[redacted]

Adsorption Analysis of Oxidized Insulin

A SPECIAL modification of adsorption chromatography on charcoal has been shown by Tiselius[1] to be a useful and selective method of analysis for mixtures containing closely related substances. The method was originally designed for the investigation of breakdown products of proteins, though it has not yet been extensively applied to relatively high-molecular polypeptides, since these are often such complex mixtures as to render any type of analysis extremely difficult.

The oxidation of insulin with performic acid yields a product in which it was believed that the molecule was split into its separate polypeptide chains[2]. This

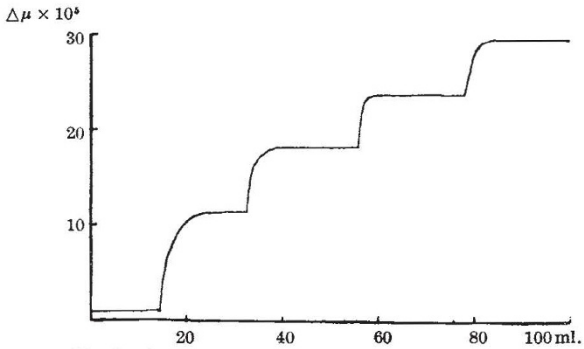

Fig. 1. ADSORPTION ANALYSIS OF OXIDIZED INSULIN

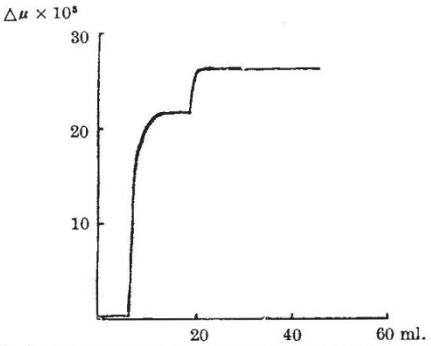

Fig. 2. ADSORPTION ANALYSIS OF FRACTION *A* OF OXIDIZED INSULIN

product should be a relatively simple mixture of polypeptides of average molecular weight 3,000, and in order to determine its complexity it was subjected to adsorption analysis. Fig. 1 shows a typical result of a frontal analysis of a 0·2 per cent solution of the oxidized insulin by the interferometric method[1], using a filter of volume 875 π c.mm. It can be seen that the mixture consists of at least four components. Considerable differences in the relative concentrations and retention volumes on the various steps were observed in different experiments, though the general appearance was the same. The front fraction was found to contain predominantly but not entirely glycyl terminal residues[3], and little of the basic amino-acids. Fig. 2 shows a frontal analysis of the crude fraction *A* [2] on a filter of volume 250 π c.mm. Both steps had only glycyl terminal residues and no arginine or lysine, suggesting that any phenylalanyl peptides present had not been eluted from the charcoal, due to their low concentration. The retention volumes of these substances is very much affected by their concentration; thus when fraction *B* was studied three steps were formed, the first large step containing predominantly phenylalanyl terminal residues.

At this stage of the work it should not be assumed that the four steps of the frontal analysis represent the four separate peptide chains of the insulin submolecule; but it is hoped that further work will help to characterize the various components.

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[1] Tiselius, A., "Advances in Protein Chemistry", 3, 67 (1947), with references to earlier work.
[2] Sanger, F., *Nature*, **160**, 295 (1947).
[3] Sanger, F., *Biochem. J.*, **39**, 507 (1945).