THE RÔLE OF CEPHALIN IN BLOOD COAGULATION.

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The process of blood clotting is very complex. As it occurs normally, it is the result of many factors not well understood. The progress made in recent years was due to the fact that ways were found to separate the many factors into groups, some of which control definite phases of the complex process of blood clotting. The phase best understood is that of the conversion of fibrinogen into fibrin. Four substances take part in this reaction. One is the substrate, and the other three combine to bring about the transformation of the substrate from a soluble into an insoluble state. Alexander Schmidt understood the process correctly. More recent workers brought out many of the details of the process. The most recent contributions were made by Bordet and his collaborators, and by Howell and his coworkers.

The terms applied to each of the factors differed with the individual author. Since the blood clotting experiments here reported were carried out by a worker of Bordet's school, the nomenclature employed in this publication is of that school. In terms of that school the process of fibrin formation is expressed by the following diagram.

<table>
<thead>
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<th>Plasma at the moment of coagulation</th>
<th>serum after coagulation</th>
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<td>Platelets or tissue extracts or certain lipoids</td>
<td>in presence of Ca ions → thrombin</td>
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<tr>
<td>cytozyme</td>
<td>+</td>
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<tr>
<td>Fibrinogen = fibrin</td>
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Howell accepts the interplay of all four of the substances in the process of blood clotting but holds a different view on the rôle of cytozyme, which, according to Howell, plays no part in the actual transformation of fibrinogen into fibrin. Howell also disagrees with other workers in his view on the chemical nature of cytozyme. Alexander Schmidt, Wooldridge, Bordet, Delange and others regarded the substance as lecithin. This opinion was based on the thermostability of the substance and on its solubility in alcohol. Howell on the contrary came to the conclusion that the active substance was another phosphatide; namely, cephalin. Howell and his coworkers have also made an attempt to associate the activity of the phosphatide with a definite peculiarity of its chemical structure. At the time of the work of Howell it was generally accepted that the unsaturated acid entering into the structure of cephalin differed from that of lecithin. From cephalin, linolic acid was isolated and from lecithin, oleic acid. According to Howell and McLean the higher unsaturation of the fatty acid is the factor which lends to cephalin its property of being an agent in the formation of thrombin.

The present communication is a mere note dealing not with the entire problem of fibrin formation but only with the chemical nature of cytozyme. Is cytozyme lecithin or cephalin? Since the work of Howell and his coworkers, the knowledge of the chemical structure of phosphatides has made considerable progress. In the light of this progress the conclusions regarding the chemical nature of cytozyme required reinvestigation.

The recent work on lecithin has brought out the fact that there exist forms of this substance which contain in their molecule a fatty acid of still higher unsaturation than the one previously isolated from cephalin. In the light of the theory of Howell and McLean, one might have expected the new form of lecithin to play the same part as cephalin in fibrin formation.

Furthermore, recent work on cephalin has brought out the fact that the material handled by the older writers under the name of lecithin was in reality a complex mixture and not a uniform substance. The components of this mixture were found to be identical in character with those of another complex mixture described by previous writers under the name of cuorin, or heparphosphatide. Cuorin and the cephalin of the older writers
differed one from another in the proportions of some of their components. Both substances were found to consist of true cephalin, true lecithin, and of the same substances in a state of partial decomposition. The character of the decomposition products varied from sample to sample. Yet, Howell and his coworkers observed that cephalin and cuorin acted in the process of blood coagulation antagonistically to one another.

On the other hand, a substance was recently prepared which was free from the decomposition product of lecithin and of cephalin and which contained 75 per cent of undecomposed cephalin and 25 per cent of undecomposed lecithin. Whether or not the substance contained impurities undetectable by the present methods of analysis, cannot be stated.

It is self-evident that it became important to compare the cytozymic function of the three substances; namely, of ordinary lecithin, of lecithin which contained the fatty acid of a high degree of unsaturation, and of the new cephalin material. In a way also the present materials were mixtures. Ordinary lecithin contains a small proportion of the new form. The new form still contained a very small proportion of the older type. The cephalin contained a small proportion of lecithin. Yet even such material was sufficient to bring out the fact that lecithin, regardless of its form and of its origin, possesses no cytozymic action. On the other hand, material containing 75 per cent of undecomposed cephalin and 25 per cent of lecithin possesses unusually high cytozymic action. It is still active in a concentration of $5 \times 10^{-7}$.

The coagulation experiments were carried out by Dr. Gratia who followed the routine customary in Bordet's school. The plan and the details of the experiments follow.

EXPERIMENTAL PART.

Oxalated plasma from which most of the platelets have been removed by centrifugation contains only a small amount of cytozyme and consequently clots very slowly when recalcified, but clots quickly if some cytozyme is given back in form either of platelet suspension, tissue juice, or lipoidic tissue extract. This offers means of testing the cytozymic properties of a given lipoid by measuring the accelerating influence of the lipoid on the coagulation of a plasma almost free from platelets.
When an oxalated plasma has been strongly centrifugalized and then recalcified, the few remaining platelets contain just enough cytozyme to react with but a small part of the serozyme and thus yield only a small amount of thrombin. The plasma clots slowly and a great excess of unutilized serozyme is found in the serum after coagulation. Such a serum is rich in serozyme and is an excellent reagent to test the cytozymic properties of a given lipoid. If cytozyme even in very small amount is added to this serum, an active production of thrombin immediately results and this mixture is able to clot an equal volume of fibrinogen or oxalated plasma in a few minutes. This is the serozyme-cytozyme reaction of Bordet and Delange.

In our researches we have submitted our different lipoids to both tests. The materials used were prepared as follows:

**Preparation of the Reagents.**

1. *Lipoidic Emulsions.*—1 per cent emulsions of our three lipoids were made in saline solution. As a control a similar 1 per cent emulsion was made with lipoidic extract of tissue which was known to possess strong cytozymic properties. When necessary, higher dilutions of these suspensions were made in the course of the experiments.

2. *Oxalated Plasma Free from Platelets.*—A rabbit was carefully bled from the carotids with a paraffined cannula. Precautions were taken to avoid the contact of the blood with tissue juice and 9 parts of blood were received in 1 part of a 1 per cent solution of sodium oxalate in saline solution, and thoroughly mixed. This 1 per cent oxalated blood was centrifugalized at high speed during 1 hour and the clear supernatant plasma removed from the cells with a pipette. For use in the experiments 1 part of this oxalated plasma (O. P.) was recalcified with 4 parts of a 0.35 per cent solution of calcium chloride in saline solution (Ca).

3. *Serum Rich in Seroxyme.*—A few cc. of oxalated plasma were recalcified as above described. When coagulation began, the recalcified plasma was defribinated with a glass rod. The serum obtained was kept at room temperature until the next day. As thrombin is very labile, the small amount of thrombin left after this very slow coagulation disappears quickly and the next day the serum containing nothing but a large excess of serozyme is ready for use.
4. Fibrinogen. - Instead of the so called pure solution of fibrinogen, “dioxalated plasma” (F) was used as a test for thrombin. This very convenient reagent was prepared according to the technique of Bordet and Delange; i.e., 1 part of 1 per cent oxa-
lated plasma was diluted with 4 parts of a 2 per cent solution of sodium oxalate in saline solution.

A. Egg Lecithin.

Experiment I.

0.25 cc. O.P. + 1 drop saline solution + 7 cc. Cu = 110'
0.25 " " + 1 " egg lecithin + 7 " " = 90'
0.25 " " + 1 " cytozyme + 7 " " = 20'

Egg lecithin exerts only a slight accelerating influence on the coagulation of recalcified oxalated plasma.

Experiment II.

0.25 cc. serozyme + 1 drop saline solution . .5'... + 0.25 cc. F = ∞
0.25 " " + 1 " egg lecithin . .5'... + 0.25 " " = still fluid after 5 hrs.; soft clot after 24 hrs.
0.25 " " + 1 " cytozyme ...5'... + 0.25 cc. F = 2'

Whereas after 5' a mixture of serum rich in serozyme together with cytozyme contains a sufficient quantity of thrombin to clot an equal volume of oxalated plasma in 2', a similar mixture of serozyme with lecithin contains only a practically negligible amount of thrombin that yields hardly a soft clot after 24 hours.

The 1 per cent emulsion of lecithin is rather viscous. Dilutions of the lecithin as well as of the cytozyme emulsions were made, 1/10, 1/100, 1/1,000, and compared.

Experiment III.

0.25 cc. serozyme + 1 drop cytozyme 1/10 ...5'... + 0.25 cc. F = 2'
0.25 " " + 1 " lecithin 1/10 ...5'... + 0.25 " " = ∞
0.25 " " + 1 " cytozyme 1/100 ...5'... + 0.25 " " = 8'
0.25 " " + 1 " lecithin 1/100 ...5'... + 0.25 " " = ∞
0.25 " " + 1 " cytozyme 1/1,000...5'... + 0.25 " " = 25'
0.25 " " + 1 " lecithin 1/1,000...5'... + 0.25 " " = ∞

Egg lecithin is thus inactive at higher dilutions. In the following series the tests were allowed to react at longer intervals.
Experiment IV.

0.25 cc. serozyme + 1 drop lecithin \ldots 5'\ldots + 0.25 cc. F = \infty
0.25 \text{ "} + 1 \text{ "} + 1 \text{ "} + 0.25 \text{ "} + 0.25 \text{ "} = \infty
0.25 \text{ "} + 1 \text{ "} \ldots 10'\ldots + 0.25 \text{ "} + 0.25 \text{ "} = \infty
0.25 \text{ "} + 1 \text{ "} \ldots 15'\ldots + 0.25 \text{ "} + 0.25 \text{ "} = \infty
0.25 \text{ "} + 1 \text{ "} \ldots 20'\ldots + 0.25 \text{ "} + 0.25 \text{ "} = \infty
0.25 \text{ "} + 1 \text{ "} \ldots 45'\ldots + 0.25 \text{ "} + 0.25 \text{ "} = \infty

0.25 \text{ cc. serozyme} + 1 \text{ drop} + 1 \text{ drop} + 1 \text{ drop} + 1 \text{ drop} + 1 \text{ drop} = \infty

The results again were negative. The following series aims to establish whether lecithin in any way affected the potency of the cytozyme.

Experiment V.

0.25 cc. serozyme + 1 drop cytozyme + 1 drop saline solution \ldots 5'\ldots + 0.25 cc. F = 2'
0.25 cc. serozyme + 1 drop cytozyme + 1 drop lecithin \ldots 5'\ldots + 0.25 cc. F = 2'
0.25 cc. serozyme + 1 drop cytozyme + 3 drops lecithin \ldots 5'\ldots + 0.25 cc. F = 2'
0.25 cc. serozyme + 1 drop cytozyme 1/10 + 1 drop saline solution \ldots 5'\ldots + 0.25 cc. F = 2'
0.25 cc. serozyme + 1 drop cytozyme 1/10 + 1 drop lecithin \ldots 5'\ldots + 0.25 cc. F = 2'
0.25 cc. serozyme + 1 drop cytozyme 1/100 + 1 drop saline solution \ldots 5'\ldots + 0.25 cc. F = 10'
0.25 cc. serozyme + 1 drop cytozyme 1/100 + 1 drop lecithin \ldots 5'\ldots + 0.25 cc. F = 10'

The results show that there was no appreciable accelerating inhibiting influence of egg lecithin on the action of cytozyme.

Conclusion.—Egg lecithin has practically no cytozymic properties. The extremely small action observed in Experiments I and II must very likely be due to traces of the active substance still present as an impurity in the egg lecithin material.

B. Liver Lecithin.

Identical experiments were repeated with the liver lecithin with similar results. Thus the liver lecithin is not more active than egg lecithin.

C. Mixtures of Pure Cephalin and Pure Lecithin.

Experiment VI.

0.25 cc. O.P. + 1 drop saline solution + 1 cc. Ca = 50'
0.25 " + 1 " cytozyme + 1 " = 11'
0.25 " + 1 " cephalin + 1 " = 10'
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The mixture containing 65 per cent of pure cephalin has a marked accelerating effect on the coagulation of recalcified oxalated plasma.

*Experiment VII.*

- 0.25 cc. serozyme + 1 drop saline solution \(\ldots 5'\ldots + 0.25 \text{ cc.} \ F = \infty\)
- 0.25 " " + 1 " cytozyme \(\ldots 5'\ldots + 0.25 " = 2'\)
- 0.25 " " + 1 " cephalin \(\ldots 5'\ldots + 0.25 " = 1'\)
- 0.25 " " + 1 " \(1/10\) \(\ldots 5'\ldots + 0.25 " = 1'\)
- 0.25 " " + 1 " \(1/100\) \(\ldots 5'\ldots + 0.25 " = 25'\)
- 0.25 " " + 1 " \(1/1,000\) \(\ldots 5'\ldots + 0.25 " = \infty\)

1 drop of 7 per cent cephalin emulsion, even diluted 1:1,000, is still able to give a positive result. The calculated amount of material contained in this drop is about 1/20,000 of a mg. This will give an idea of the extraordinary cytozymic activity of the mixture of cephalin and lecithin.
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