81. *Purification and Crystallization of Beef Interstitial Cell Stimulating Hormone (ICSH).*

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Purification of anterior pituitary interstitial cell stimulating hormone (ICSH) has been reported by C.H. Li, M.E. Simpson, and H.M. Evans¹⁾ from sheep pituitary and by B.F. Chow, H.B. van Dyke, and co-workers²⁾ from pig pituitary. The author obtained crystalline ICSH from beef anterior pituitary by a relatively simple procedure, quite different from that of above mentioned workers'. 50 g acetone-desiccated beef pituitary tissue is extracted with 500 cc 40% alcoholic acetate buffers at pH 5.0 and ionic strength of 0.15 over a period of 24 hours with constant stirring in a cold room and the residue is reextracted similarly. The supernatants are combined and filtered through coarse folded filter paper. The clear filtrate is brought to 85% alcohol concentration by the addition of 95% alcohol. All steps are carried out below 4°C. The precipitate formed is then extracted with distilled water. To this extract (pH 6.8) 1% solution of rivanol (2-ethoxy-6,9-diaminoacridinium lactate) was added until no more precipitate was formed. The rivanol precipitate is centrifuged off and the yellow filtrate is brought to 90% acetone concentration. The precipitate formed is dissolved in water again and brought to 33% acetone concentration and stored in ice box overnight. This precipitate is centrifuged off and the supernatant is brought to 50% acetone concentration gradually and stored in ice box for a few days. Then ICSH crystallized almost completely. This crude crystals obtained as above were dissolved in a small volume of water and recrystallized by the addition of

Crystalline ICSH ($\times 500$)