



In the course of investigations on the development of the reproductive system in the rabbit, we noticed that fructose appeared in the accessory glands at an early stage when there was as yet no sign of active spermatogenesis. In a four-months-old animal, both the gl. vesicularis and the prostate showed already a fairly high concentration of fructose (21 and 44 mgm. per cent, respectively) in spite of the complete absence of spermatozoa in the testis or the epididymis. When, in the sixth month of life, the spermatozoa finally made their appearance, the accessory glands were filled with secretory fluid containing the normal high level of fructose. Experiments on bull-calves gave similar results, showing that the appearance of fructose

Oxidation of Insulin by Performic Acid

FROM the determination of the terminal residues of insulin, it was suggested that the submolecule of molecular weight 12,000 is made up of four peptide chains bound together by —S—S— linkages¹. Thus if one could break the —S—S— linkages without affecting any other part of the molecule, it should be possible

to split the insulin into its separate polypeptide chains, two of which have terminal glycyl residues and the other two phenylalanyl residues. Toennies and Homiller² showed that the only amino-acids that are appreciably oxidized by performic acid are tryptophan, methionine and cystine, the latter reacting with five atoms of oxygen and presumably forming cysteic acid. Since insulin contains no tryptophan or methionine, this seemed a suitable way of splitting the —S—S— linkages.

Using the procedure of Toennies and Homiller, it was found that the oxidation of cystine to cysteic acid is complete in five minutes. With insulin an oxidation-time of 15 minutes was generally used. The oxygen consumption was the theoretical one for the cystine content, and paper chromatography³ showed no qualitative difference in the amino-acid composition except the replacement of cystine by cysteic acid. There was no destruction of the free amino-groups¹. The oxidation product was studied in the electrophoresis apparatus of Tiselius. Unfortunately, it was not possible to dialyse the material, due to its low molecular weight (about 3,000), so that the results were not always entirely reproducible. Fig. 1 illustrates a typical experiment, which shows three components and indicates that the mixture is not unduly complex.



Fig. 1. ELECTROPHORETIC DIAGRAM OF OXIDIZED INSULIN



Fig. 2. ELECTROPHORETIC DIAGRAM OF FRACTION A

By neutralization at pH 6, two fractions of about equal weight can be obtained. The solution (fraction *A*) contains predominantly (80 per cent) glycyl terminal residues, and the precipitate (fraction *B*) predominantly phenylalanyl terminal residues. By further treatment of fraction *A* a product can be obtained which has no phenylalanyl terminal residues and appears to be homogeneous by electrophoresis (Fig. 2). It contains no lysine or arginine. Estimation of the terminal glycyl residues suggested a molecular weight of about 2,500. Fraction *B* has not yet been further purified.

The work is being continued, and a more detailed report will be published later.

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¹ Sanger, F., *Biochem. J.*, **39**, 507 (1945).

² Toennies, G., and Homiller, R. P., *J. Amer. Chem. Soc.*, **64**, 3054 (1942).

³ Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).