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Insulin—Preliminary X-ray studies of citrate crystals.

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Following the preparation of orthorhombic insulin sulfate crystals (Ellenbogen, 1949) we attempted to crystallize insulin in the pH range 2·0–4·0 as selenate, formate, acetate, citrate and chloride. Insulin crystals of all but the chloride were obtained under similar conditions. It is not known whether or not the SO_4^{--} anions in insulin sulfate crystals are located at fixed sites on the molecule and thus in the unit cell. Although we consider this improbable, the study of an orthorhombic form with anions of lower electron density appeared to us desirable. Further, many inorganic sulfates are insoluble. The use of metal cations in efforts to prepare heavy atom derivatives is thus limited if insulin sulfate crystals are employed.

Crystals of insulin citrate can be grown by several slightly different procedures. The two methods described were employed for the preparation of those crystals for which quantitative data are reported here. Both give large well-developed tabular crystals approximately 1–2 mm. in length and 0·25–0·5 mm. in diameter. Insulin Citrate I. 60 mg. of beef insulin (Lilly T 2842) were dissolved in 4 ml. 0·03*M* citric acid. 0·03*M* tertiary ammonium citrate was added dropwise to persistent slight turbidity. Insulin Citrate II. 60 mg. of beef insulin (Lilly 535664) were dissolved in 4 ml. of 0·1*M* citric acid. 0·1*M* sodium hydroxide was added dropwise to persistent slight turbidity. Both preparations were placed in a cold room at 1 ± 1 °C. within a half hour after addidition of base.

The optical examination was carried out in the cold room; X-ray studies were made both in the cold room and at room temperature. The preparation of crystals for X-ray photography and the techniques employed followed those described elsewhere (Low & Shoemaker, 1959).

The procedure employed in measuring the density (D_M) of these crystals followed in all respects (choice and preparation of crystal, preparation of water-saturated bromobenzene–xylene solution, etc.) that described by Low & Richards (1952) except that after a preliminary gradient tube study the final densities were measured by flotation, and the solutions calibrated pycnometrically. The densities are close to those found for insulin sulfate crystals (Low & Richards, 1954).

X-ray photographs were taken both at 0 °C. and at room temperature. There is no observable change in minimum spacing (2·2 Å), nor is the rate of deterioration of the crystals due to X-radiation changed markedly. If we assume that the partial specific volume of the protein in dilute solution is appropriate for the wet crystals (see Low & Richards, 1954) and using values $v_p = 0{\cdot}71$ and $v_p = 0{\cdot}72$ (Oncley *et al.*, 1952), the average density of the liquid of crystallization ($1/v_{LC}$) can be calculated (Table 1).

The density of the sodium citrate buffer from which crystalline preparation II was grown was 1·012. Data for the two insulin citrate preparations are given in Table 1.

The description of the morphology and optics of Type *A* insulin sulfate crystals (Low & Shoemaker, 1959) is appropriate for insulin citrate crystals. These crystals are isomorphous with Type *A* insulin sulfate crystals; space group $P2_12_12_1$. There are two molecules (of molecular weight 5733) in the asymmetric unit. The unit-cell dimensions (Table 1) fall within the range of dimensions observed for sulfate crystals. The intensity distribution of the (0*kl*), (*h*0*l*) and (*hk*0) reflections of insulin citrate and sulfate are very similar. Indeed for crystals from some preparations they are almost identical. The greatest variations in overall distribution observed are rather between crystals, whether sulfate or citrate, from different batches grown by slightly different procedures and of different age.

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Table 1. *Data for insulin citrate preparations.*

Insulin citrate	Cell dimensions (Å)			Volume (Å^3)	D_M	Weight fraction of protein	Density of liquid of crystallization*	
	a	*b*	*c*				(1)	(2)
I	58·2 ± 0·2	51·9 ± 0·1	38·5 ± 0·1	$1{\cdot}16 \times 10^5$	1·195 ± 0·002	0·548 ± 0·005	1·01	1·02
II	58·1 ± 0·1	51·85 ± 0·1	39·1 ± 0·15	$1{\cdot}18 \times 10^5$	1·196 ± 0·002	0·540 ± 0·005	1·02	1·03

* (1) Corresponds to $v_p = 0{\cdot}71$. (2) Corresponds to $v_p = 0{\cdot}72$.