TABELLE II

Nr.		*Alter*	*BGZ*	*µA O*	*µA P*	*P/O*	*Differenz*
3a	Kontr.	wie bei		3.84	7.0	1.82	—
	K-Mangel-Tier	Nr. 3		3.82	5.77	1.51	—17%
4a	Kontr.	wie bei	Nr. 4	1.12	3.26	2.92	—
	K-Mangel-Tier	wie bei	Nr. 4	1.04	2.8	2.7	—7.5%
5a	Kontr.	wie bei	Nr. 5	2.76	4.13	1.5	—
	K-Mangel-Tier	wie bei	Nr. 5	2.79	3.32	1.19	—21%
6a	Kontr.	wie bei	Nr. 6	2.3	4.9	2.13	—
	K-Mangel-Tier	wie bei	Nr. 6	2.42	4.11	1.73	—19%

Substrat: 0.01 *M* Succinat.

Die zu den Versuchen verwendeten Mitochondrien wurden bei Vers. 1 und 2 mittels 0.25 *M* Rohrzuckerlösung, bei den übrigen mittels einer Lösung präpariert, die 0.2 *M* an Rohrzucker und 0.02 *M* an Versene war. Die Mitochondriensuspensionen zweier miteinander verglichener Ansätze enthielten, wie durch N-Bestimmungen kontrolliert wurde, gleiche Mengen Enzymmaterial. Die Atmung wurde mittels Warburgtrögen von 5 ml Inhalt gemessen, die Zusammensetzung der Suspensionsflüssigkeit usw. war die gleiche wie bei [1] angegeben.

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ON THE SIZE OF THE MONOMER OF INSULIN

by

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The question of the minimum molecular weight of insulin has recently been investigated by several authors. There seems to be general agreement about the value 12,000 for acid aqueous solutions[1–4] while the value 6000 has been suggested by HARFENIST AND CRAIG[5] for solutions of insulin in the system water-secondary butanol-dichloroacetic acid, and by FREDERICQ[6] for alkaline aqueous solutions (pH 10) and dioxan-water mixtures. It is naturally of great importance to establish as firmly as possible the size of the smallest molecular unit into which insulin may dissociate without irreversible loss of its activity. We have therefore attempted to estimate the molecular weight of the isoelectric hormone in concentrated solutions of guanidinium chloride (GuCl) in which it is soluble at pH 5. GuCl is known to cause dissociation of associated protein molecules and it does not denature insulin irreversibly (see below).

The molecular weight was measured in the micro-osmometer designed by KORSGAARD CHRISTENSEN[7,8], compare[9]. The stainless steel parts were replaced by ones of nickel since the former metal was heavily corroded by solutions of guanidinium chloride. Circular pieces cut out from Visking sausage casing (thickness 0.025 mm) were used as membranes after careful washing with GuCl. Since their permeability was low, equilibrium was but slowly reached *i.e.* in the course of 3–4 days. For the same reason the dynamic method[8,9] for measuring the pressure was rather cumbersome, and the static principle was used after establishing the approximate osmotic pressure by the dynamic method. Only experiments where the pressure was constant for several days are reported here. Bacterial growth can fortunately be excluded.

In all experiments 6 molal solutions of carefully purified GuCl were used. The special sample of pure pork insulin investigated (P 1398) was kindly placed at our disposal by Nordisk Insulin Laboratory. Its composition was: 8.39 % H_2O
On dry material: 15.47 % N
0.5 % Zn
29 internat. units per mg.

The results are seen in Fig. 1 where p is the osmotic pressure, and γ_p the number of grams of dry insulin per 1000 grams of water. The molecular weight, Mw, at infinite dilution with respect to insulin is calculated by means of the formula

$$Mw = \frac{RT}{\omega' \cdot \varphi_o}$$

where ω' is the volume (in litres) of pure GuCl solution (outer liquid) per 1000 grams of water and φ_o is the initial slope of the p-γ_p-curve[9].

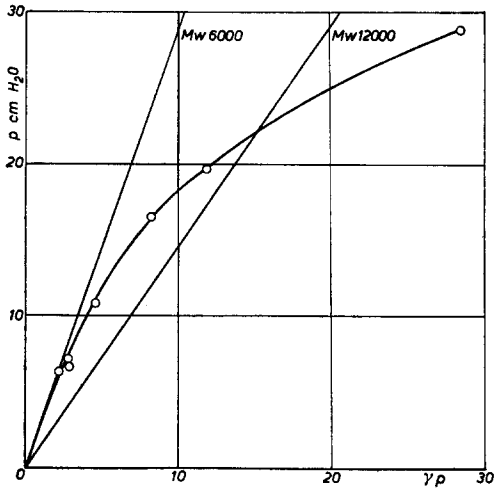

Fig. 1

The values given for γ_p are obtained from the directly weighed-in quantities of insulin corrected for water content. Indirect spectrophotometric estimations of insulin, at 276 mμ, before and after the osmotic measurements, were carried out in most cases. Their accuracy was small (10–20 %) due to the U.V.-absorption of GuCl and of toluene (which covers both outer and inner solutions in the apparatus used[9]), but within the experimental error they gave values that agreed with those found on the weight basis. No leakage could therefore be demonstrated.

As is seen from Fig. 1 the molecular weight of pork insulin is about 6000 in guanidinium chloride solution at values of γ_p below 2 (compare the theoretical initial slopes drawn for Mw 6000 and 12,000), and 6000 may therefore be taken as the weight of the monomer. The shape of the curve indicates that association occurs at higher γ_p-values. A closer analysis is very difficult on the basis of the present data but we should like to emphasize that the results are not compatible with the assumption of a simple equilibrium between monomer and dimer[4]. Higher association products must be formed.

The question about which bonds are broken in the formation of the monomer shall be left open. If these bonds are of importance for the activity of the hormone they must re-form under the conditions of the biological test on mice, since Dr. M. WEITZE, Nordisk Insulin Laboratory, who has kindly subjected a two-month-old, dilute solution of insulin in 6 M GuCl ($\gamma_p = 4$) to this test, found a value of 24 I.U. per mg insulin against the original 29. The inactivation is therefore small or not even certain.

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