Citation:

Snapper, I., On the change in the permeability of the red blood corpuscles (also in man). (A contribution to the knowledge of chlorine-retention in fever), in:
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12. In *Zygnema cruciatum* the halves of the nuclear plate develop already to daughter-nuclei within the nuclear spindle, and before they reach the poles. That part of the spindle which lies between the daughter-nuclei increases in circumference, so that the spindle becomes peculiar in shape.

The results obtained by Miss MERRIMAN, ESCOYEZ and myself differ very greatly. This may partly be ascribed to the circumstance that different species of *Zygnema* were investigated. To a much greater extent the differences must be assigned to other causes, in particular to a different interpretation of observations. The observations themselves however, are also sometimes different and perhaps not always complete. Also the fixing agent and the method of investigation may contribute to the divergence between the results of different investigators. ESCOYEZ, for example, observed a perinucleolar cavity, whilst I, using an other method of fixation observed no such cavity. Miss MERRIMAN and ESCOYEZ both believe they have seen ring-shaped nuclear-plates, whilst I came to a different conclusion, using a method by which the nuclear-plates could be observed edgeways as well as sideways.

It seems to me desirable that the various investigators should endeavour to complete their observations on *Zygnema,* and extend them to more species and also should apply different methods of investigation. Exchange of material might also be very useful. Some such action would be conducive to agreement, which will not be readily obtained by other means.

Physiology. — *"On the change in the permeability of the red blood corpuscles (also in man)". (A contribution to the knowledge of chlorine-retention in fever* [1]). By I. SNAPPER. (Communicated by Prof. HAMBURGER).

(Communicated in the meeting of April 25, 1912).

It has been known for a long time that in a number of febrile diseases an important change in the excretion of chlorine is to be observed. Under normal circumstances, all the chlorine which is taken up with the food leaves the body within 24 hours, not so, however, in the above mentioned diseases; though the patients take daily 5 or 6 grammes of NaCl, only some hundreds of milligrammes

[1]) A detailed account of these researches will be published in the Biochemische Zeitschrift, and in the Zeitschrift für Klinische Medizin.

are excreted. This means an almost absolute retention of chlorine.

Many theories have been suggested to account for this retention, without a satisfactory explanation being arrived at. Our investigations have shown that the chlorine percentage in blood and blood-serum has fallen so much, that the kidneys can no longer withdraw chlorine from the blood [1]). The lower chlorine percentage of the blood can only be explained by the supposition that chlorine is retained by the tissues, and that it does not, as in normal circumstances, pass from the tissues into the blood. The question: "what causes chlorine retention in febrile diseases" is, therefore, turned into another question: "why does not chlorine pass, in these circumstances, from the tissues into the blood?"

Is it because the permeability of the tissue-cells for chlorine has been modified?

It is difficult to investigate the permeability of human tissue-cells, even for anorganic substances. The only cells which admit of such researches are the red blood-corpuscles. Hence we have made use of these cells. But before experimenting on this valuable material, it was found desirable to investigate various questions relating to permeability, on the blood-corpuscles of animals.

If the composition of the medium in which the red blood-corpuscles are suspended is altered, interchanges between red blood-corpuscles and their medium take place. Until now it has been found possible to explain these interchanges, as an osmotic phenomenon [2]); there was no need to assume that a change in the nature of the surface of the red blood-corpuscles had taken place. It was even found that an intentional modification of this surface did not modify the osmotic interchanges.

HAMBURGER, namely, found that an addition of chloroform to red blood-corpuscles did not in the least affect their resistance to diluted salt-solutions [3]). This result had not been expected by him. Indeed there were grounds for assuming, that the lipoid substances, which form at least part of the surface of the red blood-corpuscles, are softened by chloroform. After this process this outer layer will offer less resistance to the swelling caused by hypotonic solutions. Still nothing of this decreased resistance was observed. And we too could show, in accordance with this fact, that the interchange of

[1]) I. SNAPPER, Dissertatie, Groningen 1913.

[2]) HAMBURGER, Osmotischer Druck und Ionenlehre.

[3]) HAMBURGER. Biologie der Phagozyten. 1913,p. 199.

This resistance of the red blood-corpuscles is a good criterion as regards their permeability.

substances between blood-corpuscles and medium remains absolutely unaffected by the addition of chloroform (see p. 1469).

Yet we found that there are cases when a modification in the nature of the surface, and consequently a modified permeability *must* be assumed.

From considerations which cannot be detailed here, but which are connected with the varying results obtained by HAMBURGER with intravenous injections of salt mixed with acid, on horse and calf, the following experiments were made.

I. *Experimental modification of the chlorine-interchange under the influence of acids.*

The experiments were carried out with horse's, cow's, and dog's blood. In order to find a criterion for the normal permeability of the blood-corpuscles, it was first investigated how chlorine behaved if part of the serum was replaced by a Na_2SO_4-solution. It was known that if all the serum of the blood was replaced by an isotonic Na_2SO_4-solution, chlorine passed from the blood-corpuscles into this sulphate-solution: this is an osmotic process, dependent on the normal permeability of the blood-cells. In order not to deviate too far from physiological conditions, only part of the serum was replaced by Na_2SO_4-solution; then it was investigated if the chlorine-percentage of the serum had been changed, owing to chlorine having entered into the blood-corpuscles or having left them.

In all the experiments, of 15 cm^3 of blood 1 cm^3 of serum was replaced by 1 cm^3 of isotonic or 2 $\times$ hypertonic Na_2SO_4-solution. Since part of the serum is replaced by a solution containing no chlorine. the chlorine in the serum must decrease. This decrease, however, can be calculated beforehand from the amount of serum contained in 15 cm^3 of blood. Supposing for instance, 15 cm^3 of blood contain 9 cm^3 of serum, replacing 1 cm^3 of serum by the sulphate-solution must, therefore, cause the chlorine of the serum to fall to $^8/_9$ of the original amount. *It appears now that in the above-mentioned kinds of blood, after* $\pm$ *12 %* *of the serum has been replaced by* Na_2SO_4*-solution, the chlorine in the serum falls just as much as could be calculated beforehand from the dilution of the serum with a solution containing no chlorine.*

It may not be concluded from this that under these circumstances no chlorine leaves the blood-corpuscles. The addition of the 2 $\times$ hypertonic Na_2SO_4-solution causes the blood-corpusclus to shrink, because water passes from the blood-corpuscles into the serum

Thereby the serum would be more diluted than could be calculated from the addition of the solution which contained no chlorine. That in spite of this the chlorine of the serum agrees with the value calculated beforehand, proves that, with the water, chlorine has passed from the blood-corpuscles into the serum.

The following experiment may serve as an example:

EXAMPLE.

Change in the amount of chlorine of the serum of horse's, cow's, and dog's blood, if 12 % of the serum has been replaced by sulphate-solution.

Of 15 cc. of blood 1 cc. serum is replaced by 1 cc. of 2 × hypertonic Na_2SO_4-solution The chlorine of 4 cc. of serum corresponds with:	Of 15 cc. of blood 1 cc. serum is replaced by 1 cc. of isotonic Na_2SO_4-solution. The chlorine of 4 cc. of serum corresponds with:	The chlorine of 4 cc. of normal serum corresponds with:	15 cc. of blood contain:	If no transfer of chlorine took place, the chlorine of 4 cc. of serum, after 1 cc. of serum had been replaced by a solution containing no chlorine, would correspond with:
3.95 cc. $AgNO_3$	3.96 cc $AgNO_3$	4.44 cc. $AgNO_3$	66% = 9.9 cc. $AgNO_3$	$\frac{9.9-1}{9.9} \times 4.44 =$ 3.99 cc. $AgNO_3$

It was, therefore, certain that blood-corpuscles with a normal permeability always react in the same way if part of the serum is replaced by a Na_2SO_4-solution.

When chloroform was added to the blood — so much even that slight, but distinctly visible haemolysis set in — the same rules were found to hold good: the transfer of chlorine under the influence of Na_2SO_4 is the same in normal and in chloroform-blood.

EXAMPLE.

The permeability of the blood-corpuscles is determined before and after 1.2 ‰ of chloroform has been added to the blood.

Of 15 cc. of blood 1 cc. of serum is replaced every time by:					
1 cc. of H_2O. The chlorine of 5 cc. of serum corresponds with:		1 cc. of 2 × hypertonic Na_2SO_4-solution. The chlorine of 5 cc. of serum corresponds with:		1 cc. of isotonic Na_2SO_4-solution. The chlorine of 5 cc. of serum corresponds with:	
normal blood	chloroform-blood	normal blood	chloroform-blood	normal blood	chloroform-blood
5.13 cc. $AgNO_3$	5.12 cc. $AgNO_3$	4.93 cc. $AgNO_3$	4.96 cc. $AgNO_3$	4.94 cc. $AgNO_3$	4.96 cc. $AgNO_3$

Hence the conclusion was confirmed that an addition of chloroform does not affect the permeability of the red blood-corpuscles.

Does the fact that lipolytics do not affect the permeability of the red blood-corpuscles, prove that their permeability cannot be modified?

Others have proved from analyses that the view that the stroma of the red blood-corpuscles consists exclusively of lipoids, is incorrect. Only a third part of the stromata are formed by lipoids, the other two thirds are albuminous substances [1]).

For the permeability for anorganic substances, however, the subject under consideration, the albuminous part of the stroma of the blood-corpuscles will be the most important: lipoid membranes are impermeable for anorganic substances, albuminous membranes are permeable. A priori it is, therefore, probable that lipolytic substances will not affect the permeability for anorganic matter, as they leave the albuminous part of the stroma intact.

In order to settle the question about a change in the permeability, another substance than chloroform had to be added to the blood. An acid was taken, because acids act upon albumin.

It was known that acid, added to blood, causes the blood-corpuscles to swell and effects a transfer of chlorine from the serum to the blood-corpuscles. This also takes place when acid is added to a suspension of other cells in serum [2]).

This transfer of water and chlorine was observed again when these experiments were repeated. It was, however, also observed that after an acid had been added, the permeability of the red blood-corpuscles had changed. The latter conclusion was based on the following observations:

1. Of 15 cc. of blood 1 cc. of serum was replaced by 1 cc. of an isotonic Na_2SO_4-solution, to which a trace of H_2SO_4 had been added. The transfer of chlorine, taking place now, is considerably greater than that effected by H_2SO_4 only. In normal blood an addition of the same volume of Na_2SO_4 causes no transfer of chlorine. If, however, an acid has been added to the blood, the Na_2SO_4 increases the transfer of chlorine, under the influence of the acid.

Hence Na_2SO_4 acts in different manners upon normal blood and upon blood to which an acid has been added: the permeability of the red blood-corpuscles has been affected by the acid.

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1) PASCUCCI. HOFMEISTER's Beiträge. Bd. VI. 1905.
2) HAMBURGER l.c.

EXAMPLE.

In normal blood hardly any transfer of chlorine if ± 12% of the serum is replaced by 2 × hyperton. Na_2SO_4 solution.
In blood to which an acid has been added, a considerable transfer of chlorine, under the same circumstances.

Of 15 cc. of blood 1 cc. of serum is replaced every time by:	
1 c.m³. $\frac{1}{20}$ n . H_2SO_4	1 cc. of a $\frac{1}{20}$ n . H_2SO_4-solution, which at the same time contained so much Na_2SO_4 that it was 2× hypertonic:
chlorine of 4 c.m³. serum 4.23 c.m³. $AgNO_3$	chlorine of 4 cc. of serum 3.96 c.M³. $AgNO_3$

2. As we said before, in normal blood it makes no difference if of 15 cc. of blood 1 cc, of serum is replaced by 1 cc. of isotonic or 1 cc. of 2 × hypertonic Na_2SO_4-solution. In blood to which a trace of an acid has been added, these values are different. An addition of 1 cc. of 2 × hypertonic Na_2SO_4-solution causes much more chlorine to enter the blood-corpuscles than an addition of 1 cc. of isotonic Na_2SO_4-solution.

This furnishes another proof of the changed permeability of the blood-corpuscles.

EXAMPLE.

In normal blood no difference between the transfer of chlorine, caused by an isotonic Na_2SO_4-solution, and that, caused by a 2 × hypertonic Na_2SO_4-solution.
In blood to which at the same time an acid has been added, a difference is found.

Of 15 cc. of blood 1 cc. of serum is replaced every time by:			
1 cc. of 2× hypertonic Na_2SO_4-solution (7.4%)	1 cc. isotonic Na_2SO_4-solution (3.7%)	1 cc. of a $\frac{1}{20}$ n . H_2SO_4-solution containing also 7.4% Na_2SO_4	1 cc. of a $\frac{1}{20}$ n . H_2SO_4-solution containing also 3.7% Na_2SO_4
4.15 cc. of $AgNO_3$	4.18 cc. of $AgNO_3$	3.96 cc. of $AgNO_3$	4.08 cc. of $AgNO_3$

chlorine of 4 cc. serum.

This proved that it was possible to modify the permeability of the blood-corpuscles by the addition of acids.

II. *Interchange of chlorine between blood-corpuscles and medium in human blood.*

It had to be investigated now to what extent these facts could contribute towards an explanation of the chlorine-retention in febrile diseases.

For this purpose the permeability of blood-corpuscles, of persons with a normal chlorine excretion was first examined. The results were as follows:

1. If in 10 cc. of human blood 0.6 cc. of serum is replaced by 0.6 cc. of an isotonic Na_2SO_4 solution, the amount of chlorine in the serum is greater than the dilution with the solution containing no chlorine, would lead us to expect.

Under these circumstances, therefore, chlorine passes from the blood-corpuscles into the serum. Evidently human blood-corpuscles behave in a manner different from horse's, cow's, or dog's blood-corpuscles if the medium in which they are suspended, is modified.

2. If to 10 cc of human blood 25 mgr. of crystallized Na_2SO_4 is added, chlorine passes from the blood-corpuscles into the serum.

EXAMPLE.

Transfer of chlorine in human blood if ± 12 % serum is replaced by isotonic Na_2SO_4 solution, or if crystallized Na_2SO_4 is added to the blood.

Chlorine in 4 cc of normal serum corresponds with:	10 cc of blood contain:	After 25 mgr. of Na_2SO_4 have been added to 10 cc of blood the chlorine in 4 cc of serum corresponds with:	Of 10 cc of blood 0.6 cc of serum is replaced by 0.6 of isotonic Na_2SO_4 solution. The chorine in 4 cc of serum corresponds with:	If no transposition of chlorine had taken place, the chlorine in 4 cc of serum would in the latter case have corresponded with:
4.24 cc of $AgNO_3$	6.8 cc of serum	4.44 cc of $AgNO_3$	4.05 cc of $AgNO_3$	$\frac{6.8-0.6}{6.8} \times 4.24 =$ 3.85 cc of $AgNO_3$.

In a series of cases, in which the blood was examined of people in good health and of patients, of people whose food contained much and of others whose food contained little chorine, of people with and without fever, these two results were invariably arrived at. In all these cases, however, the metabolism of chlorine was normal.

The same experiments were now carried out with the blood of fever patients showing marked retention of chlorine. The results were as follows:

1. If of 10 cc of blood 0.6 cc of serum is replaced by 0.6 cc of an isotonic Na_2SO_4 solution, the amount of chlorine in the serum is exactly the same as that which can be calculated beforehand from the serum being diluted with a solution without chlorine.

The transfer of chlorine from the blood corpuscles to the serum, found in people with a. normal chlorine-excretion, is, therefore not met with in patients with chlorine-retention.

2. If to 10 cc of blood 25 mgr. of crystallized Na_2SO_4 are added, the chlorine in the serum decreases.

Hence the chlorine now moves in an opposite direction, if this result is compared with the chlorine transfer, found in people with a normal metabolism of chlorine.

EXAMPLE.

Chlorine-transfer in the blood of patients with chlorine-retention, if $\pm$ 12 % of the serum is replaced by isotonic Na_2SO_4-solution, or if solid Na_2SO_4 is added to the blood.

Chlorine in 4 cc of normal serum corresponds with:	10 cc of blood contain:	25 mgr. of Na_2SO_4 having been added to 10 cc of blood the chlorine of 4 cc of serum corresponds with:	Of 10 cc of blood 0.6 cc of serum is replaced by 0.6 of isotonic Na_2SO_4 solution. The chorine of 4 cc of serum corresponds with:	If no chlorine-transfer took place, the chlorine of 4 cc of serum would in the latter case correspond with:
4.87 cc of $AgNO_3$	5.5 cc of serum	4.59 cc of $AgNO_3$	4.34 cc of $AgNO_3$	$\frac{5.5-0.6}{5.5} \times 4.87 =$ 4.33 cc of $AgNO_3$

It seems that from the result of these experiments no other conclusion can be drawn, except that in patients with chlorine retention a modified permeability of the red blood-corpuscles is to be observed. The question suggests itself if the permeability for the other body cells has not also undergone a change.

For the permeability of the red blood-corpuscles is very constant if the chlorine-metabolism is normal. However ill the patient may be, the permeability of the red blood-corpuscles is normal if the chlorine-metabolism is normal. Now the patients with chlorine-retention show a different permeability. This points to the fact that in this diseased organism, influences have been active which have had a powerful effect upon the permeability of the red blood-corpuscles. Since these noxious influences have been able to change the otherwise so constant permeability of the red blood-corpuscles, perhaps the supposition that the permeability of the other body-cells has also been affected, is not too hazardous. As we know the permeability of red blood-corpuscles agrees, in many respects, with that of the other body cells. We also know that acids affect the permeability of blood-corpuscles and other body-cells. Then we are not so far removed from the conclusion that, if in vivo this stable permeability of the red blood-corpuscles has been affected, the permeability of the other body-cells might also have been modified.

Conclusions.

On the ground of these facts we might form the following conception.

As in vitro the permeability of cells can be changed by an addition of acid, this can be done in vivo by other influences, in the case of some febrile diseases. In the latter case chlorine enters the cells under the same circumstances under which it leaves the cells in the normal individual. The consequence of this may be that with these patients chlorine cannot pass from the tissues into the blood.

The fact that the chlorine-transfer is constant in normal people and in patients with a normal chlorine-excretion, that the chlorine-transfer is constant in an opposite direction, in people with chlorine-retention, suggests the possibility that a modified permeability of the cells will have to be reckoned among the causes of chlorine-retention in febrile diseases.

Groningen, March 1913.

Chemistry. — *"The systems phosphorus and cyane."* By Prof. A. SMITS. (Communicated by Prof. J. D. VAN DER WAALS).

Als was already stated before [1]) it has been ascertained that the vapour tension curve of liquid white phosphorus *cannot* be the metastable prolongation of the vapour tension line of molten red phosphorus. This fact is in perfect harmony with the circumstance that it can be calculated from the determinations of ASTON and RAMSAY [2]) on the surface tension that the liquid white phosphorus must become critical at $\pm 422°$.

In consequence of the existence of this critical point the particularly interesting case presents itself that the P,T-figure for white

1) These Proc.

2) Journ. Chem. Soc. **65**, 173 (1894).