and without alcohol can hardly be compared the one with the other.

To ATWATER and BENEDICT's experiments it must be objected (as DURIG has also pointed out) that their determinations covered lengthy periods at the close of which a favourable influence may have been neutralized by a subsequent unfavourable action, so that the totals do not vary much and are not typical of the real process.

Our conclusion is that directly or indirectly alcohol not only produces energy for muscular exercise, but also that after the taking in of alcohol the latter occurs more economically at the outset, even under the unfavourable condition of a high temperature of the surroundings. This favourable influence of the alcohol gradually decreases and ultimately alters to the opposite in one of the subjects.

Utrecht. *Institute of Hygiene.*

Physiology. — *"On fibrin in sol and gel state. Likewise a contribution to our knowledge of the blood-coagulation problem."* By E. HEKMA. (Communicated by Prof. HAMBURGER).

(Communicated in the meeting of April 25, 1913).

The coagulation of blood is based, as we know, upon the transition of a coagulable albuminous substance, found in circulating blood and called fibrinogen, into a solid substance, fibrin.

Fibrin forms threads, fibrils, these fibrils may form a network which can enclose the blood-corpuscles. Hence, when the blood which flows from a wound coagulates, a plug is formed which can close the wound, whilst if for instance the blood, spouting from an opened blood-vessel, is left to itself in a glass, the fibrin-network with the enclosed blood-corpuscles forms the so-called blood-clot. If, however, the blood, flowing from a blood-vessel is not left to itself, but beaten up, then the fibrinogen is turned into fibrin in the form of a compact, white mass of fibrils. This fibrin, however, should not be looked upon as pure fibrin, that is to say as the coagulated substratum of fibrinogen. For besides the latter substance, which forms its main component part, blood-platelets and likewise red blood-corpuscles and leucocytes or rests of them, not to mention other substances, pass into the fibrin.

The study of the problem relating to the nature of blood-coagulation, in other words the formation of fibrin is mainly occupied with two questions. First with the question: what relation exists between fibrinogen and fibrin, and secondly: what agents cause fibrinogen to pass into fibrin.

Untill now next to nothing was known of the former question, whilst the latter has given rise to a great many investigations which have resulted in more or less divergent theories, without a definite solution of the problem being arrived at. The latter circumstance is naturally connected with the former; as long as the nature of the relation between fibrinogen and fibrin, the base so to speak of the problem relating to blood-coagulation, was unknown, a solution of this problem could hardly be expected.

As I believe that my investigations bearing on this subject have brought to light the nature of the relation between fibrinogen and fibrin, I beg leave to give the following short account of the researches which have led to this result.

Beforehand it should be observed that originally it was by no means my intention to occupy myself with researches in this direction. It was rather some observations, made in the course of experiments, undertaken for another purpose, which suggested to me a series of investigations relating to fibrin, with a view to the problem of blood-coagulation. I had observed, for instance, that an addition of an acid, to a solution of fibrin in strongly diluted NaOH caused a precipitate, which, after being left to itself for some time, was found to have passed into a fibrous coagulum. This observation, which naturally struck me very much, was first made with phosphoric acid and afterwards also with other acids [1]).

When subsequently it was investigated more closely under what experimental conditions coagulation in fibrin-NaOH solution took place when an acid was added, it appeared:

1. that by adding drops of HCl of a rather high concentration, to a fibrin-NaOH-solution, an acid coagulum in an alkaline medium could be obtained, which coagulum disappeared again when it was shaken and could be produced again by the addition of more HCl, as long as the fluid gave a distinctly alkaline reaction.

2. That by an addition of so much HCl, either gradually or at once, that the solution was about neutral a permanent coagulum could be obtained.

3. That likewise a permanent coagulum was obtained by an addition of so much HCl that the medium became strongly acid.

4. That an addition of so much HCl that a feebly but distinctly

1) The fibrin-NaOH-solution had been obtained by exposing ordinary fibrin to a strongly diluted NaOH-solution (e.g. 0.3%). The fibrin swells in it strongly, the swollen mass gradually passes into a liquid state and finally forms a thin liquid solution. Such a solution, after being filtered, was used for the experiments.

acid reaction of the medium set in, caused a coagulum, formed in a neutral medium, to disappear again.

5. That the coagulum, mentioned sub 1—4, consisted of a spongy mass of fibres.

From these experiments it might be concluded that a solution of ordinary fibrin in strongly diluted NaOH, contained a substance that could be obtained as a flake-like precipitate by an addition of HCl, which precipitate was found to agglutinate in fibres *a.* at an alkaline, *b.* at a neutral, *c* at a rather strongly acid reaction of the medium; these fibres, in their turn, formed a net- or spongework, a real coagulum, which coagulum could be dissolved in strongly diluted NaOH and in strongly diluted acid.

Further it appeared that this coagulum, formed at a neutral reaction, not only dissolved again in a dil. NaOH-sol., but also that in this solution a new coagulum could be obtained by neutralizing this solution, and also by acidifying it rather strongly. And conversely, if the coagulum was dissolved in strongly diluted acid, a coagulum could be obtained from this solution by neutralizing it with NaOH.

It followed that we had to deal with a coagulating substance, derived from fibrin, which could be made to pass from a sol-state into a gel-state and back again.

Now the question suggested itself what remarkable substance this might be.

Must it be assumed that the fibrin, when dissolved in NaOH, had been decomposed, forming meanwhile a coagulable substance hitherto unknown, a substance derived from fibrin itself or from one of its accompanying substances such as: blood-platelets, red and white bloodcorpuscles?

Or might it be that the fibrin when dissolved in NaOH, had *not* been decomposed, but had passed into a colloid solution, that therefore we might have to do here with fibrin in a sol-state, with an alkali-hydro-sol of fibrin, from which state it might be brought again into a gel-state by an action of acid, with or without the help of a fibrin-ferment?

It will be readily understood that I hesitated in making the latter supposition. First because it was diametrically opposed to the generally received opinion that fibrin must be looked upon as an irreversible gel. And secondly it seemed strange that fibrin coagulation should take place in a strongly acid as well as in a neutral and alkaline medium.

Yet the new problems which had presented themselves so unexpectedly, were fully deserving of attention. Not only from a general, biological or a colloid-chemical point of view, but also because it was clear that the solution of these questions might open new views on the important problem of the nature of blood-coagulation, and perhaps also on that of thrombus-formation.

These questions were therefore submitted to a further investigation.

In this investigation it had to be determined in the first place which part of the fibrin produced the coagulating substance, either the fibrin proper, as I began to suspect, or the attendant substances.

Therefore *pure fibrin* free from blood-corpuscles was experimented on. This pure fibrin was obtained from bloodplasma which was kept fluid by receiving the blood in an equal volume Na-fluoride-solution of 1—2 %, or in a citrate-NaCl-solution.

The blood taken from cow or horse, and kept fluid in this manner, was centrifugated until the fluid at the top had become clear, when the red and white blood-corpuscles and the blood-platelets were obtained as a sediment.

This clear fluid, which contained "fibrinogen", the parent substance of fibrin, was removed with a pipette and left to itself. After some time, (from a few to 48 hours) a spontaneous coagulum had been formed.

The coagulum thus obtained, was filtered off and the filtrate was again left to itself. After a shorter or longer period a new coagulum was found in the filtrate. This process of partial coagulation was repeated 3 or 4 times, the coagulum obtained from the last filtrate being used for the experiments. This somewhat lengthy method was followed in order to have the greatest possible certainty that all blood-corpuscles, including the blood-platelets, had been removed, so that the fibrin was obtained in as pure a state as possible.

The somewhat coloured coagulum thus obtained, grew white when washed with water, and consisted of a mass of elastic fibres.

It soon appeared that this pure fibrin dissolved readily in strongly diluted NaOH, Na_2CO_3 and in strongly diluted acids, which solution was preceded by a short stage when the fibres were visibly swollen. The swollen fibres were absolutely transparent, as clear as glass, not turbidly transparent as I had observed in the case of ordinary fibrin.

I shall first describe some experiments, made with solutions of this pure fibrin in diluted acids, for instance in 0,1 % orthophosphoric acid.

These and the following experiments were always carried out at room-temperature.

If to this fibrin-orthophosphoric acid-solution-strongly diluted NaOH was added, so that the fluid became about neutral, a very thin haze was formed, which after some time was found to consist of very fine threads which were dissolved again by the addition of some more NaOH. If, however, the latter solution was neutralized again by diluted acid, the coagulum of fibres appeared again; we evidently had to do here with a reversible process. The fibres were also formed if to the fibrin-orthophosphoric-acid-solution di- or trinatriumphosphate were added, both, alkaline solutions as we know.

If now the coagulum caused by Na_2HPO_4 or by Na_3PO_4 in a fibrin H_3PO_4 solution was thoroughly washed in water and exposed again to strongly diluted H_3PO_4, the coagulum was dissolved again, whilst an addition of Na_2HPO_4 or Na_3PO_4 again effected a coagulum.

Secondly some results may be mentioned, obtained in experiments with solutions of pure fibrin in strongly diluted NaOH.

Neutralization with diluted H_3PO_4 first caused a hazy precipitate which turned into fibres. With various other acids the same results were obtained. An addition of somewhat too much acid, so that the fluid became distinctly acid caused the coagulum to pass into solution again; it could, however, be produced again by neutralizing this solution.

If to a solution of pure fibrin in 0.1 % NaOH drops of NaH_2PO_4, which as we know gives an acid reaction, were added, a precipitate was formed which, often after a few minutes already, passed into a network of fibrils.

Here too it could be observed that whilst at first the fibrous substance was distributed over the whole fluid, after some time the coagulum settled upon one of the sides of the test-tube, whence we may conclude that the coagulum must possess the power of retraction.

If this coagulum, after being washed with water, was exposed to a 0.1 % NaOH-solution it was dissolved again, while the addition of an acid or of an acid phosphate of Na brought out again a very fine coagulum of fibres, often after only a few minutes.

Acid *phosphate of Ca* was found to effect coagulation in fibrin NaOH-solutions under the same circumstances as acid phosphate of Na. To a certain extent there was a difference, however, as the coagulum, obtained by acid phosphate of Ca, even after being washed thoroughly, did not dissolve so easily in dil. NaOH as the coagulum caused by acid Na-phosphate.

It seemed interesting to find out if a weak acid such as CO_2 would act in a similar way on fibrin-NaOH-solutions, as the other acids and acid salts, mentioned above. And it appeared indeed that

an addition of an equal volume of water, containing CO_2, to a fibrin-NaOH-solution, brought about turbidity and a subsequent coagulum of fibres. As a rule I saw already after some minutes that flakes were formed round the beads of CO_2, which had adhered to the side of the test-tube. These flakes formed the starting-point for further thread-formations. The beads of CO_2, being weak acid-centres with a great surface, became the starting-point of the coagulation, a mechanical factor playing undoubtedly a certain part since on the precipitate or coagulum, formed at the surface of a CO_2-bead, other precipitated colloid particles could settle.

Besides with fibrin-solutions in diluted NaOH, experiments were also carried out with solutions of pure fibrin in strongly diluted Na_2CO_3-solutions. Fibrin was found to dissolve very well in diluted Na_2CO_3-solutions, whilst on the other hand it was insoluble, or almost so, in solutions of $NaHCO_3$.

The results, obtained with solutions of fibrin in diluted Na_2CO_3, were entirely analogous to those obtained with fibrin-NaOH-solutions, as appears from the following example, which may also serve to prove that a *$CaCO_3$-solution* could effect coagulation in fibrin-Na_2CO_3-solutions, which was indeed also the case, though it was not mentioned before, in fibrin-NaOH-solutions.

Pure fibrin was put in a 0.2 % Na_2CO_3 solution in which it dissolved rather easily.

To 10 cc. of this fluid were added:

1. 10 cc water containing CO_2.
2. 10 cc water containing CO_2 + 1 cc of a 0.4% $CaCl_2$-solution.
3. 10 cc of a 0.4% $CaCl_2$ solution.

The result was as follows:

	Fluid.	Reagent.	Result.
1	Pure fibrin in 0.2 % Na_2CO_3-sol. 10 cc.	10 cc. of water containing CO_2	Coagula in flakes and fibrils which include beads of CO_2
2	the same 10 cc.	10 cc. of water containing CO_2 + 1 cc. of $CaCl_2$ sol. 0.4%	the same
3	the same 10 cc.	0.4 % $CaCl_2$ sol. 10 cc.	Seemingly jellylike coagulum; the test-tube could be turned upside down. When shaken the coagulum divides into fluid and a mass of fibrils.

The coagula obtained in 1, 2, and 3 were then put into a 0.1 %

NaOH-solution in which 1 dissolved quickly, 2 slowly, and 3 very slowly. It seemed as if $CaCl_2$, or Ca changed the coagulating substance in such a manner that it was difficult to dissolve it in strongly diluted NaOH. With regard to $CaCl_2$ it appeared, therefore, that a fibrin-NaOH-solution (and also a fibrin-Na_2CO_3-solution) had the same properties as are known of fibrinogen-solutions.

From the foregoing experiments it follows therefore:

That, like impure fibrin, also pure fibrin, free from blood-corpuscles, could be dissolved by strongly diluted NaOH or Na_2CO_3, and by diluted acids. That the fibrin-solutions in alkali contained a substance which, under the action of acids (also of CO_2) and likewise of $CaCl_2$ sol., could be obtained as a fibrous coagulum, whilst solutions of strongly diluted acids could be made to coagulate by diluted alkali.

Whilst, however, the impure fibrin could be dissolved by dil. alkali or acid, only after several days, the pure fibrin was discovered to pass into solution in a much shorter time, often after only a few minutes.

Without entering into the question if it would appear afterwards whether a coagulating substance might be obtained from attendant substances of impure fibrin more particularly from its blood-corpuscles or their component parts, these experiments justified the conclusion that at any rate pure fibrin, the coagulated substratum of fibrinogen, when dissolved in diluted acid or alkali, supplied a substance which could again be made to coagulate.

Meanwhile, I had grown more and more convinced in the course of these experiments, that I had not got to deal with a new coagulable substance, formed from fibrin when it was dissolved, but with fibrin itself in sol- and gel-state.

And as the coagulation of fibrin is generally attributed somehow to the action of a ferment (fibrin-ferment), it stands to reason that repeatedly the question suggested itself if the coagulation in the fibrin-solution, under the action of acids or alkali or $CaCl_2$, would have to be attributed to an action of these substances only, or if it might also be due to a ferment-action.

It was found that the latter, not unimportant question had to be answered in the negative, at least if it may be assumed that such a ferment would be destroyed by being boiled.

For coagulation could be effected in fibrin-alkali- and also in fibrin-acid solutions, even after they had been boiled, under exactly the same circumstances as in the case of the unboiled solutions.

The results, hitherto obtained, gave rise to the following provisional conclusions:

I. That in the solution of fibrin in strongly diluted NaOH or Na_2CO_3 or in strongly diluted acid, we have not a new coagulable substance derived from fibrin, but a transition of the gel-fibrin into the sol-state.

II. That under certain experimental conditions, fibrin from its sol-state, can be made to pass again into its gel-state whilst forming an elastic coagulum, the cooperation of a ferment being found to be unnecessary.

III. That, therefore, fibrin must be looked upon as a reversible gel, the sol-state of which can be compared, or is identical, with blood-fibrinogen in solution.

If these results might indeed be looked upon as conclusive, they would evidently furnish an entirely new foundation for the solution of the problem relating to the nature of blood-coagulation.

These preliminary conclusions, however, and more especially the third would have to be confirmed by further proofs if they were to be accepted without reserve. It seemed to me that one would be justified in considering the formulated conclusions proved, and more especially III, if it could be shown :

1. That in fibrinogen-solutions, more especially in natural fibrinogen solutions, coagulation could be effected under the same conditions as it had been effected until now, either in fibrin-alkali- or in fibrin-acid-solutions.

2. That fibrin-alkali- or fibrin-acid-solutions could be made to coagulate not only by acid or alkali, but also by those factors by which "fibrinogen-solutions" generally coagulate.

3. That saturated salt-solutions, such as NaCl or NaFl-solutions, act upon fibrin-alkali- or fibrin-acid-solutions in the same way as upon plasma which has been kept fluid or upon fibrinogen-solutions.

Ad 1. To investigate the first condition centrifugated plasma, kept fluid by a NaFl or citrate NaCl-solution, was made use of, and likewise of a transudate which did not coagulate spontaneously and was almost free from blood-corpuscles, which transudate had been obtained from a patient suffering from ascites. It appeared now that these fluids did not coagulate when strongly diluted NaOH was added. On the contrary, an addition of only slight quantities of NaOH-solution retarded the spontaneous coagulation of the fluid plasm, and if somewhat more of the diluted alkali-solution were added, spontaneous coagulation did not take place at all.

The results obtained with diluted acids and acid salts were entirely different. By an addition of a trace of acid, coagulation took place

within 15 minutes as a network or rather a spongy mass of fibres, in a NaFl or citrate NaCl plasm in which no spontaneous coagulation took place within 24 hours. The same thing was found to be the case with the ascites-fluid, a transudate which did not coagulate spontaneously. This result was obtained with all sorts of acids, also with CO_2. The coagula obtained, could be dissolved again in alkalies or acids whilst in these solutions coagulation could be effected again by neutralization of the dissolving fluid.

Plasm which was kept fluid, and likewise a transudate which did not coagulate spontaneously, were, therefore, found to present a great resemblance with fibrin-alkali-solutions and NOT *with fibrin-acid-solutions.*

Ad 2. The second condition viz. that fibrin-alkali or fibrin-acid solutions must be coagulated by those factors which coagulated fibrinogen-solutions, was also satisfied. What is the characteristic of a fibrinogen-solution? That by the action of blood-serum or by organic extracts, as containing "fibrinferment", it coagulates and passes into fibrin.

Now solutions of pure fibrin in strongly diluted NaOH also had this property. If for instance to a solution of pure fibrin in NaOH 0.05 %, an equal volume of serum was added, a coagulum was formed within a few minutes in the shape of a spongy mass, whilst within 15 minutes a jellylike coagulum seemed to have been formed so that the test-tube could be held upside down, without more than a few drops of fluid running out. This seemingly jelly-like coagulum turned out to be a very dense fibrous, spongy mass, for when it was shaken well it separated into fluid and a small clump of fibrils.

A test with the ascites-fluid, which might be looked upon as a natural fibrinogen-solution, revealed that its action on serum was identical with that of the fibrin NaOH-solution; here too a jelly-like coagulum was formed, which was in fact a fibrous, spongy mass, filled with fluid. Evidently in both cases the fibrous mass which, on the coagulum being shaken, was found to have such a small volume, was yet capable of enclosing all the fluid, so that the coagulum had the appearance of being a homogeneous jelly. Similar coagula were obtained by the addition of serum, in fibrin NaOH-solutions, the fibrin of which was supplied by the blood of horses as well as cows. Nor was the action of the serum at all a specific one, for cow- as well as horse-serum effected coagulation in solutions of cow-fibrin.

Besides, this appeared already from the fact that cow's as well as horse's serum effected coagulation in a human ascites-fluid as I observed before.

Like serum, watery organic extracts, for instance a watery extract of calf's thymus-gland, were found to effect coagulation in fibrin NaOH-solutions and in ascites-fluid.

Ad 3. Thirdly fibrin-NaOH-solutions would have to act upon saturated NaCl- and NaFl-solutions in the same way as fibrinogen-solutions, such as plasm which is kept fluid or transudates.

By the addition of an equal volume of saturated NaCl-sol. to fibrin-NaOH-solution, flakes and jelly-like strings were obtained, just as when an equal volume of saturated NaCl-solution was added to plasm which was kept fluid or to ascites-fluid. A saturated NaFl-solution immediately effected coagulation as a rule, and that in all 3 fluids. The agreement in both respects between fibrin-NaOH-solutions on the one hand, and plasm which is kept fluid and ascites-fluid, on the other, was, therefore, a striking one.

The result of these series of experiments removed all doubts as to the accuracy of the foregoing provisional conclusions. *I felt absolutely certain now that fibrin can be brought from a gel-state into a sol-state and vice versa. And moreover that the sol-state, caused by the solution of fibrin in stronqly diluted alkali and not the one obtained by the solution of fibrin in diluted acid, must be considered identical with "fibrinogen" as found in blood and body-fluids.*

How are we to conceive the transition of fibrin from the gel-state into the sol-state under the action of diluted alkali or acid, and also the return from the sol-state to the gel-state under the influence of this action?

The following experiments and considerations may supply an answer to this question. The ordinary impure fibrin, as formed when blood is beaten up and likewise pure fibrin, formed at spontaneous coagulation of plasm which is kept fluid, contains a certain amount of water, is swollen to a certain extent. By drying the washed-out fibrin, for instance by exposing it to the air, this water evaporates. The dried fibrin is brittle, hard and not elastic. If, however, the dried fibrin is placed in water, it swells again, whilst its elasticity returns.

Also if a dried fibril was *boiled* in water it began to swell, and became elastic as at first. This swelling caused by water is, however, a very restricted one. But if the ordinary, moist fibrin or the air-dry fibrin are exposed to water, to which alkali or acid have been added, the fibrin swells much more than by water only; undoubtedly we have to deal here with a swelling-process, introduced by water.

If in these experiments much fibrin is taken in proportion to alkali or acid-solution then it will be observed that the fluid is entirely imbibed by the fibrils. Every fibre is seen to swell strongly, has become jelly-like, and more or less transparent. (We mean here the ordinary, raw fibrin; pure fibrin gives less striking results because it is dissolved very quickly by alkali and acid, so that the swelling-stage is only very short).

The separate coarser fibrils are at first still plainly visible in the swollen mass as jelly-like strings.

If a coarse fibril, swollen by dil. alkali, e.g. NaOH 0.2 %, is placed in dil. acid so that the fluid becomes about neutral, then the swollen fibril gradually resumes its original form and qualities; it becomes elastic again as before. If the fibril is not placed in diluted, but in concentrated acid, the swollen fibril resumes its shape and qualities much sooner. This is also the case if the swollen fibril is put into solution of acid phosphate of sodium or a solution of acid phosphate of calcium. Hence we have to deal here with a reversible process. But not only by acids and acid salts the fibre, swollen by alkali, may be brought again into its original state, it may also be effected by a 1 % $CaCl_2$-sol. or by a saturated sol. of NaCl or NaFl, or by any other saturated salt-solution, such as $MgSO_4$ or $(NH_4)_2 SO_4$. And it also appeared that a fibril, swollen by alkali, also gradually resumes its original form and qualities, if it is placed in an excess of water, and more quickly by shaking it with much water. It is a remarkable fact that the swollen fibril retains these qualities also when *the swollen fibril is boiled first*, at least if the alkali is in a weak concentration and has not acted too long. Also after being boiled the fibril, swollen by 0.20% NaOH, returns to its original state as regards shape and qualities, when exposed to acid, salt, or an excess of water.

It follows that the process which takes place when fibrin is swollen by diluted alkali, must be of a very superficial kind, and that ordinary chemical process, or change is out of the question.

Between fibrin on the one hand and alkali on the other, only an extremely loose compound, an *adsorption-compound* can have been formed.

The logical conclusion is that all component parts of the swollen coarse fibril, first the smaller fibres, next the smallest fibres, and lastly the minutest parts of which these smallest fibres consist, have formed an adsorption-compound with the alkali, and are consequently swollen.

But it must also be inferred that the component parts and parti-

cles have not changed their places with regard to each other, as long as the coarse fibril, swollen under the influence of alkali, entirely resumes its former shape and qualities when exposed to acid, salt or water.

If the fibrin mass, swollen by alkali or acid, has absorbed all the fluid, and is left to itself, then, after some days, the swollen mass (we mean here ordinary impure fibrin) first passes into a thick and then into a thin, colloid solution. It appears to me that we must look upon this process as a continuation of the swelling-process, mentioned before. The colloid-particles retaining in the swollen fibril their coherence, their place with regard to each other, are driven apart by the continual swelling and at last pass into a colloid solution, into a sol-state.

If this view is correct, this colloid solution, this alkali-hydro-sol if I may call it thus, must have the same qualities as the swollen fibril, and we saw already that this is indeed the case.

For we saw that flakes are formed in a fibrin-NaOH-solution by neutralization, by stronger acids, and by the action of salts under the successive formation of a coagulum of fibres, of an elastic gel, which may either remain somewhat swollen, as is the case when it is treated with a saturated NaCl-solution, or a coagulum with little or no swelling, a retracting, fibrous or spongy mass may be formed as was observed under the action of acids and acid salts, of $CaCl_2$-sol, and of a saturated NaFl-sol.

The solution of fibrin in strongly diluted alkali gives rise, as we saw, to an alkali-adsorption compound. And since bloodplasm kept fluid, and also a transudate, were acted upon in a similar manner by acids and salts, as a solution of fibrin in strongly diluted NaOH, both as regards the formation of flakes and the succeeding agglutination in the form of fibrils, in other words the coagulation, it may confidently be assumed that *fibrinogen, as found in transudates, in bloodplasm kept fluid, and hence also in blood, may be looked upon as an alkali-adsorption compound of fibrin.* In other words fibrinogen as found in blood must be looked upon as an alkali-hydrosol of fibrin, as fibrin in a colloid solution in which the fibrin-particles are found in an exceedingly fine state of division, owing to the adsorbed ions of OH.

It seems to me that hereby the nature of the relation between fibrinogen and fibrin it has been determined, but likewise that it has been shown that blood contains a source of alkali in an extremely loose compound, in the form of fibrinogen. A compound so loose that it must be judged capable of giving up at any moment its

alkali to acid substances, under the formation of fibril-flakes, and, under favourable circumstances, coagulation of the colloid fibril. Perhaps we have to deal here with a fact, the importance of which to physiology and pathology goes far beyond the problem of blood-coagulation, I do not however, wish to enter into this question in this paper; I shall restrict myself to emphasize the possible significance of this fact, as a foundation for the solution of the problem concerning the nature of blood-coagulation.

It has been demonstrated that the transition of fibrin from the sol-state into the gel-state may be brought about by the following factors:

a. by acids in weak concentration, or by neutralization;
b. by acid salts, also at an acid reaction of the medium;
c. by acids in strong concentration;
d. by saturated salt-solutions;
e. by calcium-chloride solutions.

This formation of flakes in fibrinogen-solutions is only a special case of a quality, characteristic of colloids and albumens in general, at least as regards the factors mentioned sub *a*, *c*, and *d*. The colloid fibrin, however, is distinguished from other albumens, except casein, in being able to agglutinate in fibres under favourable circumstances, to form an elastic gel, a real coagulum such as is formed when blood coagulates.

Will our knowledge of the relation between fibrinogen and fibrin on the one hand, and of the factors which cause fibrin to pass from the sol-state into the gel-state, on the other, enable us to give a satisfactory explanation of the phenomenon of natural blood-coagulation?

This would indeed seem to be the case if only it could be demonstrated that in blood, substances are found or can be formed, whose action would be identical with that of one or more of the factors mentioned above.

For this purpose it might be investigated if acid salts are formed in the blood, or a hypothesis might be formed as to the importance of CO_2 in this respect, after the manner of some other investigators.

All this seems superfluous, however. Keeping in view the data to be found in the literature on the problem of blood-coagulation, it stands to reason that we should think in the first place of the *nucleoproteids* and of *calcium*. The nucleoproteids, substances derived from decomposed nucleated cells, possess as we know properties of an acid, while more especially PEKELHARING and his school have established beyond doubt that these substances can effect blood-coagulation.

The nucleoproteïds might, therefore, be ranked with acids in general, both on account of their acid-qualities and on account of their coagulating qualities, if not PEKELKARING were of opinion that the nucleoproteids, together with calcium, produce a ferment, which ferment would have to be looked upon as the cause of blood-coagulation, while likewise most other investigators attribute the coagulation of blood to a fibrin-ferment.

Since, however, it has appeared from my investigations that the relation between fibrinogen and fibrin is of such an extremely simple kind, it seems strange that the transition of the former substance into the latter, which process is in fact nothing but a withdrawal of ions of OH from fibrinogen, should require a ferment-action. The more so since, as we observed before, the action of the nucleoproteids might be readily explained by the acidity of these substances.

The conception that the nucleoproteids owe their coagulating properties to their acidity, would at once explain why nucleoproteids of such different origin, and also nucleohistons and nucleoalbumens all have the power of effecting coagulation, the reason being that all these substances act as acids.

Judging myself entitled on the ground of the investigation, of which a short summary has been given in the preceding pages, to the conclusion:

That fibrinogen, as found in the blood, must be looked upon as an alkali-adsorption compound of fibrin, in other words, as fibrin which, under the influence of adsorbed ions of OH, is in a finely divided and swollen state, in a sol state; that consequently the transition of fibrinogen into fibrin, that is to say the coagulation of blood, must be based upon a withdrawal of ions of OH from fibrinogen, I think I may add the supposition that the natural blood-coagulation must be the result of the withdrawal of ions of OH from fibrinogen by nucleoproteids in consequence of their acidity.

If this supposition is found to be correct, further investigations will have to teach if nucleoproteids act as such, or if their coagulating capacity is due to the formation of nucleinic acid or phosphoric acid, and also what part is possibly played by calcium in this process. Moreover it will have to be explained how this acid-action of the nucleoproteids can have impressed various investigators as being a ferment-action.

Groningen, April 1913. *Physiological Laboratory.*