

Sanger F. The free amino groups of insulin. *Biochemical J.* 39:507-15, 1945.
[Biochemical Laboratory, Cambridge, England]

A method for the identification and estimation of the free amino groups of proteins is described using 2:4-dinitrofluorobenzene. The results with insulin show that it contains two polypeptide chains, one with a terminal glycyl residue and the other with a terminal phenylalanyl residue. [The SC¹® indicates that this paper has been cited in over 1,645 publications since 1955.]

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After completing my PhD degree in 1943, I started working with A.C. Chibnall, who had come to Cambridge (England) as the new head of the Department of Biochemistry. His research group was mainly concerned with the analysis of proteins for their amino acid composition. Analysis of insulin had indicated that it had an unusually high content of free amino groups, showing that the polypeptide chains were particularly short. To investigate these amino groups further, I required a reagent that would react specifically with the amino groups under mild conditions to form a linkage that was stable to acid hydrolysis. Almost more important than the reagent itself, I required a fractionation procedure for separating the derivatives formed to identify and estimate them. Here I was particularly fortunate in that partition chromatography had just been developed by Martin and Synge¹ and was being used in the laboratory by G.R. Tristram.

The use of partition chromatography was probably the main factor in the success of the method. After unsuccessfully trying a number of possibilities, particularly methane sulphonyl chloride, I searched for a coloured substance because this would

make the fractionation by chromatography much easier. Abderhalden and Stix² used 2:4-dinitrochlorobenzene to prepare the 2:4-dinitrophenyl (DNP) derivatives of some amino acids, but it required high temperatures for the reaction. B.C. Saunders, who worked in the University Chemical Laboratory, was an expert on organic fluorine compounds, and we approached him about making the corresponding dinitrofluorobenzene, which we expected to be more reactive. This he kindly did, and it proved to have the required reactivity.

The method worked well for insulin and was used on other proteins in other laboratories until it was superseded by other techniques, especially the phenylisothiocyanate method of Edman.

Another possible contribution to the number of citations may be that the paper could be regarded as marking a change in the course of protein chemistry from an interest predominantly in analysis to one in the arrangement of the amino acids in the polypeptide chains. It was the first quantitative analysis of terminal residues in a protein, though not quite the first time that the position of an amino acid in a protein chain had been determined. Jensen and Evans³ had qualitatively identified the phenylalanine residue in insulin in 1935, using the reagent phenylisocyanate. My paper was the first in a series that resulted in the complete primary structure of insulin. It was the first protein to be sequenced, and I was awarded the Nobel Prize for Chemistry in 1958.^{4,5}

There was some delay in publication. The editor to whom it was sent was a distinguished organic chemist. In describing new compounds, it was customary to give a melting point and elementary analysis as evidence of their purity. I had not done this for all the DNP amino acids but had relied heavily on the chromatographic behaviour. The difficulty was eventually overcome, largely through the intervention of Chibnall with the editor concerned, by inserting the statement that their characterization would be published later.

1. Martin A J P & Synge R L M. A new form of chromatogram employing two liquid phases. *Biochemical J.* 35:1358-68, 1941. (Cited 510 times since 1955.)
2. Abderhalden E & Stix W. Die Bestimmung des Aminostickstoffs in einigen Polypeptiden nach der Methode von van Slyke. *Hoppe-Seylers Z. Physiol. Chem.* 74:505-8, 1923.
3. Jensen H & Evans E A. Studies on crystalline insulin. *J. Biol. Chem.* 108:1-10, 1935.
4. Sanger F. The chemistry of insulin. *Les Prix Nobel en 1958*. Stockholm: Norstedt & Söner, 1959. p. 134-46.
5. Chemistry of insulin. *Science* 129:1340-4, 1959. (Cited 70 times.)