

many others who depend on celestial navigation. The time of these men is precious, and astronomers should not burden their minds with facts about stars and the universe. For future navigators the study of astronomy is now an extra-curricular activity.

Professor Wylie has taken a first important step in the development of a text on war-time astronomy.

One can only hope that he will not consider the job finished with the first edition of the text. The second edition could stand drastic revision in the direction of better serving the immediate and important needs of the Army Air Corps.

BART J. BOK

HARVARD COLLEGE OBSERVATORY

SPECIAL ARTICLES

ISOLATION OF ADRENOCORTICOTROPIC HORMONE FROM SHEEP PITUITARIES

A METHOD is herein described for the isolation of a protein from the anterior hypophysis which selectively stimulates the adrenal cortex and is free from other biologically active contaminants. Sheep pituitaries were ground and extracted with acidified 80 per cent. acetone. The extract¹ was precipitated in 90 per cent. acetone and dried. The dried powder was extracted with 0.1 M Na_2HPO_4 and the extract again precipitated by bringing it to half saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was then dissolved in water and dialyzed until salt-free. The dialyzed solution was adjusted to pH 3.0 and saturated NaCl was added to 0.54 M. The precipitate formed was saved for the isolation of lactogenic hormone and the supernatant was brought to half saturation with $(\text{NH}_4)_2\text{SO}_4$. The $(\text{NH}_4)_2\text{SO}_4$ precipitate was dissolved in water and half of its volume of concentrated NH_4OH was added and the solution allowed to stand at room temperature for 4 hours. The solution was then brought to 90 per cent. acetone. The precipitate formed was suspended in water and dialyzed, first against distilled water, then against pH 7.5 phosphate buffer of ionic strength 0.10. A slight precipitate that formed was discarded. Saturated aqueous $(\text{NH}_4)_2\text{SO}_4$ was then added to the dialyzed solution to 0.4 saturation. The precipitation with $(\text{NH}_4)_2\text{SO}_4$ was repeated two more times. The final precipitate was dialyzed and adjusted to pH 3.0 and saturated NaCl solution was added to 0.54 M. The precipitate was removed and discarded and the supernatant brought to 1.35 M. The precipitation with NaCl was repeated four times.

The final precipitate obtained by NaCl at pH 3.0 was examined by electrophoretic and solubility studies. Electrophoresis experiments were carried out in a Tiselius apparatus with the Longworth scanning method. A 1 per cent. solution of protein was used at pH 6.87, 5.84, 4.60, 4.10. The potential gradient was about 6 volts per cm; the time of electrolysis was not less than 200 minutes. All these experiments indicated that the preparation contained only one com-

ponent. From these experiments the isoelectric point was estimated to be approximately pH 4.7.

In the solubility studies, the solvent employed was 1.35 M NaCl, pH 3.0. The experiments were conducted in the cold room at 2 to 3° C. When five times the amount of the solid necessary for saturation was added the solubility remained the same. The experiment indicates that protein consists of a single component.

The hormone is exceptionally stable to heat. No biological activity was lost when a 1 per cent. solution in pH 7.5 phosphate buffer of ionic strength 0.10 was placed in a water bath at 100° C. for 2 hours.

The tryptophane content was very low, approximately 0.2 per cent. It will be remembered that the tryptophane content of lactogenic hormone was found to be 2.5 per cent. when the same method of determination (glyoxalic acid) was used.

Two methods of biological standardization of adrenocorticotrophic hormone were used: (I) repair of the adrenal cortex of the immature female rat, 26 to 28 days of age at hypophysectomy, 14 days post-operative, when injected once daily for 4 days, autopsy on the 5th day, increase in width of the cortex and redistribution of the lipids being the criteria; (II) maintenance of the adrenal cortex (width and lipid distribution) in the male rat 40 days old at hypophysectomy, injected once daily for 15 days (13 injections). The dose of the homogeneous protein necessary to cause detectable repair of the adrenal cortex (Method I), was 0.05 mg total dose; the minimum daily dose for maintenance (Method II) was 0.05 mg. The hormone not only stimulates the adrenal cortex as judged by morphological but also by functional criteria. It increases the resistance of hypophysectomized and normal rats to cold, starvation and anoxia.

The chemical, physical and biological properties of the adrenocorticotrophic hormone will be described in more detail elsewhere.

CHOH HAO LI
MIRIAM E. SIMPSON
HERBERT M. EVANS

UNIVERSITY OF CALIFORNIA,
BERKELEY

¹ All subsequent procedures unless otherwise specified were performed at 2 to 3° C.