Biopsies from 40 patients with cystic fibrosis and from normal controls have been compared in this study, using histochemical methods and $^{35}SO_4^{=}$ incorporation (autoradiography) for examining mucus production; however, no consistent differences were demonstrated. An exhaustive histochemical examination of rectal mucus likewise has failed to reveal any chemical abnormality in cystic fibrosis mucus[6].

The potential of this method for investigating a variety of other problems appears to be considerable, and at present it is being used for studying the toxic effects of gluten digests on duodenal and rectal biopsies from patients with treated coeliac disease[7].

Zusammenfassung. Bei der zystischen Pankreasfibrose ist eine abnorme Schleimabsonderung vorhanden. Bei der einfachen Rektummucosabiopsie sind keine sicheren Befunde zu erheben. Wichtige Veränderungen wurden jedoch an der Duodenalmucosa mit Hilfe einer Organzüchtungsmethode festgestellt.

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[7] Acknowledgement. I should like to thank Mrs. R. KAY for her technical assistance in this project. This work was supported in part by a grant from the National Cystic Fibrosis Research Foundation.

Crystallization and Properties of L-Asparaginase from *Escherichia coli*

EC II L-asparaginase from *E. coli*[1] is crystallizable from fractionated cell extracts[2]. Favourable conditions for crystallization are protein concentrations of 3–5%, low ionic strength of buffer solution, pH ranging from 5.0–6.5 and cold room temperature. Ethanol is slowly added until a slight turbidity becomes apparent, which is immediately removed by centrifugation. Crystallization then starts and is completed after several hours. The yield of crystalline L-asparaginase is about 80% of the starting material. Cations such as Mg, Ca, Sr, Ba, Zn and Co are not necessary for crystallization, which even starts in the presence of EDTA. Rhombs originate at pH 6.5, whereas at pH 5.0 slender rods are formed (Figures 1 and 2). This phenomenon is to be explained by a favoured growth of one dimension at pH 6.5. When crystallization is performed, starting with a rather impure material, the contaminating proteins which migrate in PAA-gel electrophoresis faster towards the anode than L-asparaginase, remain in the supernatant (Figure 3). The specific activity of 300 IU/mg is reached by repeated crystallization (Figure 4, sample 1).

Purified L-asparaginase gradually loses up to 90% of enzyme activity on exposure to 37 °C for several days. However, the inactivated product is as easily crystallized as the enzyme, from which it has originated. The loss of activity is represented in PAA-gel electrophoresis by a splitting into 3–4 components of minute higher mobility than the active enzyme which exhibits a single component only (Figure 4). In the ultracentrifuge the Schlieren patterns of two 37 °C treated samples are entirely homogenous. Sedimentation constants $S^{\circ}_{20,w}$ are identical with $S^{\circ}_{20,w}$ of the active enzyme (Table).

The molecular weight of L-asparaginase, inactivated at 37 °C, was determined from equilibrium sedimentation

[1] D. A. CAMPBELL, L. T. MASHBURN, E. A. BOYSE and L. J. OLD, Biochemistry *6*, 721 (1967).

[2] P. K. HO and E. B. MILIKIN Fedn. Proc. *28*, 728, Abstr. 2625 (1969).

[3] T. SVEDBERG and K. O. PEDERSEN, *The Ultracentrifuge* (Oxford University Press, New York 1940), p. 262.

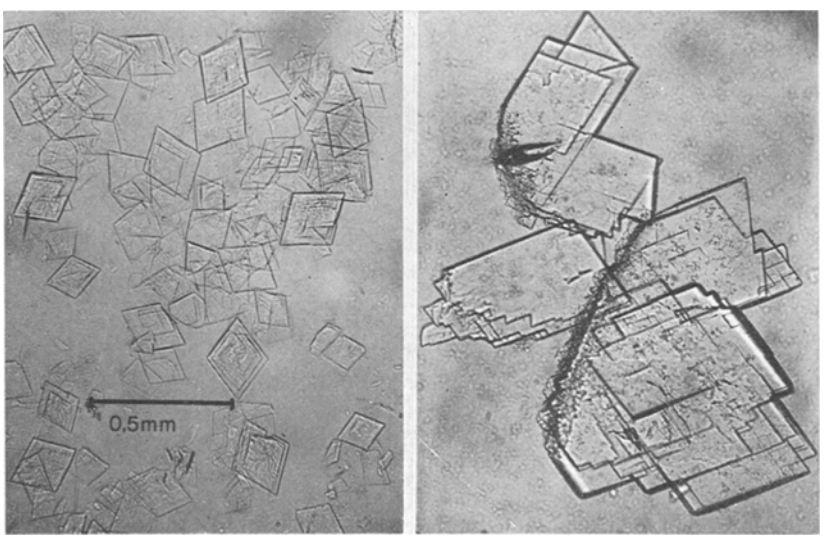

Fig. 1. Rhombs of crystallized L-asparaginase from *E. coli*. Conditions are: 4.8% protein in 0.025 *M* sodium phosphate, pH 6.5; addition of approx. 20% v/v ethanol at 4 °C. Left: fast crystallization within 5 min. Right: slow crystallization with 24 h. The scale applies to both exposures.

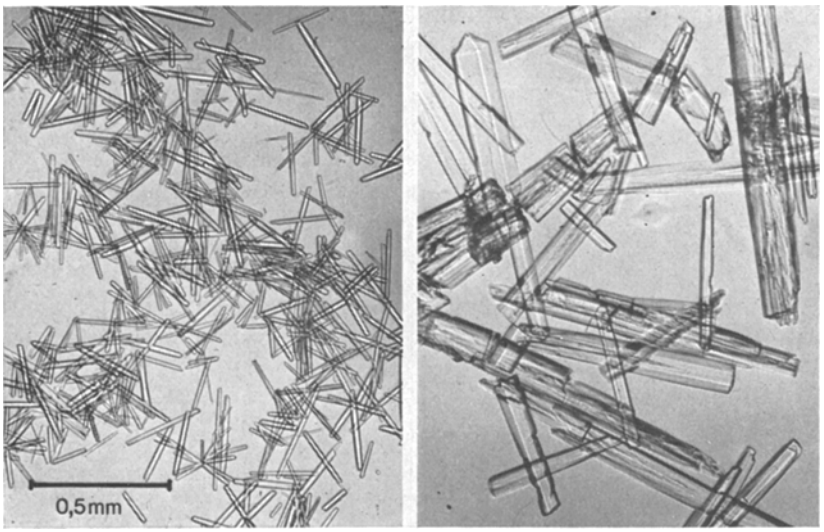

Fig. 2. Rods of crystallized L-asparaginase from *E. coli*. Conditions are: 4.8% protein in 0.025 *M* sodium acetate, pH 5.0; addition of approx. 17% v/v ethanol at 4 °C. Left: fast crystallization within 5 min. Right: slow crystallization within 24 h. The scale applies to both exposures.

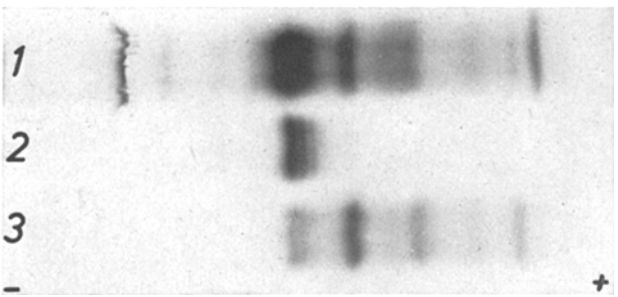

Fig. 3. Purification of L-asparaginase by crystallization represented by PAA-gel electrophoresis, pH 8.5, 10 V/cm, 3 h. (1) Starting material, 80 IU/mg. (2) Crystallized L-asparaginase, 170 IU/mg. (3) Supernatant.

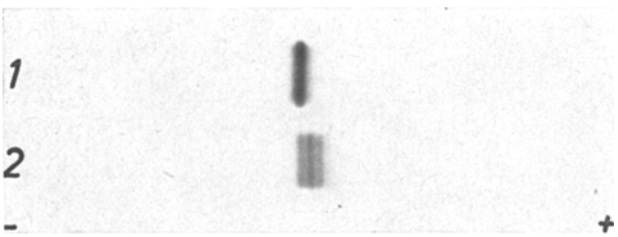

Fig. 4. Inactivation of L-asparaginase at elevated temperature represented by PAA-gel electrophoresis, pH 8.5, 10 V/cm, 3 h. (1) Twice recrystallized preparation, 300 IU/mg. (2) Sample from 1, kept at 37 °C for 10 days, 70 IU/mg.

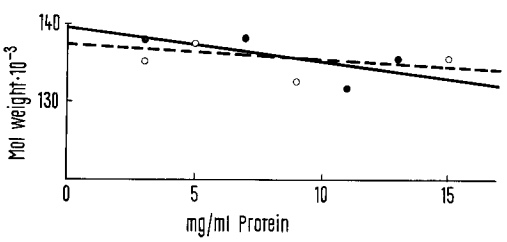

Fig. 5. Molecular weight of L-asparaginase determined by multichannel equilibrium sedimentation runs according to YPHANTIS[5]. The sample, inactivated at 37 °C, has a specific activity of 30 IU/mg. $\frac{du}{dc} = 0.184$, $v = 0.73$, as found by the method of SCHACHMAN[6]. ○--○, Values of molecular weight from sedimentation in buffer, pH 6.8[3]. ●—●, With addition of 1% sucrose. Lines are drawn according to calculation $y = A + Bx$ with application of the minimum square of the error.

Preparations of L-asparaginase	Specific activity (IU/mg)	$S^{\circ}_{20,w}$ at pH 6.8 SVEDBERG units[3]
Purified by crystallization	270	7.5
Kept 3 days at 37 °C, then recrystallized	150	7.4
Kept 15 days at 37 °C, then recrystallized	30	7.5

analysis (Figure 5). It is fairly constant within the range of concentration measured. In plain buffer no increase is observed as reported by WHELAN[4].

Crystallization of L-asparaginase is applicable for its purification arriving at high specific activity. Care must be taken to avoid the kind of inactivation which was demonstrated by exposure to elevated temperature, since the inactivated product exhibits physical properties very similar to those of the fully active enzyme. It is assumed that the purification of partially inactivated enzyme by chromatography procedures would not be successful either[7].

Zusammenfassung. L-Asparaginase aus *E. coli* ist bei hoher Proteinkonzentration zwischen pH 5,0 und 6,5 und geringer Ionenstärke durch Zugabe von Äthanol in der Kälte leicht kristallisierbar. Bei pH 5,0 und pH 6,5 entstehen unterschiedliche Kristallformen. Das durch Kristallisation gereinigte Enzym lässt sich bei erhöhter Temperatur inaktivieren, wobei das Produkt der Inaktivierung ebenso kristallisiert werden kann. Es zeigt das gleiche Verhalten in der Ultrazentrifuge wie das aktive Enzym.

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