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* Present address: Department of Pharmacology, Washington University School of Medicine, St. Louis. Mo.
† Present address: Graduate Department of Biochemistry, Brandeis University, Waltham, Mass.

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Purification and Properties of an Interstitial Cell-Stimulating Hormone from Sheep Pituitaries

Two groups of investigators have previously reported procedures for the isolation of interstitial cell-stimulating hormone (ICSH) from both sheep (*1*) and swine pituitary glands (*2*). Recently, reexamination of these procedures has led us to the development of a new method utilizing some of the newer techniques for purification of proteins that have been developed since the earlier purifications were described. In the course of this work, it was noted that more than one protein component in the extract from sheep pituitaries possesses ICSH activity. Moreover, one of these active components, the isolation of which is described herein, has properties different from those reported for either of the earlier preparations (*1, 2*).

Acetone-desiccated sheep pituitaries (1 kg) were extracted (*3*) with a 0.5 percent NaCl solution at *p*H 4.6. The extract was adjusted to *p*H 7.15, and ethanol was added at −15°C until the concentration of ethanol reached 40 percent by volume. The resulting precipitate was extracted with water at 0°C, and the insoluble portion was removed by centrifugation. Solid NaCl and a 0.2*M* sulfosalicylate buffer (*p*H about 3.6) were added to the supernatant fluid to give a final concentration of 0.15*M* with respect to sodium chloride and 0.02*M* with respect to sulfosalicylate. The *p*H was then adjusted to 3.60 ± 0.03 by addition of 1*M* HCl. The precipitate that formed was of low activity and consequently was discarded; the supernatant fluid was adjusted to *p*H 6.9 and fractionated with $(NH_4)_2SO_4$. The fraction that precipitated at concentrations of $(NH_4)_2SO_4$ between 1.3*M* and 2.1*M* was collected, dialyzed, and lyophilized. This fraction (designated as fraction B) was next subjected to chromatography on a polycarboxylic acid resin, Amberlite IRC-50 (XE-97), which had been equilibrated with 0.2*M* potassium phosphate buffer of *p*H 5.88. The active fraction (fraction C) which was eluted from the column with 0.2*M* phosphate buffer of *p*H 6.9 was recovered and further purified by chromatography on an IRC-50 resin column under the conditions shown in Fig. 1. It was noted that materials in tubes 126 to 143 and in tubes 144 to 159 were equally active; these were designated as fractions D-2 and D-3, respectively.

Further purification of fraction D-3 was achieved by zone electrophoresis on starch. The contents of segments No. 19 to 29 (fraction E-3, Fig. 2) were combined, and the protein was recovered and subjected again to chromatography on IRC-50 resin under the same conditions as those shown in Fig. 1. The active fraction (obtained in a yield of 65 mg/kg of pituitary glands) obtained in this manner behaves as a homogeneous protein according to chromatography on IRC-50 resin and carboxymethyl cellulose columns, in zone electrophoresis on starch, in boundary electrophoresis by the conventional method, and in the ultracentrifuge. It represents one of the ICSH-active components in the glandular extract and is hereafter designated as β-ICSH.

The interstitial-cell stimulating activity of β-ICSH was estimated by its ability to increase the weight of the ventral prostate in hypophysectomized rats (*4*). It was found that a total dose of 0.002 mg causes an increment of ventral prostate weight of from 8.5 to 18.6 mg when it is injected intraperitoneally over a period of 4 days into 23-day-old hypophysectomized rats (Long-Evans strain, 2 days postoperatively). It is essentially free from lactogenic, somatotropic follicle-stimulating, thyrotropic, and adrenocorticotropic activities.

The highly purified β-ICSH was subjected to biophysical investigation. The electrophoretic mobility was determined at 1°C in buffers of 0.1 ionic strength over a wide range of *p*H by means of the free boundary technique. At *p*H 4.2 in acetate buffer, the mobility was found to be $+1.84 \times 10^{-5} cm^2 sec^{-1} v^{-1}$; the isoelectric point was at *p*H 7.3. The sedimentation constant and diffusion coefficient were measured in a buffer consisting of 0.010*M* K_2HPO_4, 0.010*M* KH_2PO_4, and 0.200*M* NaCl. The respective values, corrected to zero protein concentration and 20°C, were found to be $s^0_{20} = 2.47$ *S* and $D^0_{20} = 7.54 \times 10^{-7} cm^2 sec^{-1}$. From these constants, together with an assumed value for partial specific volume (0.73 ml/g) and a solvent density of 1.010, the molecular weight of β-ICSH was computed to be 30,000. Chemical characterization of β-ICSH is in progress (*5*).

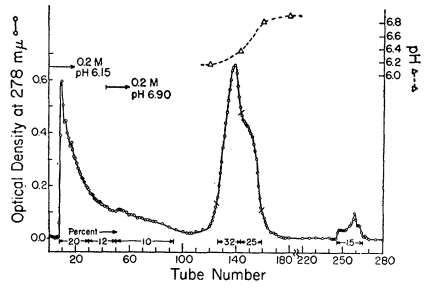

Fig. 1. Chromatography of fraction C on IRC-50 resin. One and two-tenths grams of the fraction was applied to the column containing 33 mg of resin equilibrated with 0.2*M* potassium phosphate at *p*H 6.13. Elution with buffer of *p*H 6.9 was started at tube 43. The final peak, tubes 244 to 270, was eluted with 0.2*N* NaOH. Fraction D-2 was recovered from tubes 126 to 143, and fraction D-3 from tubes 144 to 159. Percentage of recovery of protein nitrogen is given above the corresponding tube numbers. The *p*H during elution of the peak is represented by a dashed line.

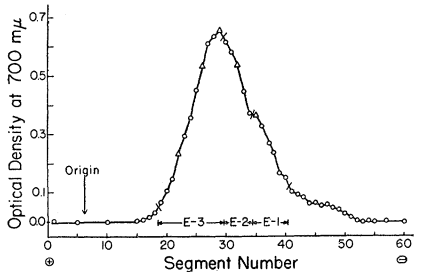

Fig. 2. Zone electrophoresis of 150 mg of fraction D-3 in acetate buffer of *p*H 4.2 and 0.10 ionic strength for 72 hours at 3.0 v cm^{-1}.

PHIL G. SQUIRE
CHOH HAO LI
Hormone Research Laboratory and
Department of Biochemistry,
University of California, Berkeley

References and Notes

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