granted that the creatinin excreted by the kidneys, represents only a part of this creatin. Yet, stating the average excretion of nitrogen and creatinin per hour, the fact is remarkable that on the tonus-days the proportion of the amount of nitrogen to that of the creatinin is largest in the afternoon in the period of 3—10, after the creatinin has reached its maximum in the preceding period.

So the above experiments confirm the conclusion, drawn from the content of creatin in the muscles of vertebrates, that chemism in the muscular tonus is totally different from that in the contraction of the muscles. In the first case a nitrogenous metabolite, creatin, is formed, in the second non-nitrogenous products are consumed.

In performing mechanical labour the influence of the tonus is greater proportionally to a more or less careful control of the movements. Consequently we might admit the supposition that intense muscular labour will produce an increase of the excretion of nitrogen, if not only powerful contractions are called forth, but the movements are regulated with great care by tonic contraction of the antagonists, as is often the case with athletic performances.

Physiology. — "*The effect of substances which dissolve in fat on the mobility of Phagocytes and other cells.*" By Prof. H. J. HAMBURGER and J. DE HAAN.

The investigations which will be described in the following treatise, are a continuation of those published in the Proceedings of March 25th 1911 [1]).

It will be remembered that their starting-point was formed by an investigation relating to the favourable effect of Iodoform on the treatment of wounds, and that we arrived at the result that even a slight quantity of this substance (a dilution of 1 to 5000000) has the faculty of accelerating the amoeboid motion of the white blood-corpuscles and of promoting at the same time their phagocytarian capacity. In order to explain this property of Iodoform we assumed that the outer layer of the phagocytes consists of a fatty (lipoid) substance. Now, when Iodoform is dissolved in it, this fatty substance is softened and the amoeboid motion is facilitated. If this view was the correct one, then other substances, soluble in fat, such as Choroform, Chloral, Benzene, Camphor, Turpentine must likewise increase

1) HAMBURGER, DE HAAN and BUBANOVIC: On the influence of Jodoform, Chloroform and other substances dissoluble in fats on Phagocytosis.

the mobility of these cells and consequently their phagocytarian capacity. This was indeed found to be invariably the case.

In order to test this view by means of further experiments we have continued our investigations in three directions.

In the first place the effect of other substances, soluble in fat, viz. of Alcohol, Butyric acid, Propionic acid and also of Peruvian Balsam on the phagocytarian power was investigated.

Secondly we examined to what extent the amount of these substances, necessary to bring about a just perceptible increase of Phagocytosis, is governed by and proportionate with the degree of solubility of these substances in fat

And finally we asked ourselves whether other cells namely plant-cells were affected, as regards their mobility, by the substances soluble in fat.

I. Effect of alcohol, butyric acid, propionic acid and peruvian balsam on phagocytosis.

The experimental method adopted here was identical with the one we had applied before. We investigated namely the percentage of leucocytes which had taken up carbon from a suspension, to which slight quantities of the substance to be investigated had been added in one case, and not in another.

First preliminary experiments had to be carried out to establish how much of the substance would have to be added.

Table I may serve as an answer as regards alcohol,

a. Effect of Alcohol on Phagocytosis.

TABLE I.

The leucocyte-suspension has been in contact for one hour, at the ordinary room-temperature, with the fluids to be investigated. Thereupon the leucocytes have been enabled to take up carbon-particles for 25 minutes.

Fluids.	Percentage of leucocytes having taken up carbon.
NaCl 0.9%	$\frac{404}{834}$[1] $\times 100 = 48.4\%$
1 cc. Alcohol to 10 ccm. NaCl-solution	$\frac{0}{322} \times 100 = 0\ \%$
1 cc. Alcohol to 100 ccm. NaCl-solution	$\frac{168}{543} \times 100 = 30.9\%$

[1]) This quotient denotes that 834 leucocytes were examined and that of these 404 had taken up carbon.

Hence we see that Alcohol in a dilution of.1 to 100 has still a pernicious effect on phagocytosis. Therefore it was advisable to experiment also on weaker alcoholic solutions.

The following table gives the result of this experiment.

Effect of Alkohol on Phagocytosis.

TABLE II.

Fluids.	Percentage of leucocytes having taken up carbon.
NaCl 0.9%	$\frac{389}{1179} \times 100 = 32.9\%$
1 cc Alcohol to 500 cc NaCl sol.	$\frac{454}{1092} \times 100 = 41.5\%$
1 » » » 1000 » » »	$\frac{447}{1091} \times 100 = 40.9\%$
1 » » » 5000 » » »	$\frac{463}{1113} \times 100 = 41.5\%$
1 » » » 20000 » » »	$\frac{434}{1263} \times 100 = 34.3\%$

Hence we see that alcohol in a dilution of 1 to 500 causes phagocytosis to increase from 32.9 to 41.5, an increase of 27%. A dilution of 1 to 20000 has still a favourable effect on phagocytosis, but then it has already become slight.

For shortness' sake we shall not give an account of other experiments; we may state, however, that they gave the same results.

b. Effect of Butyric acid and Propionic acid on phagocytosis.

Effect of Butyric acid on Phagocytosis.

TABLE III.

Fluids.	Percentage of leucocytes having taken up carbon.
NaCl 0.9%	$\frac{446}{1173} \times 100 = 38.1\%$
1 cc butyric acid to 100.000 cc NaCl sol.	$\frac{43}{603} \times 100 = 7.1\%$
1 » » » 500.000 » » »	$\frac{630}{1341} \times 100 = 46.9\%$
1 » » » 1000.000 » » »	$\frac{457}{1239} \times 100 = 36.8\%$
1 » » » 5000.000 » » »	$\frac{287}{759} \times 100 = 37.8\%$

We see from this table that in a dilution of 1 to 100000 butyric acid has a pernicious effect on phagocytosis; in a dilution, however, of 1 to 500000 the phagocytarian capacity rises from 38.1 to 46.9, to return again to its normal value at a dilution of 1 to 1000000.

Similar results have been acquired with propionic acid, as is seen from the following table.

Effect of Propionic acid on Phagocytosis.

TABLE IV.

Fluids.	Percentage of leucocytes having taken up carbon.
NaCl 0.9%	$\frac{201}{432} \times 133 = 46.5\%$
1 propionic acid to 1000 NaCl sol.	$\frac{0}{256} \times 100 = 0$
1 » » 20000 » »	$\frac{0}{316} \times 100 = 0$
1 » » 100000 » »	$\frac{3}{256} \times 100 = 1\%$
1 » » 500000 » »	$\frac{102}{375} \times 100 = 27.2\%$

As we see from the preceding table the phagocytosis in NaCl 0.9% amounts to 46.5 %. Already, an addition of propionic acid of 1 to 100000 prevents it almost entirely, and a dilution of 1 to 500000 still causes a considerable decrease. Therefore experiments were also made with slighter amounts of propionic acid. The following table contains the results of these experiments.

Effect of Propionic acid on Phagocytosis.

TABLE V.

Fluids.	Percentage of leucocytes having taken up carbon.
NaCl 0.9%	$\frac{356}{933} \times 100 = 38.1\%$
1 propionic acid to 500.000 NaCl sol.	$\frac{314}{992} \times 100 = 31.6\%$
1 » » 1000.000 » »	$\frac{425}{902} \times 100 = 47.1\%$
1 » » 5000.000 » »	$\frac{456}{673} \times 100 = 52.8\%$
1 » » 10.000.000 » »	$\frac{389}{934} \times 100 = 41.6\%$

Here too it appears that a solution of propionic acid 1 to 500000 has still a deleterious effect on phagocytosis. A solution of 1 to 1000000 causes it to increase from 38.1 to 47.1, which value rises even to 52.8 % when the solution is 1 to 5000000, an increase therefore of $\frac{52.8 - 38,1}{38,1} \times 100 = 38,5\ \%$.

Hence it appears that fatty acids impede phagocytosis even in a very slight dilution, whereas still weaker dilutions have a directly opposite effect.

On comparing the effect of fatty acids, with that of ordinary mineral acids, on phagocytosis, *the fact will be impressed upon us how poisonous the fatty acids are.* For as we saw before [1]), in a concentration of 1 to 7000 the impeding effect of sulfuric acid has already become very slight, whilst in a dilution of 1 to 500000 propionic acid has still an even more hurtful effect. To some extent the cause of this difference will have to be traced to the greater penetrative power of the fatty acids, owing to their solubility in the lipoid surface. In this connection we are reminded of the symptoms of autointoxication which characterize acidosis, so well known in pathology.

c. *Effect of Peruvian Balsam on Phagocytosis*

Considering that also in Peruvian Balsam an organic acid is found, viz. cinnamic acid, we asked ourselves if perhaps Peruvian Balsam might not likewise increase phagocytosis.

The experiments have fully confirmed this supposition.

Therefore the following experiment was carried out: a big drop

Effect of Peruvian Balsam on Phagocytosis.

TABLE VI.

Fluids.	Percentage of leucocytes having taken up carbon.
1. NaCl sol. 0.9%	$\frac{470}{1057} \times 100 = 44.4\%$
2. Extract of Peruvian balsam in NaCl sol.	$\frac{565}{893} \times 100 = 63.2\%$
3. 1 vol. extract (2) and 3 vol. NaCl sol.	$\frac{622}{1062} \times 100 = 58.5\%$
4. 1 vol. extract (2) and 49 vol. NaCl sol.	$\frac{521}{1089} \times 100 = 47.8\%$

[1]) HAMBURGER and HEKMA. On Phagocytosis, Proceedings of June 29 1907.

of Peruvian Balsam was shaken with 50 cc. of a NaCl-solution of 0,9 % and then filtrated. The filtrate had a nicely aromatic smell. Then various dilutions were made of this filtrate with 0,9 % NaCl-solution, viz. dilutions of 1 to 4, 1 to 10 and 1 to 50 NaCl-solution. We subjoin one of the series of experiments.

This table shows that whilst in NaCl 0.9% the number of white blood-corpuscles having taken up carbon amounts to 44%, this value has become 63.2% under the influence of the undiluted extract. This means a considerable increase of phagocytosis. This increase is still plainly observable at a tenfold dilution; even in a fiftyfold dilution a slight increase could still be established.

There is no doubt but the remarkably favourable results obtained with Peruvian balsam in the treatment of infected wounds, which has hitherto not been explained, must be attributed, partly at least, to an increase of phagocytosis [1]). Undoubtedly chemotaxis will be promoted likewise, based as it is upon an increased mobility of the phagocytes. Indeed we have observed before, when studying the results of the action of calcium, that promotion of phagocytosis and promotion of chemotaxis go hand in hand. But the salutary effect of Peruvian balsam will probably not be restricted to the phagocytes only. It will very likely also affect the granular tissue, and the activity of other cells, which play a part in the healing-process.

II. Phagocytosis and Distribution-coefficient.

In order to test by further observation our views on the cause of the greater mobility of cells under the influence of substances dissolving fat, we have asked ourselves whether there is perhaps some connection between the quantities of these substances to be added to the watery suspension, and the solubility of these substances in the lipoid membrane of the cells. If for instance a solution of Iodoform in NaCl-solution is added to leucocytes, then the Iodoform will soon distribute itself between the lipoid of the leucocytes and the NaCl-solution. The proportion between the concentration of Iodoform in the fat and in the NaCl-solution (water) is called distribution-coefficient as we know. It is obvious that the greater the solubility in fat and the slighter the solubility in water, the more of the Iodoform will pass into the cells. In general it may, therefore, be expected that of a substance which is not so very well soluble in fat, but more so in water, a greater quantity will have to be added to the watery solution, if

[1]) More detailed communications on this subject will be published in the "Feestbundel voor Prof. Hector Treub".

one wishes a sufficient amount of this substance to enter into the exterior of the cells.

Now the question is: Is this corroborated (in the present instance) by experiments? In other words is a weaker solution of Iodoform than of Alcohol sufficient to bring about an increased phagocytosis? For we know, that Alcohol is dissolved in fat much less easily than Iodoform. This is indeed the case. It was found for instance that a solution of 1 Iodoform to 5000000 water promoted phagocytosis, whilst the amount of Alcohol necessary to do so should certainly be no less than 1 to 20000.

Camphor dissolves easily in fat or oil, but in water with some difficulty. Hence the fatty surface of the phagocytes will be able to extract from a very weak, watery camphor-solution the required amount of camphor. It is indeed found that a watery camphor-solution in a dilution of 1 to 1000000 greatly increases phagocytosis.

Chloral dissolves pretty easily in fat, but also in water. And what do we find? That a much stronger solution of Chloral in NaCl-solution is necessary than of Camphor. And like this we might continue. The greater the distribution-coefficient of the substance between oil and water, the weaker the concentration of the watery solution may be.

It goes without saying that a mathematical proportion cannot be expected here. In the first place it is very doubtful whether the same molecular amount of different substances dissolving fat, brings about the same weakening of the lipoid membrane. And secondly another factor comes into play, viz. the noxious effect of the penetrating substance on the movement of the protoplasm, which effect will most probably be different in the case of different substances. Moreover the exterior of the cells is a fatty substance, but no fat. *Nevertheless a manifest relation is found to exist between the relative solubility (distribution coefficient) of the substances in oil and water on the one hand, and the concentration necessary to bring about a just perceptible increase of phagocytosis on the other hand.* We shall revert to this subject more explicitly elsewhere. For the present we would point out another remarkable phenomenon deserving mention in the same connection.

When the weakest concentrations are sought of the substances dissolving fat, which cause paralysis of the phagocytes, then it appears that these concentrations correspond with those, which according to H. MEYER *and* OVERTON *are necessary to cause narcosis, consequently to paralyze the ganglion-cells.* And it has been established by these investigators

that the concentration of the fluids, necessary to produce narcosis, runs parallel to the distribution coefficients between water and oil.

We shall give a few examples.

	Paralysis of Phagocytosis.	Narcosis. (ganglioncells.)
Chloroform	$^1/_{6000}$	$^1/_{6000}$
Chloral	$^1/_{250}$—$^1/_{1000}$	$^1/_{800}$—$^1/_{1200}$
Alcohol	$^1/_{10}$—$^1/_{100}$	$^1/_{80}$

III. Effect of Chloroform on the Germination of wheatgrains.

Already in our former paper we pointed out that the facts, discovered with respect to phagocytes, correspond entirely with various phenomena, observed in other cells. Hence we remarked that just as phagocytes show a greater phagocytarian capacity when a slight quantity of a narcotic is added, (and are paralyzed by a greater quantity), the excitementstage in narcosis will probably have to be explained by an increased activity of the ganglion-cells.

Eggs of star-fishes and sea-urchins can be brought to a parthenogenetic development, according to J. Loeb, by an addition of slight quantities of substances which dissolve fat. This too agrees with our views of what has been observed in the case of the phagocytes. For according to our interpretation this development may be explained by a softening of the egg-membrane, of which a more rapid cell-division must be the consequence. If moreover we bear in mind that ciliated epithelium is stimulated by traces of alcohol or ether, then we are inclined to think that the influence of substances dissolving fat, on the mobility of cells is a widespread phenomenon in nature. For this reason we have investigated whether this influence might also be traced perhaps in *plant-cells*.

For this purpose 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 substance dissolving fat we took chloroform.

A number of wheat grains (seed corn) were selected and soaked for some time in watery solutions containing slight quantities of chloroform. Another number of wheat-grains were left for some time in distilled water. Then the swollen seeds were allowed to germinate. For this purpose they were placed on a horizontal piece of gauze, which is used for dust-filters, and which was stretched over a square aluminium frame. This frame was placed in a square glass basin

on the surface of the distilled water with which this basin was partly filled. The grains were therefore placed, as it were, on the surface of the water. They were just moistened by it and were yet sufficiently at the surface to get plenty of air, necessary for their germination. Now it was observed regularly, after certain intervals, how many of the wheat-grains showed distinctly that their germination had set in.

The seed was considered to have germinated when the white germ became visible through the broken seed-coat.

We shall not detail the particulars we had to take into account in these experiments, but summarize one of the experiments in a table.

Effect of Chloroform on the Germination of Wheat-grains.

TABLE VII.

The seeds have been soaked for 18 hrs. in:	Of 200 seeds have germinated after	
	15 hours	27 hours
Distilled water Exp. A	56 = 28%	87 = 43.5%
» » » B	54 = 27%	83 = 41.5%
Chloroform 1 to 1000	42 = 21%	77 = 38.5%
» 1 » 10000	53 = 26,5%	84 = 42%

From this table it appears that Chloroform 1 to 1000 and 1 to 10000 are detrimental to the rapidity of the germination, for after 15 hours the percentages are 21 and 26,5 instead of 27.5% (the average between 28 and 27), whilst after 27 hours the injurious effect, at any rate of Chloroform 1 to 1000, still makes itself felt.

Therefore an experiment was made with weaker Chloroform-solutions.

Effect of Chloroform on the germination of wheat-grains.

TABLE VIII.

The seeds have been soaked for 18 hours in the following fluids:	Of the 242 seeds have germinated after			
	16 hours	19 hours	23 hours	28 hours
Distilled water	51 = 20.8%	74 = 30 %	84=34.6%	100=41.2%
Distilled water, after that 1 hour in chloroform $^1/_{250}$	12 = 5 %	26 = 10.6 %	44=18.1%	57=23.5%
Chloroform $^1/_{10.000}$	58 =.23.9%	75 = 31 %	88=36.3%	101=41.7%
» $^1/_{100.000}$	72 = 29.7%	92 = 38 %	104=42.9%	111=45.8%

Hence we see that the Chloroform-solution $^1/_{250}$ has worked unfavourably on the germination. Evidently the protoplasm has been paralyzed to a certain extent. In the weak chloroform-solution 1 to 100000, however, the germination has been promoted. It appears that in this Chloroform-solution 72 grains have germinated after 16 hours, whilst in the same time only 51 grains have germinated of those which were soaked in water. After 28 hours this accelerating effect of the Chloroform-solutions is still visible, but by no means so plainly as after 16 hours. After 40 hours, as was shown by other experiments, no traces of this favourable effect of Chloroform were any longer perceptible.

We shall not add any more of these experiments. *It need only be observed that the same results were obtained if the grains were allowed to germinate not on water, but in humus.* Also in plant-cells therefore, we are led to think of an increased mobility of the cells, caused by Chloroform ; for without such mobility no division and germination can be conceived.

It may be expected that also *other* substances dissolving in fat, will bring about the same phenomenon in plant-cells. We are extending our researches in this direction. It has already appeared, however, that various factors have to be considered. First the rapidity with which the substances dissolving fat, enter the seed and leave it again. Secondly the noxious effect of this substance on the protoplasm. In other words, care should be taken that the amount of the substance dissolving fat, which enters the cells is just sufficient to increase their mobility, but not large enough to impair, seriously at least, the vital functions of the protoplasm. On the other hand it should be contrived that the fat dissolving substance which has penetrated into the cell, does not pass too soon into the water, leaving the cell entirely before it has had time to effect an acceleration.

We may add that our experiments lend support to the supposition of CZAPEK, that the superficial layer of the protoplasm of plant-cells is of a lipoid nature[1]).

Summary.

The opinion put forth in the preceding treatise, that the acceleration of phagocytosis by substances dissolving fat, must be attributed to a weakening of the fatty surface with the result that the amoeboid

[1]) F. CZAPEK, Ueber die Oberflächenspannung und den Lipoïdgehalt der Plasmahaut in lebenden Pflanzenzellen. Ber. d. Deutschen Botan. Gesellsch. Vol. 28 Dez. 1910.

motion is facilitated, finds an additional support in the following 3 arguments:

I. Also other substances dissolving fat, taken at random, and which were formerly not experimented upon, viz. Alcohol, Butyric acid, Propionic acid and also Peruvian Balsam, were found to accelerate phagocytosis.

Alcohol was found to increase phagocytosis in a concentration of 1 *to* 500—20000; *Propionic acid in a concentration of* 1 *to* 10.000000.

On comparing these results with the effect of mineral acids such as HCl and H_2SO_4 we are struck by the noxious effect which mere traces of Propionic acid and Butyric acid have on phagocytosis. For whilst the bad effect of Propionic acid commences already at 1 to 1000,000, that of H_2SO_4 manifests itself only at 1 to 7000.

That *Peruvian balsam* should increase phagocytosis was to be expected, as it contains cinnamic acid, likewise an organic acid.

The remarkably favourable, hitherto unexplained, effect of Peruvian balsam on infected wounds, may be explained, partly at least, by an increased mobility of the phagocytes and of other cells playing a part in the healing-process.

II. *If the weakest concentrations of the fat dissolving substances are sought in which the phagocytes show a plainly perceptible acceleration of phagocytosis, then these are found to run parallel to the degree of solubility of these substances in fat*; in other words to the distribution coefficients of these substances between water and oil.

Moreover the remarkable fact is observed that the same concentration of substances which dissolve fat, necessary to cause paralysis of phagocytosis, also effects narcosis of tadpoles and of mammals. As we know the concentrations necessary to bring about narcosis are, according to the investigations of H. MEYER and OVERTON governed by these distribution-coefficients.

III. Not only animal cells (phagocytes, ganglion-cells, eggs of lower marine animals, ciliated epithelium) show an increased mobility under the effect of slight quantities of substances dissolving fat, but *also in plant-cells the same fact is observed.* Under the effect of Chloroform 1 to 100000 an important acceleration in the germination of wheat-grains was observed. Chloroform 1 to 1000 on the contrary, impairs the generation, evidently because a second factor makes itself felt, viz. paralysis of the protoplasm.

Groningen, September 1911.