

We have tested this view experimentally, for instance by allowing

chloroform and nitrogen to act together under various conditions as regards time and concentration. But we shall omit giving an account of these experiments to restrict the size of this paper. Moreover a detailed report will, as we said before, be published elsewhere.

And now the excitement-stage in narcosis.

If we let the various narcosis-theories pass in review, then it appears that not a single one *has even attempted* to give an explanation of the excitement-stage. Our researches on phagocytosis, and the agreement in the conduct of respiratory centre and phagocytes enable us to do so.

When MAX VERWORN in his article "Narkose" in the "Handbuch der Naturwissenschaften" B. VII, 1912, has explained that. in his opinion, narcosis is nothing but a consequence of *acute asphyxia*, and adds a few words on the attendant symptoms in narcosis, he expresses himself as follows:

"Es ist nicht wahrscheinlich, dass diese Nebenwirkung (Excitationsstadium) ebenfalls aus dem einem Punkte der Oxydationslähmung in der Zelle entspringt, *doch fehlt für die Genese dieser Nebenwirkung bisher noch jede Analyse*".

Our investigations of the origin of an increased phagocytosis by oxygen-withdrawal, have shown that also the excitement-stage in narcosis is in perfect agreement with the fact stated by VERWORN in his narcosis theory.

We need only conceive that at the beginning of the narcosis, owing to a decrease in the amount of available oxygen, the sensibility of the higher nervecentres is heightened.

If the chloroform-inhalation is continued, this sensibility will decrease, owing to a further decrease of the potential O-percentage, and finally narcosis will set in. Whether the state of complete narcosis is partly due to other factors, for instance to a semi-coagulation of the protoplasm in the sense of CLAUDE BERNARD, or to a decrease of dispersity of enzymes etc. need not be considered here. First the higher centres which are, as we know, very sensitive to oxygen withdrawal, are paralyzed, then the spinal centres and after that the respiratory centre.

We may add that in the first stage of narcosis not only the higher cortical centres and the spinal centr s pass through an excitement-stage, but according to researches of KNOLL and of ARLOING the respiratory centre is also in a state of heightened irritability.

The question which first suggests itself, is the following: how is it that a decrease of the available oxygen-percentage heightens the irritability of the phagocytes (and ganglion cells).

We might suppose that — as regards the phagocytes — the withdrawal of oxygen affects in the first place the surface of the cells; owing to this fact the surface layer will plunge into their inner part which contains more oxygen; thus the amoeboid motion would be accentuated. The phenomenon would remind of the chemotactical motion of bacteria to an airbubble. In the case of phagocytes we might speak of an "entochemotaxis", if I may be allowed to call it so.

But it might also be assumed that a withdrawal of oxygen causes a decreased viscosity in the cells.

[I have indeed found that if yolk is treated with oxygen, the viscosity increases, whence it follows that the viscosity is indeed affected by the oxygen percentage. Albuminous solutions were not so affected; we must, therefore, think of lipoid substances, and in this we are strengthened by observations of THUNBERG, which were amply confirmed by WARBURG, viz. that lecithin in the presence of iron can bind oxygen in relatively great quantities. They think that an oxydative decomposition of lecithin takes place, but could find no oxydation products In my opinion we have to deal here with a compound of lecithin iron, which, like haemoglobin, can bind oxygen in a dissociable form.

In this way oxygen might be supplied in a concentrated form to the oxydable substances in the cell. It is the task of the red blood corpuscles to supply on their way through the capillaries, and by means of plasma and lymph, the oxygen required for the tissue-cells]. [1]

In this direction my investigations are continued. More problems suggest themselves which will not be discussed now.

SUMMARY.

1. *If phagocytes are exposed during half an hour to a medium from which O has been almost entirely removed, they display a considerable acceleration of phagocytosis.*

If the cells are left for a longer time, e. g. 5 hours, in this solution, then the acceleration of the phagocytosis will give way to a retardation.

2. For this *acceleration of the phagocytosis* by lack of O, which may seem strange at a first glance, and which was indeed unexpected, an *analogy may be found in the respiratory centre.* Here too lack of O heightens the irritability (dyspnoea), the respiration ceasing entirely when the amount of O is further decreased.

3. This view is confirmed *by the corresponding behaviour of both cellspecies when exposed to KCN.*

[1] [ ] Note added to the translation.

It is well-known that this substance checks the O-consumption. When applied in traces, which renders the check imperfect, KCN was found to accelerate phagocytosis considerably. On the respiratory centre the effect of slight quantities of KCN is the same. Violent respiratory movements set in. Greater quantities cause paralysis in both cases.

4. *Also as regards carbonic acid an analogy is found between phagocytes and respiratory centre.* Traces of CO_2 were discovered to promote phagocytosis, whilst greater quantities decreased it. As we know the irritability of the respiratory centre is likewise increased by CO_2, but the centre is paralyzed by an excess of CO_2 in the blood.

5. The facts and views set forth here, supply an obvious answer to the question which formed the starting-point of the present investigation: *why do traces of chloroform and other fat-dissolving substances cause an acceleration of phagocytosis?*

The numerous researches of VERWORN and his pupils on narcosis have established the fact that narcotics such as chloroform have the property of impeding the O-consumption by the cells (spinal centres, nerve-fibres, amoebae etc.). Now it is obvious that as long as mere traces of chloroform are acting, only part of the available oxygen will be rendered useless, in other terms, the blockade of the oxygen will be incomplete. And then the phagocytes are in the case of the experiments mentioned sub 1, where partial removal of oxygen by nitrogen or hydrogen causes an acceleration of the phagocytosis. This acceleration gradually passes into a retardation in proportion as the store of oxygen of the cell becomes more exhausted; an exhaustion which sets in quickly when, for instance by the administration of larger amounts of chloroform, the oxygen-consumption has fallen to a minimum or has ceased altogether.

b. The explanation given sub 5 of the acceleration of phagocytosis by traces of chloroform is in perfect agreement with the fact that *in the first stage of chloroform-narcosis the irritability of the respiratory-centre is increased. Likewise the excitement-stage is explained, which manifests itself at the beginning of the narcosis, and which hitherto none of the narcosis theories have so much as attempted to explain.* (Cf. note 3 p. 1326).

Here too, with the higher nerve-centres, the explanation must be sought in a heightened sensitiveness in consequence of an incipient

lack of oxygen, which increases if the chloroform inhalation is continued, and finally leads to a paralysis of consciousness. When this sets in, the respiratory centre has not been paralyzed yet. It is indeed a well-known fact that the higher brain-centres are more sensitive to oxygen-withdrawal than all other cells of the body.

Probably the increased sensitiveness, as a result of a partial oxygen-withdrawal, must be looked upon as a general phenomenon. The sensitiveness of the vomit-centre for instance decreases, just like that of respiratory centre and phagocytes, if more oxygen is supplied. Hence the inclination to vomit may be subdued to some extent by frequent and deep breathing, whilst it is stimulated by lack of oxygen.

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