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Clotting of Plasma in the Absence of Lipoid.

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Cephalin is known to accelerate clotting of blood. In Howell's theory[1] of blood coagulation a thromboplastic substance (or thromboplastein) is liberated from the platelets or tissues when the blood is shed. This thromboplastic substance is supposed to combine with anti-prothrombin (heparin?), setting free prothrombin which is converted by calcium into thrombin. The thromboplastic substance is fat-soluble and appears to be cephalin. The theories of Morawitz[2] and of Bordet[3] are essentially similar to that of Howell. The thrombokinase of Morawitz is only a different name for the same substance as the thromboplastein of Howell, while the cytozyme of Bordet is believed to be a lipoprotein. In Mill's theory[4] of blood clotting, thrombin is a protein-calcium-cephalin compound and the fibrin is a protein-calcium-cephalin-fibrinogen compound. If this view is correct, clotting is impossible in the absence of cephalin, while according to the other theories clotting can still occur in the absence of lipoid, once the prothrombin has been converted into thrombin.

Hardy and Gardiner[5] found that plasma which had been freed from lipoids by extraction with alcohol in the cold clotted normally. This seems to suggest that lipoid is not necessary for clotting. However, this observation of Hardy and Gardiner was only incidental to their study of fat-free serum proteins and they did not mention how the clotting was induced. Their clotting mixture must have contained some lipoid, because the clotting agent, be it serum or thrombin, prepared in the usual way, could not be lipoid-free. It would be desirable to study the clotting phenomenon in the entire absence of lipoid.

Preparation of material. Lipoid-free plasma and serum were prepared essentially according to Hewitt's method[6] described in detail elsewhere.[7] Oxalated plasma was used. In one experiment the oxa-

[1] Howell, W. H., *Bull. Johns Hopkins Hosp.*, 1928, **42**, 199.

[2] Morawitz, P., *Ergeb. Physiol.*, 1905, **4**, 307.

[3] Bordet, J., *Ann. L'Inst. Pasteur*, 1920, **34**, 561.

[4] Mills, C. A., and Guest, G. M., *Am. J. Physiol.*, 1921, **57**, 395.

[5] Hardy, W. B., and Gardiner, S., *J. Physiol.*, 1910, **40**, lxviii.

[6] Hewitt, L. F., *Biochem. J.*, 1927, **21**, 216.

late was removed by dialysis against 0.8% NaCl before the removal of lipoid. The result was essentially the same.

For coagulation experiments, the plasma or serum powder was dissolved in 0.8% NaCl solution to give a concentration of 7%. The solutions were centrifuged to remove any insoluble (denatured) protein.

The tests were carried out as follows: 28 cc. of 0.8% NaCl were mixed with

a. 1 cc. natural serum
b. 1 cc. fat-free serum.
c. 1 cc. 2.5% $CaCl_2$
d. 5 drops of 5% etheral solution of plasma lipoid. This was recovered from the ether-alcohol filtrate in the preparation of fat-free plasma and serum. Most of the lipoid was precipitated when the etheral solution was added to the saline. The precipitate was removed by filtration.
e. 1 cc. 2.5% $CaCl_2$ and 5 drops of 5% etheral solution of plasma lipoid.

To each of the above mixtures 1 cc. of fat-free plasma was added. In a similar series of experiment, natural plasma was used. The results are shown in the accompanying table.

TABLE I.
Coagulation of natural plasma and lipoid-free plasma.

Clotting agent	Natural plasma	Fat-free plasma
Natural serum	+	+
Lipoid-free serum	+	+
Calcium	+	—
Lipoid	—	—
Calcium-lipoid	+	+

+ Indicates clotting.

The clotting of fat-free plasma with natural serum or natural plasma with fat-free serum is to be expected, since in these mixtures all the plasma constituents are present. The clotting of the fat-free plasma with fat-free serum demonstrates that lipoid is not essential for the formation of the clot. However, the fat-free plasma does not clot on calcification as the natural plasma does. Only when both calcium and lipoid are added was the clotting of the fat-free plasma induced. Addition of lipoid alone to the plasma could not induce clotting.

Conclusion. From these observations we must conclude that lipoid as well as calcium is essential for the activation of the thrombin, but once this is formed the presence of lipoid is not necessary.

[7] Wu, H., *Chinese J. Physiol.*, 1933, **7**, 125.