oxidase* was followed at 265 mμ. Conditions of the test and relationship between enzyme concentration and changes in optical density are recorded in Table I. As can be seen, proportionality is satisfactory for an assay method, and in fact the turnover number calculated from initial velocities is more than three times the value obtained by the manometric test. The purified oxidase has also been used for the spectrophotometric determination of ascorbic acid, by subtracting the small residual absorption at 265 mμ after oxidase action, from the initial absorption.

TABLE I

SPECTROPHOTOMETRIC ASSAY OF ASCORBIC ACID OXIDASE

Enzyme added micrograms of protein	*Log $\frac{I_o}{I}$ (265 mμ) for first minute*
0.060	0.320
0.045	0.260
0.030	0.180
0.015	0.090

Disappearance of dihydroxymaleic acid was measured at 290 mμ in the presence of peroxidase from horse radish or cabbage and 1 micromole of hydrogen peroxide. The tests were carried out in a final volume of 3 ml and 0.1 *M* KH_2PO_4. Proportionality between enzyme concentration and density changes was less satisfactory than in the case of ascorbic acid oxidase. Relatively lower activity values were obtained with increasing enzyme concentration and corrections had to be applied. An enzyme system was found in extracts of cabbage which catalyzes the oxidation of dihydroxymaleic acid without addition of hydrogen peroxide. After fractionation with ammonium sulfate and dialysis, the enzymic activity was lost. Addition of a heat stable factor obtained from cabbage fully restored enzymic activity to the dialyzed preparation.

Into quartz cells (1 cm light path) of a Beckman spectrophotometer the following solutions were pipetted: 2.8 ml of citrate-phosphate buffer pH 5.6 (containing 0.025 *M* citric acid and 0.05 *M* Na_2HPO_4); 0.1 ml of a solution containing 0.05% ascorbic acid and 1% neutralized. Versene: 0.05 ml of 1% bovine serum albumen and 0.05 ml of the ascorbic acid oxidase solution. All dilutions of the enzyme were made in 0.01 *M* phosphate buffer pH 7.4 containing 0.1% bovine serum albumen. Density readings were made at 265 mμ.

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THE PRODUCTS OF THE ACTION OF THROMBIN ON FIBRINOGEN

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Analyses for N-terminal residues by the fluorodinitrobenzene (FDNB) method[1] show that the end-groups of bovine fibrinogen are tyrosine and glutamic acid, while the fibrin derived from it after clotting with a small amount of purified thrombin has tyrosine and glycine N-terminal groups.

Thus, in addition to the appearance of glycine end-groups reported previously[2], there is a disappearance of glutamic acid end-groups from the protein on clotting. Quantitative estimations of the end-groups indicate the most likely values to be 3 or 4 residues of N-terminal tyrosine and 2 of glutamic acid per molecule of fibrinogen, and 3 or 4 of tyrosine and 4 of glycine in an approximately equal weight of fibrin.

The N-terminal glutamic acid lost from fibrinogen can be found quantitatively after clotting in material which does not become insoluble on treatment with FDNB under SANGER's conditions, and is therefore not present in any insoluble dinitrophenyl-protein (DNP-protein) fraction. This material, which is found in solution in the clot liquor separated from fibrin by purely mechanical means, is peptide in nature; it dialyzes through cellophan, is not precipitated by trichloroacetic acid, and contains most of the common amino-acids. On treatment with FDNB and subsequent hydrolysis it yields, besides DNP-glutamic acid, a roughly equimolecular amount of ε-N-DNP-lysine. The conclusion that peptide material is liberated during clotting was reached independently by LORAND[3], who found that non-protein nitrogen is formed during clotting.

In order to obtain the peptide material free of the salt necessary to bring the fibrinogen into solution, clotting has been carried out in the presence of no salt other than ammonium acetate, which is volatile. When clot liquor thus obtained is taken to dryness, however, the product contains considerably less than the expected amount of N-terminal glutamic acid capable of reacting with FDNB. Such a disappearance of free α-amino groups might result from internal condensation to form a pyrrolidone ring, which is known to occur readily when the γ-carboxyl group is amidized[4]; there is no direct evidence, however, to show that the reaction involved here is of this type. The extent of the change can be diminished by drying the clot liquor from the frozen state rather than at ordinary temperatures.

It will be noticed that there is a discrepancy between the number of glycine end-groups in fibrin—the "scars" left behind when the peptide material is split off—and the number of glutamic acid end-groups which are lost from fibrinogen. Several possibilities have been considered to account for this. It has now been found that the peptide material in an aqueous extract of freeze-dried ammonium acetate clot liquor can be fractionated into two ninhydrin-reactive components by paper electrophoresis[5] in acetate buffer, pH 4.15. The small amount of unclotted protein present moves towards the cathode, while both the peptides move towards the anode. The faster-moving component, peptide A, contains all the N-terminal glutamic acid of the original peptide material; the slower-moving component, peptide B, contains all the lysine ε-amino groups, but apparently no N-terminal residue capable of reacting with FDNB, for it has not been possible to detect an α-N-DNP-amino-acid in hydrolysates of its DNP derivative. The existence of this peptide may therefore account for the excess of the number of glycine end-groups in fibrin over that of glutamic acid end-groups in fibrinogen. Since the amount of peptide lysine (estimated as ε-N-DNP-lysine) corresponds most closely to 2 residues per molecule of fibrinogen, it is possible, by assuming these two residues not to be in the same chain, to correlate each of the presumptive 4 glycine end-groups liberated per molecule of fibrinogen with a chain of peptide split off—2 with peptide A and 2 with peptide B. However, it should be pointed out that the quantitative aspect of the analyses involving DNP derivatives has to be regarded with caution.

Both peptides contain aspartic acid, glutamic acid, glycine, threonine, valine, leucine, phenylalanine, proline and arginine, but peptide B also contains alanine and tyrosine of which peptide A appears to be free, and peptide A contains serine of which peptide B contains none or very little. It thus seems definitely established that peptide molecules of at least two distinct types are split off from fibrinogen when it is clotted by thrombin.

A full account of this work will be published later. One of us (F.R.B.) wishes to acknowledge a grant from the Medical Research Council.

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