Physiology. — "*The effect of fatty acids and soaps on phagocytosis*".[1]) By Prof. HAMBURGER and J. DE HAAN.

(Communicated in the meeting of February 22, 1913.)

In our former paper[2]) we drew the attention to the particularly noxious effect of fatty acids on phagocytosis.

Already at a concentration of 1 : 1000,000 the pernicious influence of propionic acid became manifest. The law of division-coefficients, obeyed by all the other fat-dissolving substances, examined by us, did not lead us to expect such a poisonous effect of propionic acid.

How could this abnormal action of propionic acid, and likewise of butyric acid, which was also examined by us, be explained?

Is it caused by a noxious effect of ions of H, or perhaps also by a specifically injurious effect of the anion of fatty acid?

At that time we failed to supply an answer to this question.

In order to determine to what extent the ions of H are responsible for the noxious effect of the fatty acids we exposed the

TABLE I.

Comparison of sulphuric acid- and propionic acid solutions with equal percentages of ions of H. The solutions act upon the leucocytes during 5/4 hours; the leucocytes are brought into contact with carbon during 25 minutes.

	NaCl-solution in which has been dissolved:	Number of leucocytes examined	Number of leucocytes having taken up carbon	Percentage of phagocytosis
	nothing	349	101	29 %
1	H_2SO_4 $^1/_{100\,000}$	208	0	0 »
	Proprionic acid $^{1.5}/_{100\,000}$	301	0	0 »
2	H_2SO_4 $^1/_{500\,000}$	194	13	6.7 »
	Proprionic acid $^{1.5}/_{500\,000}$	180	0	0 »
3	H_2SO_4 $^1/_{2500\,000}$	148	33	22.2 »
	Propionic ac. $^{1\,5}/_{2500\,000}$	215	52	24.2 »

[1]) A more detailed account will be published in the Archiv. f. (Anat. u.) Physiologie.

[2]) The effect of substances which dissolve in fat on the mobility of Phagocytes and other cells. These Proceedings Vol. XIV p. 314.

leucocytes to the action of fatty acid and of sulphuric acid-solutions *containing the same percentage of ions of H*, and determined subsequently its phagocytarian power.

The table on p. 1290 will need no further explanation.

It follows from this series of experiments that the noxious effect of aqueous sulphuric acid- and propionic acid-solutions manifests itself at the same concentration of ions of *H*.

This renders it in a high degree probable that the noxious effect of a strongly diluted solution of propionic-acid must be attributed to the action of ions of H.

If this view was the correct one, if it was not *the anion of propionic acid*, but the ion of *H* which had to be reckoned with, it might be expected that the propionate of sodium, in the corresponding dilution, would have *no* bad effect.

This was indeed not the case, as appears from the following table.

TABLE II.

Effect of Na-propionate on phagocytosis. The propionate acts upon the leucocytes during half an hour The leucocytes are brought into contact with carbon during half an hour at 37°.

NaCl-sol. 0.9% in which has been dissolved:	Number of leucocytes examined	Number of leucocytes having taken up carbon	Perc. of phagocytosis
nothing	768	373	48.5%
	323	163	50.4 »
Na-propionate 1 : 100 (i.e. 1 gr. propionate dissolved in 100 ccm. NaCl)	923	535	57.9 »
Na-propionate 1 : 250	549	332	60.4 »
» 1 : 1000	781	460	58.6 »
» 1 : 5000	412	247	59.9 »
	344	83	24.1 » ?
» 1 : 25000	891	437	49 »
» 1 : 100.000	633	321	50.7 »

A hurtful effect of anions of *H*, even in much greater concentrations than those in which the anion was used in the propionic acid experiments, is evidently out of the question. The propionate 1 : 25000 and 1 : 100000 leave the phagocytarian power intact; propionic *acid* in this concentration destroys all the leucocytes.

But what is much more remarkable than this result is the favourable effect of still higher concentrations of propionate (1 : 100; 1 : 250; 1 : 1000) *on phagocytosis.*

By dissolving for instance 1 gramme of propionate in 250 ccm. of NaCl 0.9 % the phagocytosis is found to increase by 100 %.

This increase, which was also caused by *the Na-salts of butyric acid and formic acid,* was all the more remarkable, as the fluid was made strongly hyperisotonic by the addition of these soaps, and as was shown hyperisotony has nearly always a highly injurious effect upon phagocytosis.

This is clearly confirmed by the following experiment in which isosmotic NaCl-solutions, with and without propionate, are compared with each other.

The comparison relates to the following isosmotic solutions:

NaCl 0,9% and NaCl 0,9%
NaCl 1, % „ NaCl 0,9% + Na-Propionate 0,165%
NaCl 1,1% „ NaCl 0,9% + „ „ 0,33 %
NaCl 1,2% „ NaCl 0,9% + „ „ 0,5 %
NaCl 1,3% „ NaCl 0,9% + „ „ 0,66 %

These fluids acted for half an hour upon fresh leucocytes; then

TABLE III.

Effect of isosmotic NaCl and NaCl-Propionate-solutions.

Solution	Percentage of leucocytes having taken up carbon	NaCl-solution 0.9% +	Percentage of leucocytes having taken up carbon
NaCl 0.9%	$\frac{132}{465} \times 100 = 28.2\%$	nothing	$\frac{68}{266} \times 100 = 25.5\%$
» 1 »	$\frac{113}{457} \times 100 = 24.7$ »	Na-Propionate 0.165%	$\frac{42}{325} \times 100 = 12.9$ »
» 1.1 »	$\frac{62}{400} \times 100 = 15.5$ »	» 0.33 »	$\frac{113}{316} \times 100 = 35.9$ »
» 1.2 »	$\frac{69}{524} \times 100 = 13.1$ »	» 0.5 »	$\frac{193}{643} \times 100 = 30$ »
» 1.3 »	$\frac{6}{272} \times 100 = 2.2$ »	» 0.66 »	$\frac{116}{428} \times 100 = 27.1$ »

the suspensions were brought into contact with coal for $^1/_2$ hour at 37°, and the preparations were made.

This result is indeed interesting, for we find that when by the application of a strongly hyperisotonic NaCl-sol. (1.1 %) the phagocytosis has been reduced by 50 % (from 28 % to 15,5 %) a NaCl-solution, isosmotic with the former, in which, however, part of the NaCl has been replaced by propionate, promotes phagocytosis to a considerable extent (to 35.7 %).

A similar result was obtained with leucocytes which had been left in serum containing citrate of Na during one night, and which had consequently lost part of their phagocytarian power.

After the results obtained with the propionate it might be expected that also the butyrate and the formate would give the same results.

This was indeed the case.

We subjoin a table, showing the results obtained with butyrate.

This table shows that Na-butyrate in a dilution of 1:1000 has

TABLE IV.

Effect of butyrate of Na on phagocytosis. The NaCl-solutions containing butyrate have acted upon the leucocytes for half an hour at room-temperature; then they were brought into contact with carbon for half an hour.

NaCl-solution 0.9% +	Percentage of leucocytes having taken up carbon
nothing	$\frac{132}{449} \times 100 = 29.3\%$
nothing	$\frac{132}{488} \times 100 = 27$ „
Na butyrate 1:100	$\frac{130}{448} \times 100 = 29$ „
„ 1:250	$\frac{138}{479} \times 100 = 28.8$ „
„ 1:1000	$\frac{321}{841} \times 100 = 38.1$ „
„ 1:5000	$\frac{306}{808} \times 100 = 37.8$ „
„ 1:25000	$\frac{260}{554} \times 100 = 39.7$ „

increased phagocytosis (from 28% to 38%), and that this increase is still more obvious in a dilution of 1 : 25000.

As regards the *formiate*, here too a dilution of 1 : 1000 caused an important increase, which continued at 1 : 2000, and which was still clearly visible at 1 : 10000.

An attempt at an explanation of the facts observed.

How must the favourable effect of propionate and of other soaps on phagocytosis be explained?

Is the cause the same as that which we adduced to explain the effect of lipoid-dissolving substances such as iodoform, chloroform, chloral, etc.?

Also in the case of these soaps we might think that propionate — for convenience sake we shall only mention propionate when we should also name the other two soaps which were experimented upon — dissolves in the lipoid surface of the phagocytes, softens them and facilitates in this way the amoeboid motion.

Numerous experiments, however, showed that propionate is absolutely insoluble in olive-oil.

We have then tried to find another explanation, and it occurred to us that *soaps have in a high degree the property of lessening the surface tension of oil.*

The reader knows GAD's experiment: if oil is brought into contact with a soap solution, an extremely fine emulsion is formed.

As far as we know these experiments have only been carried out with soaps of higher fatty acids (sapo medicatus or olive-oil containing some fatty acid).

Therefore we have repeated them with soaps containing a smaller number of C atoms in their molecules.

It appeared indeed that the propionate, butyrate and formiate of Na have an emulgent effect on olive-oil. The formiate of Na was more active than the two others.

We may conceive that the soaps lay themselves against the surface of the phagocytes, reduce the surface-tension, and in this way facilitate the amoeboid motion.

The following observations point in the same direction.

By way of an illustration we beg the reader to glance at Table III.

In this series of experiments the leucocyte suspensions, after having been in contact with carbon for $^3/_4$ hours at 37°, were suddenly cooled down by water at 13°. Then the phagocytes were fixed by means of a drop of an osmium-solution.

Microscopical examination showed that in the NaCl-solution of 1,1%, 1,2%, and 1,3% all the leucocytes had regained their round shape, while in the isosmotic NaCl-propionate solution nearly all the cells still had pseudopodia.

Even in the NaCl-solution 0,9% relatively few leucocytes with pseudopodia were found, and yet the phagocytosis had reached about the same stage as in the latter fluid, which contained much propionate (12,7% and 15% respectively).

It follows from this that propionate has the property of influencing the amoeboid motion of the leucocytes in a favourable sense; one might be inclined to say that they are made more resistant.

For what was observed to take place?

In the NaCl-solution 0,9% the leucocytes drew back their protrusions owing to the lower temperature, but in the propionate-sol. with the same degree of phagocytosis they remained, notwithstanding this low temperature.

Similar results were arrived at in the experiments of Table II: in NaCl 0,9% no pseudopodia, in NaCl combined with propionate 1:100, 1:250 and 1·1000 many pseudopodia, in propionate 1:5000 fewer, and in 1:25000 and 1000.000 none.

Now it would be incorrect to look upon the promotion of phagocytosis and the capacity of resistance of the pseudopodia as being identical.

First there are a number of leucocytes which protrude pseudopodia, but which show no phagocytosis, and secondly it appeared from another series of experiments with propionate and $CaCl_2$ where both substances equally promoted phagocytosis, that after being cooled down and fixed, the microscopic pictures were entirely different. In the $CaCl_2$-solution namely the lower temperature had caused the pseudopodia to disappear almost entirely, in the propionate-solution on the other hand, this was not the case.

But since the formation of pseudopodia is *one of the conditions* for phagocytosis, it may be concluded from the observation with propionate that propionate by influencing the formation of pseudopodia in a favourable sense has contributed to the promotion of phagocytosis.

That the effect of propionate is due to a surface-action and not to a direct action on the contents of the cells appears from *volumetrical determinations,*

The volumes of two equal amounts of blood corpuscles, exposed to the action of isosmotic solutions, are equal, as we know, *but only on condition that the substances do not penetrate into the blood corpus-*

cles and that therefore the phenomenon remains restricted to an interchange of water between the cells and the surrounding fluid.[1])

Conversely, it may be concluded that if two isosmotic solutions give the same volume to the blood-corpuscles, the latter are impermeable to these substances[2]).

Therefore we have investigated to what extent a certain amount of blood-corpuscles in a solution of NaCl 1,2 % had the same volume as a solution, isosmotic with the former and which contained 0,9 % NaCl and 0.5 propionate of Na.

If the volumes were equal then it might be concluded that propionate did not penetrate or hardly into the cells.

The experiments showed that only traces of propionate could have penetrated into the blood-corpuscles.

Consequently Na-propionate acted upon the red blood corpuscles like for instance NaBr and other anorganic Na-salts.

Now it might be objected that the permeability of the red and the white blood-corpuscles need not be alike. As regards this we may observe that none of the many researches carried out in this direction, have established any difference.

The agreement goes even so far that the same hyperisotonic salt solution causes the same relative decrease in volume in the red and in the white blood corpuscles[3]). And this also applies to the hypisotonic one.

The analogy also appears from the way in which anisotonic salt-solutions act upon phagocytosis[4]).

We arrive, therefore at the conclusion that until now we have discovered three causes which may increase phagocytosis.

1. *Traces of a calcium-salt*; there can be hardly any doubt but here we have to do with an action of Ca on the cell-protoplasm. It has not been verified as yet whether the Ca also acts upon the surface.

2. *Fat-dissolving substances* such as iodoform, chloroform, chloral, turpentine, etc. When applied in homoiopathic quantities (e.g. Chlo-

[1]) *Perfectly* equal when the isosmotic solutions are isotonic. *Hedin*, PFLUGER's Archiv **60**, 198, p. 300.

[2]) Only urea, as appears from investigations by GRIJNS and myself, makes an exception.

[3]) HAMBURGER. Archiv. f. (Anat u.) Physiol. 1898 S. 317; Osmot. Druck u. Ionenlehre I. S. 337.

[4]) HAMBURGER and HEKMA. Biochem. Zeitschr. **7**, 1907, 102. Further HAMBURGER, Physik. Chem. Unters. uber Phagocyten u. s. w. Wiesbaden, J. F. BERGMANN. 1912.

roform 1:500000, Propionic acid 1:10000000) they restrict their action to the lipoid surface, which they weaken thus facilitating the amoeboid motion.

When applied in somewhat greater quantities a second factor becomes of importance viz. the noxious effect of these substances on the protoplasm. All these substances indeed penetrate easily into the cells, thus causing paralysis.

3. *Soaps*, such as propionate, butyrate and formiate. These substances, unlike the fat dissolving substances, do *not* enter into the phagocytes. Their action upon the phagocytes is therefore entirely different from that of the fat-dissolving substances, for even when applied in high concentrations (1:250), in concentrations in which the fat dissolving substances would inevitably kill the cells, *they have a very favourable effect upon phagocytosis.*

When applied in still greater quantities their action is a pernicious one, but this may be due to the solution being too hyperisotonic.

Further it is a remarkable fact — and in this respect the soaps are distinguished from calcium as well as from the fat dissolving substances — that within rather wide limits, the degree to which phagocytosis is promoted is independent of the amount of soap, found in the solution. (Cf. Tables II and IV.)

The researches, described above, have given rise to different questions, which, owing to the present circumstances we cannot enter into now.

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Astronomy. — "*A proof of the constancy of the velocity of light*". By Prof. W. DE SITTER.

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In the theory of RITZ light emitted by a source moving with velocity u is propagated through space in the direction of the motion of the source with the velocity $c + u$, c being the velocity of light emitted by a motionless source. In other theories (LORENTZ, EINSTEIN) the velocity of light in always c, independent of the motion of the source. Now it is easily seen that the hypothesis of RITZ leads to results which are absolutely inadmissible.

Consider one of the components of a double star, and an observer situated at a great distance Δ. Let at the time t, the projection of