thenate biosynthesis. Since the organisms are genetically endowed with the enzymic make up to synthesize pantothenate, this provides another example of the fact that environment plays an important role in genetic expression.

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A Method of Crystallization of Human Myoglobin.* (26157)

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Myoglobin was first crystallized by Theorell(1) using material of equine origin. Human myoglobin was first crystallized by Drabkin(2). Additional methods of crystallizing human myoglobin have been described by Theorell and de Duve(3) and by Rossi-Fanelli(4). In these methods hemoglobin and other proteins are salted out of aqueous extracts of muscle by lead acetate, phosphate, or ammonium sulfate. Concentration of phosphate or sulfate is then gradually increased by dialysis(3) against concentrated solutions of these salts or by evaporation(4) and crystallization is thereby induced. These steps are performed at a constant hydrogen ion concentration near pH 6.6, the isoelectric point of human myoglobin(5).

We have had difficulty in regularly obtaining crystalline human myoglobin by these methods. We have developed a simple method of isolation and crystallization of human myoglobin that depends on precipitation of contaminating proteins from aqueous extracts of muscle at pH 8 by saturation with ammonium sulfate. Myoglobin remains in solution under these conditions. After precipitation of these contaminating proteins and adjustment of the pH to 7, myoglobin crystallizes out of solution.

Procedure. Skeletal muscle from surgical or autopsy sources was frozen within one hour of operative removal or post mortem examination, and stored at −60°C. Cases with a post mortem interval before autopsy of more than 4 hours or with debilitation were avoided. While still frozen, samples weighing approximately 100 g were chopped into small pieces and mixed in an electric blender with 2 ml of distilled water per gram of tissue. These and all subsequent steps were performed at 4°C. After blending, the mixture was adjusted to pH 8 with 2N sodium hydroxide and extracted for one hour with constant stirring. Insoluble materials were then removed by centrifugation at 16,000 g for 30 minutes and the supernate filtered through coarse filter paper.

The filtrate was then made 80% saturated by addition of solid ammonium sulfate, C. P. grade. During this and subsequent additions of ammonium sulfate, the solution was maintained at pH 8 ± 0.05 by additions of 2N sodium hydroxide or 2% acetic acid. The material was then centrifuged at 16,000 g for 30 minutes.

The supernatant fluid was brought to

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100% saturation by addition of excess ammonium sulfate and allowed to stand overnight with constant stirring. The material was centrifuged at 39,000 g for 30 minutes, precipitate discarded and supernate again stirred for 4 to 8 hours with excess ammonium sulfate. Centrifugation was repeated for 30 minutes at 39,000 g and any precipitate discarded.

The supernate was adjusted to pH 7.0 with 2% acetic acid and allowed to stand in beakers at 4°C. Crystallization began after 24 to 48 hours or occasionally longer. The initial precipitates were sometimes amorphous and were discarded after separation by centrifugation. The resultant supernate was again allowed to stand in beakers at 4°C. The crystals were collected by centrifugation or filtration.

Results. The resultant crystals were needle-shaped and doubly refractile. Crystals were dissolved in distilled water or phosphate buffer, pH 7, 0.1 ionic strength. The solutions were reduced with sodium hydrosulfite and converted to carboxymyoglobin. Absorption maxima in the 540 mμ and 580 mμ regions were determined in a Beckman DU spectrophotometer and were compared to a carboxyhemoglobin standard. The peaks corresponded to those given in the literature.

Discussion. Morgan(6) in his studies of the solubility of equine myoglobin in concentrated ammonium sulfate solutions and in concentrated phosphate buffers noted that at pH 6.6 myoglobin is much more soluble than hemoglobin and that ammonium sulfate or phosphate solutions may be utilized for quantitative separation of these proteins at this pH. Previous methods(3,4) for purification of human myoglobin have utilized unsaturated phosphate or ammonium sulfate solutions to precipitate hemoglobin from myoglobin solutions at a constant pH of about 7. In Theorell's method(3) proteins other than hemoglobin and myoglobin were first precipitated by basic lead acetate. In these methods(2,3,4) myoglobin was crystallized after removal of hemoglobin by gradually increasing ammonium sulfate or phosphate concentration at constant pH. In Theorell's method(3) additional purification by electrophoresis was required before crystallization was accomplished.

The present method of purification and crystallization depends on our observation that human myoglobin is soluble in saturated ammonium sulfate at pH 8 and that hemoglobin is insoluble under these conditions. After removal of other proteins, including hemoglobin, and adjustment to pH 7, myoglobin crystallizes out on standing. In our hands this method has proved simple and effective.

Summary. A method of purifying and crystallizing human myoglobin is described. This method depends on the observation that myoglobin is soluble in saturated ammonium sulfate solutions at pH 8.

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