acid spot as Fig. 1 indicates; but no other growth-active region of the chromatogram was found. Since we thought some loss of indole-3-acetic acid might occur on running chromatograms in air, this was repeated in a nitrogen atmosphere without any different result. This may not establish with certainty the absence of auxin *a* as there is the possibility of its having extreme instability on a chromatogram; but in a nitrogen atmosphere this would seem less probable.

In plant material, for example, etiolated sunflower seedlings, indole-3-acetic acid was clearly detected on biological analysis of a chromatogram of the anionic fraction of the ether extract, see Fig. 2. Here it may be noted that active extension growth was obtained from the eluate of the indole-3-acetic acid region only. Indication of the presence of an inhibitor having an R_F slightly larger than indole-3-acetic acid is also shown.

This technique affords a ready characterization of indole-3-acetic acid far more specific than diffusion measurements or tests of stability in acid or alkaline media, because the separations effected are due to partition coefficient or solubility differences and may be checked by a reasonably specific spot colour reaction. Simultaneously with this separation and characterization, indole-3-acetic acid and other growth promoters and inhibitors may be quantitatively assayed.

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Mechanism of Blood Coagulation

None of the theories of blood coagulation can explain some important anomalies without the introduction of hypothetical factors which have yet to be isolated. Their disadvantages are most apparent when an attempt is made to correlate:

(*a*) the one- and two-stage method of prothrombin estimation;

(*b*) the difference between the use of Russell viper venom plus lecithin and brain thromboplastin in the one-stage prothrombin estimation;

(*c*) the apparent increase in prothrombin concentration in hæmophilic blood after clotting;

(*d*) an apparent auto-catalytic generation of thrombin after clotting has commenced.

Electrophoretic studies upon normal plasma freed from prothrombin or fibrinogen, or both, suggested that, although these substances exist in plasma in a free state, a greater proportion exists as a 'prothrombin' – fibrinogen complex. It also appears that the fibrinogen of this complex clots rapidly with thrombin, but the 'prothrombin' part of the complex is inactive until the fibrinogen is acted upon by a part of a thrombin complex (which I have previously called 'thrombin *A*'), formed from free prothrombin in the plasma. The substance which forms the complex with fibrinogen is called 'prothrombin' here, but may or may not be identical with the free form of prothrombin.

The following observations are presented in support of the above conception.

(1) If the above is true, the serum from the one-stage prothrombin method of Quick (immediately after the formation of a clot) should show increased prothrombin instead of none as always accepted. This increase in prothrombin can be shown by a prothrombin consumption test. If further prothrombin conversion is checked by the addition of excess oxalate (*p*H 7·3) immediately after a clot is formed, thrombin present clots a prothrombin-free plasma in 16–20 sec. If physiological saline is added in place of oxalate to the serum from the one-stage prothrombin test, a clotting time of 7–8 sec. is found when the prothrombin consumption test is performed within 20 sec. This is similar to the readings found in hæmophilia; but, unlike in hæmophilia, the residual prothrombin of normal blood can be converted to thrombin rapidly.

(2) When fibrinogen is removed from plasma by the addition of dilute thrombin (clotting time 45 sec.), the expressed serum shows a greater prothrombin concentration than the original plasma. The prothrombin concentration measured by the addition of prothrombin-free plasma, brain thromboplastin and calcium ions gives a prothrombin time of 6–8 sec. The use of Russell viper venom plus lecithin as a thromboplastic agent gives similar results with whole plasma. Other experiments also indicate that Russell viper venom can split the suggested 'prothrombin'-fibrinogen complex.

(3) If normal plasma is treated with Russell viper venom and fibrinogen is then prepared by precipitation, it is found that this fibrinogen clots much more slowly with thrombin than fibrinogen prepared from untreated plasma.

(4) The prothrombin time using Russell viper venom plus lecithin as a thromboplastic agent gives similar results to: (*a*) the prothrombin consumption test in hæmophilia (8 sec.), (*b*) the two-stage prothrombin estimation method, and (*c*) tests using the 'serum prothrombin conversion accelerators'. Yet the same prothrombin activity can be shown in plasma from which fibrinogen has been removed by negligible amounts of thrombin, even though previously the prothrombin estimated by the one-stage method was far less. (The standardized thromboplastin used in all these experiments was not as potent as Quick's, as it gave a 14–15 sec. prothrombin-time compared with Quick's 11–12 sec.)

(5) In hæmophilia there is the enigma of the constant 8-sec. prothrombin consumption-time in many cases. This can be explained by the liberation of prothrombin once clotting has occurred, since there is little thromboplastic activity in hæmophilia.

I believe that the free prothrombin is responsible for the prothrombin-time as estimated by Quick's one-stage method, whereas the splitting of the prothrombin – fibrinogen complex is responsible for many anomalies noted in various prothrombin estimation tests.

The experiments which support this theory of blood coagulation, together with some on pathological plasma, will be published in full elsewhere.

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