Gilford optical density converter, and recorded with a Texas Servoriter No. 500. Units of activity are the changes in optical density/min./mgm. protein. Typical results are shown in Table 1. These results show that chloretone, like sodium amytal, exerts a considerable inhibition of DPNH cytochrome *c* reductase in a brain homogenate or in a preparation of dog liver mitochondria.

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Terminal Amino-Acid Analysis of Vasculokinase-activated Fibrin

In recent experiments in this laboratory, a substance has been demonstrated in human aortæ which is capable of coagulating fibrinogen independently of thrombin[1]. This factor, vasculokinase, appears to have no esterase, amidase or gelatinase activity. Nevertheless, its capability of catalysing fibrinogen to fibrin suggests that it may be a proteolytic enzyme. In previous experiments by Lorand and Middlebrook[2], it has been shown that the *N*-terminal amino-acids of the fibrin monomer catalysed by thrombin are tyrosine and glycine. It has been suggested that there is a specific peptide site in the fibrinogen molecule which must be split in order for the monomers to polymerize[3-4].

In order to test this hypothesis, various fibrins were formed and coupled with 2,4-dinitrofluorobenzene according to Sanger's technique[5]. Thrombin-catalysed fibrin was made from Parke-Davis thrombin and partially purified fraction I (Armour). A similar product was prepared from Parke-Davis thrombin and a purified fibrinogen prepared by the Blomback's technique[6] (kindly provided by Dr. U. Westphal, Ft. Knox, Kentucky). Similarly, two vasculokinase-catalysed fibrins were prepared by the activation of Armour fibrinogen and Blomback fibrinogen with purified vasculokinase[7].

Various digestion processes of the coupled fibrin were utilized. These included digestion with 6 *N* hydrochloric acid under nitrogen, digestion with perchloric and acetic acids under nitrogen and reflux digestion with 6 *N* hydrochloric acid.

The coupled amino-acids were subjected to paper chromatography on Whatman No. 1 filter paper. 2 chloroethanol, pyridine, toluene and ammonia (6 : 3 : 10 : 6) were used in the organic dimension and phosphate buffer, 1·5 *M*, was used in the polar dimension.

The observation that the *N*-terminal amino-acids of thrombin-catalysed fibrin are tyrosine and glycine was confirmed. The *N*-terminal amino-acid of vasculokinase, however, was found to be phenylalanine. Inasmuch as the *N*-terminal amino-acids of fibrinogen are glutamic acid and tyrosine[8], it must be concluded that vasculokinase splits the fibrinogen molecule at a different site from thrombin. Although the liberated peptide has not as yet been recovered and evaluated for its size, these results suggest that vasculokinase is indeed a proteolytic enzyme. The results also suggest that there is more than one locus in the fibrinogen molecule which may be cleaved and still permit polymerization to fibrin fibrils.

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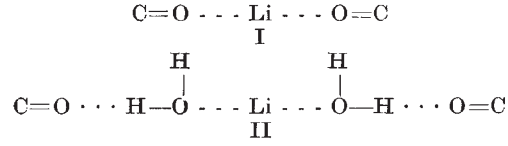
Evidence from Model Peptides relating to the Denaturation of Proteins by Lithium Salts

In an earlier communication[1] we reported that mixtures of *N*-methylacetamide (NMA) or *N*,*N*-dimethylacetamide (DMA) with strong aqueous lithium bromide show viscosities much higher than those calculated on the assumption of ideal mixing. This was termed 'relative viscosity', which is different from the usual use of this term. It was proposed that the high viscosity is caused by aggregation arising from carbonyl–lithium **(I)** and carbonyl–water–lithium (II) associations.



Dots indicate hydrogen bonds, and dashes ion-dipole interactions. These associations were also considered to occur in protein denaturation. We now report evidence for the existence of both types of association, as well as peptide-anion associations.

We had reported[1] that on mixing NMA and 13 *M* lithium bromide (incorrectly reported as 12 *M* before) in the volume ratio 4 : 1 (also molar ratio), respectively, a crystalline precipitate was obtained. The crystals dissolve in their mother liquor at about 53° C. The crystals contain NMA and bromide in the molar ratio of 4 : 1, by analysis. The structure of the crystal has been determined by means of X-ray diffraction by Dr. David Harker and Mr. David Haas, to whom we are grateful for permission to describe some of their results. The detailed structure will be published elsewhere after additional refinement. The main features of the structure that are of interest with regard to protein denaturation are as follows: each lithium is surrounded by four carbonyl oxygens and two water oxygens; each bromide is surrounded