dilute aqueous solution at pH 6.6–6.8. The crude nonapeptide amide IX was purified by counter-current distribution in the system 2-butanol–0.017 *N* acetic acid[6]. Redistribution of Ser[4]–Ile[8]–oxytocin (IX) in the same solvent system gave a material having at 25° a partition coefficient of 0.53. The experimental curve, obtained from the Folin color values[7], coincided with the theoretical curve. The purified product showed a correct quantitative amino acid composition and possessed an oxytocic activity of approx. 130 I.U./mg pure peptide, when assayed on the isolated rat uterus[8].

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Note added in proof: Since this paper was submitted for publication, GUTTMANN *et al.*[9] described in a brief report a synthesis and some pharmacological effects of Ser[4]–Ile[8]–oxytocin. According to the authors the peptide possesses an oxytocic activity on the isolated rat uterus[8] of 150 ± 12 I.U./mg. (Received November 29th, 1962)

PN 1189

On the interaction of insulin with raney nickel

In a former survey[1] we studied the interaction of methionine with Raney nickel, establishing a quantitative removal of sulphur and the formation of methane and α-aminobutyric acid. On this basis a method was developed for the determination of methionine by determination of the quantity of methane by an infrared spectrometric procedure.

In the present survey, the behaviour to Raney nickel of products with a peptide structure and a higher molecular weight has been studied. We took note of the observations made by COOLEY AND WOOD[2], namely that the treatment of egg albumin with Raney nickel results in an almost complete removal of sulphur without a noticeable fragmentation of the protein molecule. In order to follow with greater ease and assurance the interaction of proteins with Raney nickel we selected insulin since its chemical structure is known. We were further inclined to use this protein because of various data in the literature on the reactivity of its disulphide bonds under differing conditions. Thus CECIL AND LOENING[3] have studied the interaction of the disulphide groups of insulin with sodium sulphite, establishing that the intra-chain disulphide

bond 6–11 in the A-chain is apparently unreactive under ordinary conditions. On the other hand, LINDLEY's[4] results show the intra-chain disulphide bond to have greatest reactivity when insulin is reduced by lithium thioglycollate at pH 5. Other authors likewise discuss the peculiar behaviour of this bridge, suggesting that it might be intimately involved in the biological action of insulin[3,5,6].

We used in our research crystalline insulin produced by the Organon (Oss, Holland), which we treated with Raney nickel[7] (1 ml suspension per 10 mg of insulin) at pH 8–9 for 3 h at room temperature, and finally 1 h at 35–45°. The solution was continuously and intensively stirred. The product was then filtred and lyophilized, while a similarly treated parallel sample was analysed in order to determine the amount of sulphur removed[8]. Under these conditions this varied around 2.0%. No essential change in this value was observed when the pH was decreased to 5. Complete removal of the sulphur contained in the insulin was obtained by treating the latter with 5 M urea over a period of 24 h followed by treatment according to the above procedure.

The lyophilized product obtained after 4-h treatment with Raney nickel at pH 8–9 was subjected to an electrophoretic and chromatographic analysis. For comparison we used samples of the original insulin and insulin oxidized by performic acid according to SANGER[9]. The sample treated with Raney nickel separated into two components, observed on the chromatogram as 2 spots using the system *n*-butanol–pyridine–acetic acid–water[10] (Fig. 1) as well as on the horizontal high-voltage electropherogram[11] (Fig. 2). Under descending electrophoresis (pH 5.6)[12] the product remained insoluble at the start, while the insulin oxidized by performic acid under these conditions separated into its component A and B chains.

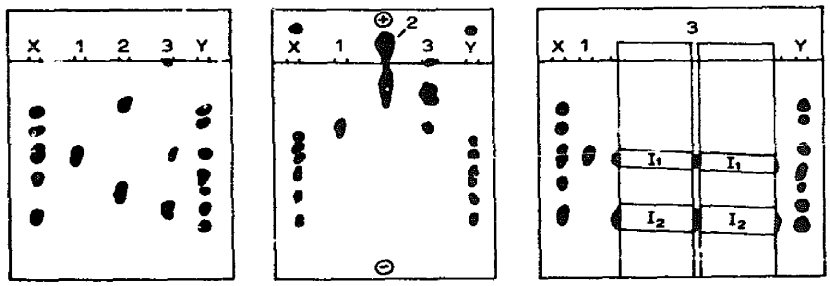

Fig. 1. Paperchromatography of: 1, original insulin; 2, insulin oxidized by performic acid; 3, insulin treated with Raney nickel. X and Y are standard solutions of amino acids.

Fig. 2. High-voltage electrophoresis of preparations shown in Fig. 1.

Fig. 3. Preparative paper chromatography of insulin treated with Raney nickel. Key as in Fig. 1.

The preparative separation of the mixture was carried out by the above-mentioned chromatographic method (Fig. 3). Dinitrophenylation[13] of aliquots of both fractions established the presence of only DNP-Gly and DNP-Phe in the ratio of 1:1 in each fraction. Chromatograms of the total hydrolysates of either fraction showed the presence of all amino acids forming part of the insulin molecule. Visual inspection of these chromatograms showed that in the I_2 fraction we have a considerable increase in the quantity of alanine and a reduction of the quantity of cysteic acid, compared with the I_1 hydrolisates and with those of the original insulin. This gave us grounds to assume that in I_2 desulphurization affected more of the cystine residues of the molecule

than in I_1, as the splitting of 1 cystine residue produces 2 alanine residues[14,15]. No convincing proof was obtained by these experiments whether the smaller fraction I_1 was at all affected by the Raney nickel. The answer was obtained by determining the molar ratio of amino acids of these fractions, according to MOORE AND STEIN[16].

TABLE I

AMINO ACID COMPOSITION OF FRACTIONS ISOLATED FROM INSULIN TREATED WITH RANEY NICKEL

*Amino acid residues**	*Asp*	*Thr*	*Ser*	*Glu*	*Pro*	*Gly*	*Ala*	*Val*	*Ile*	*Leu*
Fraction I_1	3.36	0.95	2.77	7.25	0.96	4.04	3.10	4.87	0.88	5.54
Fraction I_2	3.28	0.84	2.89	7.36	0.90	4.07	6.97	5.15	0.76	6.20
Insulin	3	1	3	7	1	4	3	5	1	6

* The other amino acids contained in the insulin molecule have not been quantitatively determined.

The results given in Table I clearly show that Fraction I_1 contains intact insulin, which is in conformity with the respective chromatograms and electropherograms. On the contrary, the I_2 fraction represents insulin deprived of 2 disulphide bonds (increase of the alanine residue by 4). One of the split bonds in I_2 is undoubtedly the intra-chain bond 6–11. These results also come to show essential differences between the reaction of the disulphide bonds of insulin with sodium sulphite under ordinary conditions and with Raney nickel. Therefore, when removing about $^2/_3$ of the sulphur contained in the insulin by desulphurization (*i.e.*, by destroying 2 of the 3 disulphide bonds) no separation of the insulin molecule into A and B chains takes place. This is made evident by the informative chromatograms and electropherograms, by the composition of the total hydrolysates of the resulting fractions and by the results of dinitrophenylation.

Additional research will be necessary to establish which of two inter-chain bonds had been effected by desulphurization. This shall form the subject of our next study.

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