RESEARCHES ON PHAGOCYTOSIS.

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I. INTRODUCTION.

THE investigations on the life of phagocytes, which during recent years have occupied myself and my collaborators, are a continuation of researches begun several years ago (1883) with the object of ascertaining the influence of salt solutions of various concentrations upon the red blood corpuscles, and the significance of these results with regard to the functions of the body. These researches on the red blood corpuscles, which introduced physical chemistry into medical science, were confined to the study of their chemical and volumetrical alterations.

But the influence of the salt solutions on the very life of the cells could not well be tested on those particular objects, although systematically investigated. We have felt this from the beginning, and repeatedly asked ourselves whether the solutions producing the chemical and volumetric changes of the red blood corpuscles had or had not seriously damaged these cells, and thus, whether it was possible to apply the conclusions arrived at to problems regarding life itself. Therefore, wherever it was possible, we examined whether the phagocytes, being submitted to the same agencies, kept their phagocytic power. This was always the case. This conclusion enhances the value of our researches on red corpuscles, the more so because the chemical and volumetric alterations of the white cells corresponded with those of the red. But a systematic study of the life of leucocytes had not, previous to our researches, been undertaken, and this is rather surprising.

The phagocytes are a suitable object for studying life phenomena. In the first place, they are not such complicated organs as the muscles, heart, brain, etc.; they are simple cells, easily isolated. Secondly, it is possible to follow quantitatively the effect on their life of slight alterations of their natural medium.

It is obvious that the phenomena produced by the agency of solutions not dangerous to life are, in fact, nothing else but the effect of reactions which finally will help us to penetrate further into the physical and chemical structure of the living cell.

Yet not only from a theoretical but also from a practical point of view the life of these cells deserves our interest; but until now it has not been, as such, the object of a systematic investigation, a fact Metchnikoff himself regretted in a paper he read some years ago to the students of the University of Amsterdam, on "Réactions Phagocytaires":

Nous ne sommes qu'au début. Lorsqu'on connaîtra mieux la physiologie des phagocytes, 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.

Now I must confess that the importance of the phagocytes for the defence of our body against bacteria has not been the first and principal reason of my investigations with regard to these cells: the principal reason was that they were an excellent object for researches of a general biological nature.

I think a physiologist in the first place must practise science for its own sake, not asking whether his work may be of immediate practical use; but should he, however, accidentally stumble upon an idea which may be directly used for the bodily welfare of mankind, this should not prevent him from giving his attention thereto—in fact, it is his duty to do so.

II. METHOD OF INVESTIGATION.

Our method has been very simple. White corpuscles (leucocytes) from the blood of a horse are transferred to various media mixed with very small particles of carbon. After having stood some thirty minutes in an incubator at body temperature a great many of the cells have eaten up carbon. Microscopical preparations are made, and it is ascertained what percentage of the leucocytes has taken up carbon. *This percentage is the measure of the degree*

of phagocytosis, and gives the value of the influence of the various agents used on that function of life.

Suppose in examining 600 leucocytes lying in their natural medium I find that 300 of them have taken up carbon, the percentage of phagocytosis can then be expressed by $\frac{300}{600} \times 100 = 50$ per cent. In order to examine, for instance, the influence of small amounts of acid, I add this substance to the serum and repeat the same experiment under the same conditions. I now find that of the 540 examined leucocytes only 200 have taken up carbon. The phagocytosis is now expressed by $\frac{200}{540} \times 100 = 37$ per cent. Thus the addition of acid to the serum has lowered phagocytosis.

This calculation is based on the principle that the phagocytic power of the individual phagocytes present in a suspension is unequal, a fact which cannot be wondered at when we take into consideration that they are of different ages. The more detrimental the action of the agent the smaller should be the number of phagocytes which take up carbon in the same space of time.

Our selection of an indifferent substance such as carbon instead of bacteria was based upon the fact that we feared our work would otherwise become too complicated. We here refer to the fact established by Sir Almoth Wright and his school that most kinds of bacteria, before they can be taken up by the phagocytes, must undergo a certain amount of sensitization. Hence it follows that the intensity of phagocytosis will not only be influenced by the agent, as such, but also by the degree of "sensitization" the particles have undergone. Another fact which had to be borne in mind was that bacteria sometimes secrete poisons, which have an injurious effect on the phagocytosis. Even dead bacteria contain poisons.

The selection of carbon as an indifferent substance instead of the usual grains of carmine was based upon the greater facility and accuracy with which the taking up of carbon can be ascertained. I shall not give here a detailed description of the method of obtaining the leucocytes or of preparing the carbon, or of the method of determining the percentage of cells which have eaten carbon. I prefer to give some of the more striking results. A glance on the figures in the tables will give an idea of the exactitude of the method, an exactitude which cannot be obtained with bacteria.

III. RESULTS.

1. The Influence of Small Amounts of Calcium.

In examining systematically the influence of the addition of small amounts of various salts (ions) to the blood serum, it was found that very small quantities of calcium increased the phagocytosis to a considerable extent. An addition, for instance, of 0.005 per cent. of calcium chloride (CaCl_2) to the natural medium (serum) caused an increase of about 22 per cent. in the phagocytic power. This favourable effect becomes even more strongly manifest when, instead of being added to the suspension of phagocytes in serum, the calcium is added to a suspension of phagocytes in a solution of sodium chloride (common salt) in which phagocytosis occurs almost to the same extent as in serum.

These investigations have been continued in two directions. In the first place, we asked ourselves whether the influence of calcium would also manifest itself in the living body. All experiments had hitherto been made outside the body. It will be remembered that on placing under the skin (for instance, on the inside of the thigh of a rabbit) small capillary tubes, closed at one end and filled with an extract of bacteria in a solution of sodium chloride, phagocytes enter these tubes. Chemiotaxis is based upon the creeping movement of the phagocytes towards the excretions and secretions produced by the bacteria.

After a certain lapse of time the open part of the capillary tube is filled with a white column consisting of phagocytes. If, in fact, calcium promotes the activity of the phagocytes not only in the phagocytic process described above, but also in the living body, we argued it might be expected that by calcium that mobility of the cells, which here finds its expression in chemiotaxis, would be accelerated. Therefore we determined the length of the white column formed in the capillary tube

with animals, either treated with calcium or not so treated.

We expected to find that in rabbits treated with calcium the white column would, after the same space of time, be longer than in animals not having been treated with calcium. We have used two methods, which gave the same result. I will mention only one. It consisted in injecting into the rectum of two series of animals a solution of sodium chloride mixed for one series with calcium, and for the other without calcium, and then examining the length of the column of phagocytes penetrating into the capillary tubes.

The difference between the two series was considerable. The calcium rabbits showed the greater degree of chemiotaxis, although the amount of calcium chloride used was very small—namely, 0.06 gram—a quantity corresponding with that given to man in mineral waters rich in calcium. Let us add that a natural mineral water, rich in calcium, used in our experiments gave the same results as the calcium chloride solution.

What can be said of the mechanism of this effect? The calcium, on being absorbed by the mucous membrane of the rectum, enters the blood circulation and by this is carried into the lymph of the tissue spaces, where the glass capillary tubes are placed. The phagocytes which are there will soon undergo the stimulating influence of the calcium thus introduced—that is, their mobility will be increased and chemiotaxis will be promoted.

Even without measuring the length of the phagocyte columns, we may convince ourselves that our conclusion as to calcium promoting the activity of the phagocytes is a correct one. On opening the skin wound it is immediately seen that in the mineral water rabbits a much thicker mass of phagocytes is gathered round the tubes than in the pure sodium chloride rabbits. The same thing we observed invariably in all experiments in which NaCl solution containing calcium chloride was injected.

Taking it for granted that phagocytosis plays a part in the defence against microbes, we may infer from the above that an enrichment of our blood, and through this also of the lymph spaces, with small amounts of calcium, must have a favourable effect on local and other infections.

There are many experiences supporting this view. I will mention only two examples. In the first place, Sir Lauder Brunton has used with great success calcium chloride in pneumonia. Other clinicians have also met with success along the same lines; for instance, Crombie, who some years earlier, in the hospital of Calcutta, saw the mortality decrease from 38.6 to 5 per cent. He was inclined to attribute the influence of calcium to an action on the poison excreted by the pneumonia bacteria. But for this there is no evidence. Sir Lauder Brunton has recommended calcium for getting amelioration of the heart action. In recovery from pneumonia the amelioration of the heart action is of the greatest importance. So it was quite rational that the famous clinician and pharmacologist advised calcium for this purpose—in the first place, because it was pointed out by the work of Sydney Ringer that calcium is absolutely necessary for the heart function, and, on the other hand, Langendorff and Hueck had found that by the injection of calcium into the circulation of a cat the action of the heart was considerably strengthened.

Sometimes it happens that during surgical operations the heart beat is stopped by the inhalation of chloroform. The idea occurred to me that in this case calcium might be able to be of good service. Therefore I tried to improve the heart action by intravenous injection of calcium in animals which had purposely been given too much of the anaesthetic. I had no success, so I began to doubt whether calcium was really capable of stimulating even the normal heart. In a number of experiments I found that injection of calcium had no effect on the heart beat of a normal animal. Studying the original article of Langendorff with renewed attention, I saw that he had performed only one experiment. His great reputation had given his assertion general acceptance, and so it had become a fact in the literature. Obviously the amount of calcium in the normal blood serum provides already just the optimum quantity for the heart function.

Thus this favourable effect of calcium in pneumonia not being explained by the influence of calcium on the heart, we had to look for another reason. It was therefore most

natural to remember the stimulating action of calcium on phagocytes, which in pneumonia play such a preponderant part.

My second argument for the probability that calcium is of importance in infectious diseases is this: Labourers in limekilns do not get tuberculosis; in Holland these workmen cover any wound they receive with lime.

The significance of calcium has for some time past been occupying the special attention of biologists and pathologists in an increasing degree, and I shall speak of it further now, and of the cause of the promotion of the activity of the phagocytes in phagocytosis and chemiotaxis by calcium, only to make one remark. We might be inclined to attribute the increase of the plasticity to a modification in the aggregation of the colloidal protoplasm particles as a consequence of the electric charge caused by the entering of a number of bivalent ions into the cells. This explanation, however, can scarcely be the correct one here, for experiment shows that other bivalent cations—namely, barium, strontium, and magnesium—do not augment the amoeboid motion—that is, of phagocytosis and chemiotaxis. It must be assumed, then, that the action of calcium in this case is based upon a hitherto unknown specific biochemical property of this metal.

2. Influence of Iodoform on Phagocytosis.

Every one knows that for the last thirty years iodoform has been successfully applied in the treatment of wounds and chronic inflammations. At first it was thought that this favourable effect was based upon an antiseptic action, but the idea was relinquished when it was found that lower organisms develop fully in a medium containing iodoform. Then other hypotheses were suggested, which need not be dwelt on. They were founded mainly on iodine being split off. None of these suggestions have proved satisfactory. For reasons unnecessary to state here, we have raised the question whether the favourable effect of iodoform on local infections may not be due to the stimulating effect of this substance on phagocytosis. To answer this question, iodoform solutions of different concentrations in a common salt solution of 0.9 per cent. were prepared and mixed with a thick suspension of white blood corpuscles. After having added carbon particles, the mixtures were left to themselves for half an hour in an incubator at 37° C., and then cooled down. Finally, microscopic preparations were made and examined to determine what percentage of the total numbers of white blood corpuscles had taken up carbon particles. The following table contains the results of one of the experiments:

Concentration of Iodoform Solutions in which the Effect on Phagocytosis is still Perceptible.

Fluids.	Percentage of Leucocytes having taken up Carbon.
NaCl 0.9 per cent. (common salt solution of 0.9 per cent.)	$\frac{171}{388} \times 100 = 44.0$ $\frac{151}{345} \times 100 = 43.7$ } 43.8%
1 iodoform to 100,000 NaCl 0.9 per cent.	$\frac{221}{374} \times 100 = 58.6$ $\frac{256}{377} \times 100 = 59.9$ } 58.2%
1 iodoform to 500,000 NaCl 0.9 per cent.	$\frac{228}{371} \times 100 = 61.4$ } 61.4%
1 iodoform to 2,000,000 NaCl 0.9 per cent.	$\frac{208}{398} \times 100 = 52.2$ $\frac{251}{445} \times 100 = 51.9$ } 52.0%
1 iodoform to 5,000,000 NaCl 0.9 per cent.	$\frac{222}{621} \times 100 = 46.9$ $\frac{176}{372} \times 100 = 47.3$ } 47.0%

This table shows that, in the pure salt solution, of the 388 examined leucocytes 171 have taken up carbon—that is, 44 per cent. In a parallel experiment the percentage was 43.7 per cent. Thus the average was 43.8 per cent. In the salt solution containing iodoform in a proportion 1 grain to 100,000 grains NaCl solution, the percentage in two parallel experiments was 58.6 and 59.9, average 59.2 per cent. Evidently the iodoform promoted phagocytosis.

These and other parallel experiments illustrate also the exactitude of the method. As a matter of course, control

experiments have been made in all cases. Further, it appears that a weaker solution of iodoform (1 in 500,000) yields a still higher phagocytosis (61.4 per cent.). The explanation of this phenomenon will afterwards be given. It is seen that in still weaker concentrations the phagocytosis is diminished, but even in a solution of 1 iodoform to 5,000,000 salt solution, the promoting effect of iodoform is still perceptible, for it is 47.1 per cent., whereas in a pure NaCl solution it was $\frac{1}{2}$ ($44 + 43.7$) = 43.9 per cent.

The favouring effect of iodoform is still more evident when the leucocytes are placed for several hours in a pure NaCl solution. Then the phagocytosis falls to a considerable extent. Sometimes the phagocytes are quite paralysed. The addition of iodoform in a concentration of 1 in 5,000,000 then raises the phagocytic power by 30 per cent. or more. Thus there is no doubt that iodoform promotes phagocytosis.

Is it the iodine or the iodoform as such? We have found that iodine is noxious in a high degree. We must conclude, therefore, that iodoform as such causes the increase of the phagocytic power.

The question now suggests itself: How can the favourable effect of the iodoform be explained? It may be accepted from numerous investigations that the outer layer of the cells consists of a fatty substance, a so-called lipid surface. Now, iodoform is soluble in fat, and it is quite obvious that such a surface will grow more soft and more flexible after having absorbed iodoform, and that consequently the plasticity and mobility will be facilitated.

If this interpretation be correct, then other substances soluble in fat, such as chloroform, chloral, alcohol, benzene, camphor, fatty acids (propionic and butyric), should affect phagocytosis in a similar way. This is found to be invariably the case.

3. Effect of Chloroform, Alcohol, Camphor, Benzene, and other Substances Soluble in Fat on Phagocytosis.

I shall not give an account of all our experiments made in this direction. I only give as an example an experiment with chloroform, from which one can at once get an idea as to the exactitude of the method. It was soon found that dilutions 1 in 2,000, 1 in 6,000, and also 1 in 10,000 paralysed the phagocytes. The following table contains some experiments with weaker solutions:

Effect of Chloroform on Phagocytosis.

Fluids.	Percentage of Leucocytes having taken up Carbon.
NaCl 0.9 per cent.	$\left(\begin{array}{l} \frac{174}{423} \times 100 = 40.6 \\ \frac{288}{562} \times 100 = 41.2 \end{array} \right) 40.9\%$
Chloroform 1 in 20,000	$\left(\begin{array}{l} \frac{244}{480} \times 100 = 50.8 \\ \frac{225}{451} \times 100 = 49.8 \end{array} \right) 50.3\%$
Chloroform 1 in 100,000	$\left(\begin{array}{l} \frac{254}{510} \times 100 = 60.6 \\ \frac{382}{455} \times 100 = 60.1 \end{array} \right) 60.3\%$
Chloroform 1 in 500,000	$\left(\begin{array}{l} \frac{332}{582} \times 100 = 57.0 \\ \frac{249}{370} \times 100 = 53.1 \end{array} \right) 58.0\%$
Chloroform 1 in 5,000,000	$\left(\begin{array}{l} \frac{379}{868} \times 100 = 43.6 \\ \frac{298}{651} \times 100 = 45.0 \end{array} \right) 44.3\%$

From this table it appears that chloroform 1 in 20,000 raises the phagocytosis power from 40.9 per cent. to 50.3 per cent.; further, that phagocytosis is considerably increased by chloroform 1 in 100,000, in which case it rises to 60.5 per cent. Evidently in the chloroform solution 1 in 20,000 the paralysing influence also makes itself felt. In a dilution of 1 in 500,000 the favourable effect remains to about the same extent as in 1 in 100,000, and finally in a solution of 1 chloroform to 5,000,000 NaCl solution this favourable effect is still visible, though slight.

Similar results were obtained with benzene, camphor, turpentine, alcohol, chloral, fatty acids (propionic and butyric), and also Peruvian balsam. These all, without exception, promoted phagocytosis. Hence our hypothesis for explaining the effect of iodoform was fully confirmed. Another strong support to our hypothesis was obtained by

comparing the concentrations of the named substances which were able to increase phagocytosis. It appeared that the relation between these concentrations corresponded with that existing between the solubility of these substances in fat.

Further, all the substances applied in very small doses showed a stimulating effect, but paralysed when given in greater quantities. This can be explained by distinguishing two factors: First, slight quantities of the substance by dissolving in the outer layer of the cell weaken it and increase the plasticity. Then, greater quantities having entered the cell, a second factor, the paralysing effect on the protoplasm, comes into play. As the amount of chloroform is increased the paralysing effect gets the upper hand, and no carbon at all is taken up.

4. Other Phenomena seen in the Light of the Foregoing Facts.

The effect of fat-dissolving substances on phagocytosis throws a new light on several facts which hitherto have been, for the most part, entirely unexplained. In the first place we may refer to the power of turpentine to cause local exudations, and the favourable effect of a subcutaneous injection of the same substance in the treatment of pneumonia in horses and in the mastitis of cows, which for some time has been practised with great success in France, Denmark, and Holland. Experiments made by us in this direction have shown that there is much reason to attribute this effect to very small quantities of turpentine dissolving in the lymph of tissue spaces, and afterwards entering the blood circulation and promoting the phagocytic power of the phagocytes in lung and udder.

In the same way we can explain the marked effect of camphor treatment in inflammatory processes of the uterus and its adnexa, an effect not understood hitherto. Then there is the great effect of covering wounds with balsam of Peru, the therapeutic value of inhalation of turpentine vapour in tuberculosis and other infectious processes in the respiratory organs.

In all these cases a stimulating action on the phagocytes, and probably also on other cells (granulation cells), which play a part in the healing process, may come into play.

Yet more becomes clear to us from the above researches. It is well known that various narcotics applied in small doses have a stimulating effect and paralyse in greater quantities. Engelmann observed this many years ago on ciliated cells, but he did not try to explain it. Also, as regards the nervous system, we know with regard to chloroform and ether that, when administered, they first cause excitement and afterwards insensibility. So far as we know, this contrast has never been explained, but when viewed in connexion with our experiments, the phenomenon becomes clear. In the beginning of narcosis only small quantities have entered into the nerve cells. The consequence is a softening, in the first place, of the outer layer. This weakened state of the cells produces an increase of activity (amoeboid motion?). Later, when a greater amount of the substance has been taken in, the paralysing effect gets the upper hand. With alcohol the same experience is well known.

In following this point we are led to think of the remarkable influence of a sojourn in some high mountain regions on metabolism, on sleep, and on the state of the mind. The cause is entirely unknown. It has been proved that these phenomena are not due to the height as such, for in balloons they do not occur, nor are they found in all high mountain regions, but when examining where they are observed, we find that it is in the neighbourhood of coniferous woods. So we are inclined to think that it is due to the stimulating effect of the odour of resin (turpentine, etc.) on the nerve cells.

Professor Sherrington and Miss Sowton, in their studies on the dosage of the mammalian heart by chloroform, found that, especially with greater amounts of the drug, it was usual for the first effect of the administration to be a distinct though slight increase in amplitude of the contractions. After cessation of the administration of the drug in moderate dose, it was not unusual, before the recovery, for the beat of the heart to pass over for a short time into a condition of super-activity. These phenomena suggest an irritative excitatory effect of small doses of the substance on muscle or nerve fibres (or both) of the heart.

These results agree with those previously obtained by Professor A. D. Waller in his experiments with isolated nerve.

There are experiments, also, with other organs which may be considered in the same light. Thus, Professor J. Loeb discovered that substances dissolving fat have the power of rendering possible the parthenogenetic development of eggs. Now we may imagine, with Loeb, that the substance dissolves in a lipid of the outer layer of the egg, thus giving rise to the formation of a fertilizing membrane. We think we may safely go one step further, and assume that it is owing to the weakening of the membrane that the movement of the protoplasm underlying every cell division manifests itself. This view is confirmed by the observation of Professor R. S. Lillie, who saw also that by a short transitory raising of the temperature in the eggs of starfishes a typical fertilizing membrane may be formed, which formation is followed by the development of part of the eggs into larvae. J. Loeb was able to confirm this for Medusae.

After consideration of the foregoing facts and suggestions we were inclined to think that the stimulating effect of traces of substances which dissolve fat on the activity of cells is a widespread phenomenon. Hence we have investigated whether this influence might also be seen in *plant cells*. To do so we chose the germination of seeds, a process in which a considerable division and growth of cells manifests itself. The seeds we chose were grains of wheat, and for the fat-solvent we took chloroform. With chloroform, 1 part in 100,000, an important acceleration in the germination was observed; chloroform 1 in 1,000, on the contrary, impaired the germination, evidently because a second factor made itself felt—namely, paralysis of the protoplasm. Later I heard that in practice the growth of snowdrops is promoted by ether. In recent times Chiari succeeded in forcing blossoms to a considerable extent by ether vapour, and Mansfeld the germination of pumpkin seeds by vapour of alcohol and ether.

Hence it is clear that the observations on phagocytosis deserve our attention on general grounds as well as special. We do not at all mean to say that all substances dissolving fat have the same effect on cells of all kinds; far from it. I would emphasize that in order to be brief and to avoid complexity of detail, I have here presented the matter as somewhat simpler than it really is. Nor have all the suggestions here made towards explaining facts regarding other cells than phagocytes been actually proved. Without hypothesis science cannot progress. Fortunately we need not stay in error for long, for we can always appeal to the supreme judge—experiment.

5. Effect of Oxygen and Carbonic Acid on Phagocytosis. Resemblance between Phagocytes and Respiratory Centre.

In continuing these researches we asked ourselves, for a reason we shall not dwell upon here, to what extent phagocytosis depends 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 rice-flour. The principle for determining the degree of phagocytosis was the same as for the taking up of carbon. It was determined what percentage of the leucocytes counted had taken up starch grains after a certain time.

By this method we compared the degree of phagocytosis in a common salt solution, treated with air, with oxygen and with nitrogen. The result of these experiments was quite unexpected; it appeared that the phagocytosis is greatest where the slightest amount of oxygen was present.

Repeated repetition of the experiment when only the NaCl solutions were compared which were not treated at all and treated with nitrogen, yielded similar results.

Effect of Treatment with Nitrogen on Phagocytosis. Degree of Phagocytosis.

The fluid not treated :	The fluid treated with nitrogen :
22.9 per cent.	26.1 per cent.
23.8 "	33.0 "
23.2 "	27.8 "
20.5 "	31.2 "

It is obvious that the treatment with nitrogen has caused an increase of phagocytosis.

However, the possibility remained that the nitrogen used contained substances which had accelerated the phagocytosis. This was not very probable, since the nitrogen supplied by the company "Oxygenium" at Schiedam had been prepared by fractionated distillation of liquid air. It still contains 1 per cent. of oxygen and gases of the helium group. At any rate, it seemed desirable to carry out experiments with hydrogen likewise. These experiments all resulted invariably in phagocytosis being promoted by the action of hydrogen. Hence it may be concluded that expulsion of oxygen from the medium causes an increase of phagocytosis.

However, the expulsion of oxygen from the cells in the foregoing experiments could not have been extensive, for their contact with the fluid from which the oxygen previously was removed had lasted only half an hour. So we repeated the experiments, with this difference, that the fluid treated with nitrogen and hydrogen could act for five hours instead of half an hour on the phagocytes. In this way the cells had every opportunity to consume the greatest part of their own oxygen. The results were striking; instead of an increase always a decrease of phagocytosis occurred.*

If we submit the results obtained to a close examination we are struck by the agreement between the effect which 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 the action of CO₂ the respiratory centre is also stimulated by a withdrawal of oxygen. The respiratory centre increases its activity when oxygen is very scarce (dyspnoea) and is paralysed when O₂ continues to be withdrawn (suffocation, asphyxia); whereas by frequent deep respiration, by which the O₂ percentage of the blood is increased, the activity of the respiratory centre is lowered in such a degree that the respiration may be stopped for some time without the animal showing any need of it (apnoea).

In view of the 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 CO₂ on phagocytosis. The experiments plainly demonstrated that in weak concentrations (for instance, 3½ volumes per cent. of CO₂ and less) carbonic acid increases the phagocytosis, and that in higher concentrations (for instance, 10 per cent.) it has a paralysing effect.

Involuntarily we are inclined to think here of the favourable effect of the therapeutic application of *venous congestion in inflammatory processes of microbial nature* (Bier). Probably the increasing of the amount of CO₂ accelerates phagocytosis. It is well known that a too energetic use of artificial venous congestion has an unfavourable effect on the inflammatory process, probably because the cells are paralysed.

Another substance having a highly stimulating effect on the respiratory centre before paralysis sets in is, as we know, potassium cyanide; a violent dyspnoea manifests itself. We found that KCN has the same effect on phagocytes—stimulating in slight doses, paralysing in somewhat greater quantities.

Now, it is all but certain that these phenomena must be connected with the property this substance has of obstructing the oxygen consumption of the cells.

6. Explanation of the Excitement Stage in Chloroform Narcosis.

As we have seen under heading 2, there is much evidence to justify the assumption that the stimulating effect of iodoform, chloroform, turpentine, and other fat-dissolving substances on phagocytosis must be connected with a softening of the outer layer of the cells. The present researches on the influence of O₂ withdrawal suggest the existence of a second factor.

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 O₂ consumption by the cells (spinal centres, nerve fibres, amoebae, etc.). Now, it is obvious that as long as mere traces of

* We shall not give figures here; we only refer to the article in the *Proceedings of the Royal Society of the Netherlands* meeting of Friday, April 23rd, 1915, p. 1325, and also to more extensive articles on the subject which will appear in the *Internationale Zeitschrift für physikalisch-chemische Biologie*, vol. II.

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 under 5, 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.

The Excitement Stage in Chloroform Narcosis.

If we let the various narcosis theories pass in review, it appears that no one has even attempted to give an explanation of the excitement stage.

In his article "Narkose" Verworn says: Für die Genese des Exzitastadiums fehlt bisher jede Analyse."

Our researches on phagocytosis and the agreement in the conduct of the respiratory centre and phagocytes may be useful to lighten the difficulty.

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

If the chloroform inhalation is continued, this sensibility will decrease, owing to a further decrease of the potential oxygen percentage, and finally narcosis sets 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 the dispersity of enzymes, etc.—need not be considered here. First, the higher centres, which are, as we know, very sensitive to oxygen withdrawal, are paralysed; then the spinal centres; and, after that, the respiratory centres.

We may add that in the first stage of narcosis not only the higher cortical centres and the spinal centres 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.

Probably the increased sensitivity as a result of a partial oxygen withdrawal must be looked upon as a general phenomenon. The sensitivity of the vomiting centre, for instance, decreases, just like that of the 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.

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)?

The foregoing accounts of results and views, the fruit of some years' work with several collaborators (Hekma, De Haan, etc.),² being of necessity incomplete, I have attempted to select what may specially interest every medical man.

We are continuing our study of the biology of phagocytes, and each day we become more convinced of their excellence as a simple object for the study of problems of a general biochemical nature, and of some of practical interest as well.

It is a satisfaction to me that other workers have begun to cultivate this field of research and to follow our method of investigation.

REFERENCES.

¹ *Handbuch der Naturwissenschaften*, vol. vii, 1912. ² A full account of the researches down to the year 1912 can be found in my monograph, *Physiologisch-chemische Untersuchungen über Phagozyten. Ihre Bedeutung vom allgemein biologischen und pathologischen Gesichtspunkt*. (Wiesbaden: J. F. Bergmann, 1912.) Cf. also some articles in the *Proceedings of the Royal Society of the Netherlands* (Amsterdam)

MR. CLEMENT LUCAS left property of the value of £33,487, the net personalty being £20,976.

AT a meeting of the K.k. Gesellschaft der Aerzte in Vienna, Dr. A. v. Hauer, in giving an account of eight months' experience in the field, discussed the subject of self-mutilation; soldiers with wounds of the left hand were, he said, at once interrogated with this possibility in view. Inoculation against cholera was repeated every three months; active immunization against typhoid fever and small-pox was also undertaken.

An Address

ON THE

ABSENCE OF PROPER FACILITIES FOR THE TREATMENT OF MENTAL DISORDERS IN THEIR EARLY STAGES.

DELIVERED AT A MEETING OF THE YORKSHIRE BRANCH
OF THE BRITISH MEDICAL ASSOCIATION.

BY

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ONE hundred years ago, whilst the campaign in Belgium which ended at Waterloo was in progress, a Select Committee of the House of Commons was engaged in inquiring into the conditions of madhouses in England. Its report, presented in July, 1815, disclosed a terrible state of things, and did much to awaken the public conscience. Amongst those who gave evidence was William Tuke, the founder of the Retreat, then 83 years of age, and frequent reference is made to the *History of the Retreat*, published in 1812, which, largely owing to an article by Sidney Smith in the *Edinburgh Review*, entitled "Mad Quakers," had much influence in promoting improved treatment of the insane. Among the institutions reported upon were some spoken of in the highest terms, in particular Dr. Fox's house at Brislington near Bristol, and Dr. Finch's, Lavestock House, Salisbury, but in the main the state of affairs disclosed was horrible beyond description. One case investigated became notorious—namely, that of a man named Norris, who was restrained for fifteen years at Bethlem with a triple arrangement of bars and chains.

I do not wish, however, to discuss the abuses that existed at that time, and continued more or less for another thirty years, until the appointment of the Lunacy Commission finally put an end in this country to the era of straw and chains in the treatment of the insane.

My object in referring to the report of 1815 is to point out that, in spite of the grave condition of public affairs caused by Napoleon's escape from Elba and his success in raising an army of 250,000 men in less than three months, a committee of the English House of Commons week after week calmly listened to evidence dealing with the management of madhouses. The nation is again passing through a serious crisis in its history, but unless the British character during the past century has seriously altered, it will not be long before the country will be willing to give attention to social questions. To refer to a subject which involves the expenditure of public money at the present time would seem futile, yet it did not discourage our ancestors, exhausted and almost ruined by the Napoleonic wars. On the Friday before the battle of Waterloo the Select Committee considered designs for the construction of two asylums in London and Yorkshire respectively, estimated to cost together £36,596.

The lull of political controversy would seem to afford a singularly opportune time for considering whether any alteration in the law is required in the interests of persons threatened with mental disease. I therefore do not hesitate in bringing to your notice a subject which I believe to be of great importance—namely, the absence of proper facilities for treatment of nervous and mental disorders in their early stages. Its importance was long ago realized by the British Medical Association, which, with the Medico-Psychological Association and the Medico-Legal Society, repeatedly advocated a change in the law. Immediately before the outbreak of the war the Medico-Psychological Association adopted the report of a special committee advocating the establishment of clinics for the treatment of early cases. In the medical press recently the subject has again been discussed, and general public attention was called to the matter, when it was realized that, apart from certification, there was no legal method of treating soldiers temporarily disordered in mind: It was at once seen that the existing procedure was impossible for men temporarily broken down in the service of their country, yet a bill introduced into Parliament to meet the case failed to pass. Although the difficulty in respect to soldiers has to a large