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Coagulative Power of "Activated" Chick Extract in Absence of the Calcium Ion.

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It has been reported that the addition of 1 vol. rabbit plasma to 60 to 100 vol. chick extract greatly increases the coagulative power of the extract.[1]

According to the theory of Mills[2] there are two coagulants which may cause the clotting of blood fibrinogen, thrombin, which acts in the absence of ionized calcium, and tissue fibrinogen which causes coagulation only in the presence of the calcium ion.

The above criterion is utilized in an attempt to establish the nature of the substance causing the marked increase in the coagulative power of an extract which has been activated by the addition of a small amount of plasma.

Chick extract is made by extracting a 10-day chick embryo in 10 cc. of Ringer fluid. It is activated by adding 1 vol. citrated rabbit plasma to 20 vol. of extract. The same extract without plasma addition serves as control. Coagulation time is determined as described in an accompanying paper.

The results with and without calcium are shown graphically in Fig. 1. The extract was activated at 11:35 and tested for coagulative power at subsequent intervals as indicated. The line labelled "Tissue Extract" shows the coagulative power of the control extract on recalcified citrate plasma; without recalcification of the plasma this extract causes no coagulation in 24 hours.

The line labelled "Tissue Extract Activated" shows the coagulative power of the activated extract on recalcified citrate plasma; the solid line gives the power of the same extract when the plasma is not recalcified.

Comparison of the power of the activated and control extract, in the presence of the calcium ion, shows that the former is much more powerful than the latter. According to the work of Mills[3] a decrease in coagulation time from 1¼ minutes to 20 seconds indicates an increase in the concentration of the coagulant of about 64 times.

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[1] King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

[2] Mills, C. A., *Chinese J. Physiol.*, 1927, **1**, 435.

[3] Mills, C. A., *Am. J. Physiol.*, 1922, **60**, 193.

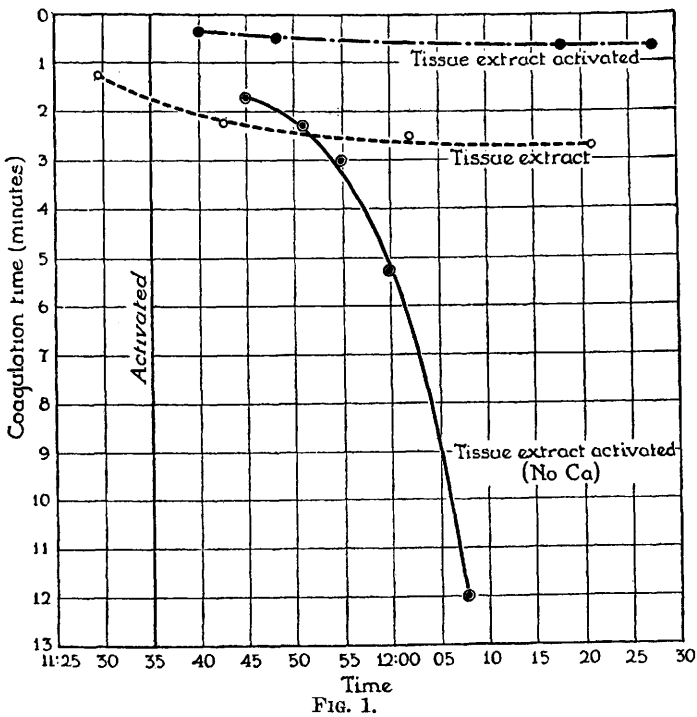

FIG. 1.

The coagulative power of "activated" chick extract in the absence of the calcium ion.

The fact that the activated extract shows coagulative power on citrate plasma without recalcification, whereas the control extract shows no clotting in 24 hours, indicates that a coagulant has appeared in the process of activation which, like thrombin, can cause coagulation independent of the presence of the calcium ion.

The rapid decrease in the coagulative power of the activated extract in the absence of calcium is consistent with the work of Loucks and Scott[4] showing that thrombin is rapidly destroyed in the presence of oxalate. I have found (unpublished experiments) that citrate acts similarly. It must be assumed that the coagulant is exposed to a citrate excess in these experiments (when the plasma is not recalcified) since normal extract fails to show coagulation in 24 hours under these conditions.

The above data indicate that the coagulant involved in the process of activation behaves like thrombin in relation to the calcium ion.

[4] Loucks, M. M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.