tion behaviour in polymers, and also its applicability over a wider temperature-range than most other established methods. Strain birefringence limits the use of the method at large degrees of supercooling where poor heat transfer is also significant[2].

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BIOCHEMISTRY

Synthesis of Succinate from Acetate

IN a previous communication[1], evidence was presented that an enzyme system, isolated from pig heart, catalysed the oxidation of acetate to succinate. Aconitase, assayed by the method of Racker[2], was not detectable and the addition of malate to the incubation mixture did not increase the incorporation of acetate into succinate. Consequently, the formation of succinate was attributed to a Thunberg condensation and the slow rate (7 mμmoles/hr./mgm. protein) was explained by the unfavourable equilibrium[3].

This explanation has been tested by attempting to measure the formation of acetate-^{14}C from succinate-2.3-^{14}C with preparations capable of incorporating acetate-^{14}C into succinate. Such experiments failed to demonstrate any significant formation of acetate, acetyl co-enzyme A or acetyl phosphate (when a preparation of transacetylase was added to the incubation mixture). These results indicate the absence of the Thunberg reaction and suggest that succinate is formed via the established reactions of the Krebs cycle—the slow rate being due to the extremely low activity of aconitase. Support for this view comes from the finding (Dr. T. M. Sutherland, private communication) that the incorporation of acetate into succinate disappears after dialysis of the enzyme system, but is restored by the addition of oxaloacetate.

It thus appears that the interpretation of the data in terms of a Thunberg condensation, for which I was solely responsible, was erroneous.

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Conversion of Fibrin to Desmofibrin

IN experiments on fibrin stabilization great difficulty has been encountered in obtaining a substrate free from fibrin-stabilizing factor (FSF)[1–5].

Such a substrate can be obtained from a fibrinogen preparation made by various methods, for example, from Kekwick's preparation[6] or, from oxalated plasma clotted with citrate thrombin[7]. From a clot mechanically released from a solvent or serum, a 2–4 per cent fibrin solution in urea solution at *p*H 5·2 (20 gm. urea in 100 ml. acetic buffer adjusted to *p*H 5·2 with acetic acid) is prepared.

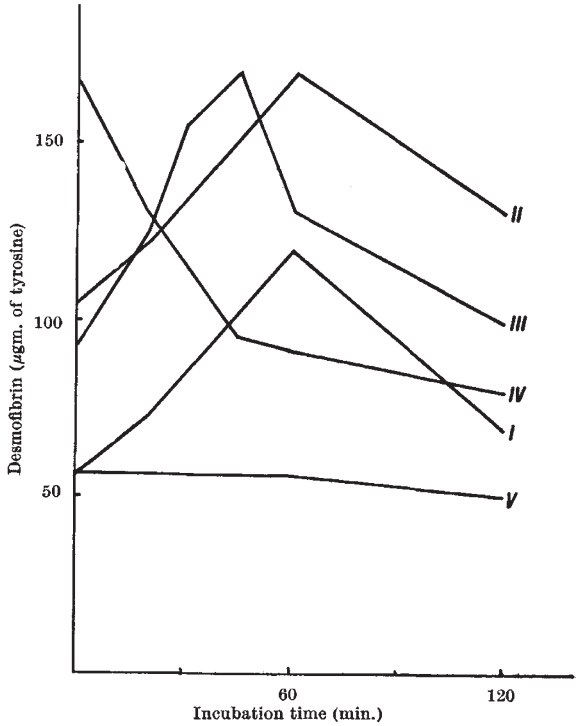

Fig. 1. Thrombinic activation of fibrin stabilizing factor in the presence of calcium. Incubated mixture: thrombin + FSF + calcium chloride. Curves I, II, V, 0·05 unit thrombin/ml.; curve III, 0·1 unit thrombin/ml.; curve IV, 1·0 unit thrombin/ml.; curves I, III, IV, V, 20 min.; curve II, 45 min. reaction of fibrin with incubated mixture; curve V, incubated mixture: thrombin + FSF without calcium chloride (calcium chloride was added just before mixing with fibrin monomer)

A fibrin solution from which the precipitates, regarded as being denatured, had been removed by centrifugation, was diluted 25–50 times in Palitzsch buffer solution[8] at *p*H 7·6, in which a fibrin network was immediately formed. This procedure of dissolving and repolymerizing of fibrin was repeated ten times.

Traces of thrombin could be identified in the diluent after the third repolymerization and removal of the clot. In subsequent operations, it was not possible to demonstrate the presence of thrombin, though it is known to be absorbed on the fibrin[9].

The product finally obtained is considered to be a fibrin monomer and it possesses the following properties: it clots immediately after dilution and neutralization, the time of polymerization depends on the concentration of salt and is lengthened if distilled water is used for dilution, the addition of thrombin has no effect on polymerization-time, provided that the concentrations of salt are constant.

The clot, that is, the fibrin polymer obtained from the monomer described here, is soluble in urea solution and in mono-chloracetic acid even when thrombin and calcium are added.

Stabilized fibrin polymer or desmofibrin is obtained by adding FSF, thrombin and calcium.

The fibrin polymer stabilization time and the amount of desmofibrin obtained in a definite period of time (for example, 20 min.) are proportional to the amounts of FSF added. With a constant FSF fibrin polymer, stabilization time and the amount of desmofibrin formed depend on the amount of thrombin added.

The experiment shown in Fig. 1 was carried out in two stages. In the first stage, three test-tubes con-