but slowly under existing limitations, and speed is urgently needed; for the medical profession confidently predicts a malaria peak this year or next.

Those individuals, organizations, institutions and manufacturers who are willing to cooperate by supplying the information suggested in the foregoing pages are requested to communicate with the chairman of the committee, whose address until September 20 will be Belgrade Lakes, Maine; after that, Havemeyer Hall, Columbia University, New York.

As this committee has been for some time in touch with many others interested in antimalarials both in this and in other countries and has accumulated considerable information of value, it seems to us wise to publish this statement, in order that confusion and duplication of effort may be avoided and national preparedness advanced more speedily, for we have been informed that other committees and groups have been or are about to be organized to cover much the same field, evidently unaware of the existence of our committee and its activities.

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SPECIAL ARTICLES

THE ISOLATION IN PURE FORM OF THE INTERSTITIAL CELL-STIMULATING (LUTEINIZING) HORMONE OF THE ANTERIOR LOBE OF THE PITUITARY GLAND

SHORTLY after proof of the secretion of gonadotrophic hormones by the anterior lobe of the pituitary body had been offered by the work of Smith,[1] Zondek and Aschheim,[2] and a number of later investigators,[3] it was suggested that the effects of implants or crude extracts of the pituitary on the gonads should be attributed to at least two hormones (follicle-stimulating and luteinizing hormones). This view-point was supported by Fevold, Hisaw and their collaborators and, more recently, by work in this laboratory and in that of H. M. Evans. However, some investigators are not satisfied that more than one gonadotrophic hormone can be extracted from the gland and have awaited the isolation of pure hormones which should satisfactorily correct the existing confusion with regard to the possible number and effects of the gonadotrophic hormones postulated by others. In New Brunswick,[4] considerable progress had been made in isolating a hormone[5] stimulating the interstitial cells of the male or female gonads. This material was not chemically pure but was found to be free of other pituitary hormones. The work here reported describes the isolation of this hormone as a pure protein.

[1] P. E. Smith, *Anat. Rec.*, 32: 221, 1926.
[2] B. Zondek and S. Aschheim, *Klin. Wschr.*, 6: 248, 1927.
[3] H. B. van Dyke, "The Physiology and Pharmacology of the Pituitary Body," Chicago, I, 1936; II, 1939.
[4] R. O. Greep, H. B. van Dyke and B. F. Chow, *Jour. Biol. Chem.*, 133: 289, 1940.
[5] Evans, Korpi, Simpson, Pencharz and Wonder (*Univ. Calif. Pub. Anat.*, 1: 255, 1936) isolated an interstitial cell-stimulating fraction which was considered not to cause luteinization. Later, Evans, Simpson, Tolksdorf and Jensen (*Endocrinology*, 25: 529, 1939) concluded that the same fraction can bring about both interstitial cell-stimulation and luteinization.

The initial extraction of gonadotrophic hormones from the pituitary glands of swine was undertaken by a method already described.[6] The interstitial cell-stimulating extract free from other hormones was then isolated by a second procedure.[4] Electrophoretic studies were carried out on this preparation in a Tiselius apparatus,[7] using the scanning method of Longsworth[8] for obtaining the electrophoretic patterns. Experiments were performed over a range of pH values from about 4.5 to 8.0 at a constant ionic strength of 0.05, using the monovalent buffers, acetate, cacodylate and diethylbarbiturate. The results showed the presence of three components (Fig. 1 A). After

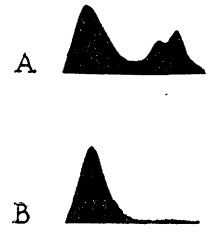

FIG. 1. Electrophoretic patterns at pH = 7.85. A—Before final purification. B—After final purification.

sufficient separation of the electrophoretic boundaries it was possible to isolate two fractions, one containing the major component, the other the two minor ones. The biological activity was found to be entirely in the main component. A plot of mobility against pH showed the isoelectric point of this main component to be at pH 7.45. The isoelectric points of the contaminating, inert components fell between pH 4 and 5.

This information made it possible to secure the pure hormone by isoelectric precipitation. To a concen-

[6] B. F. Chow, R. O. Greep and H. B. van Dyke, *Jour. Endocrinol.*, 1: 439, 1939.
[7] A. Tiselius, *Tr. Faraday Soc.*, 33: 524, 1937.
[8] L. G. Longsworth, *Jour. Am. Chem. Soc.*, 61: 529, 1939.

trated solution of the material thus studied, ammonium sulfate was added to one third saturation and the pH was adjusted to 7.3–7.4, as measured by the glass electrode. The precipitate, after the separation of the solvent by centrifugation and the removal of the solvent, was dissolved in water; saturated ammonium sulfate solution equal to one half of the volume of water used was added, and the pH was again adjusted to 7.3–7.4. These steps were repeated seven times, and the final precipitate was dissolved in a small volume of water; the solution was then dialyzed free from ammonium sulfate. (The supernatant fluids contained considerable amounts of hormone and were set aside for further recovery.) The product obtained appeared as a single component (Fig. 1 B) in electrophoretic analysis of solutions having different pH values, with mobilities of -3.85×10^{-5} at pH = 4.58, -2.01×10^{-5} at pH = 6.21, and 0.66×10^{-5} at pH = 7.86. The material was found to show the phenomenon of "reversible boundary spreading" which is usually observed with globulins. That is, after electrolyzing for a certain length of time, reversal of the current results in a sharpening of the boundary. This indicates that the spreading of the boundary observed with increased time of electrolysis is not due entirely to diffusion but results from the fact that the material consists of molecules corresponding to a range of mobility within relatively narrow limits. If one takes the view that native proteins exist as folded molecules, such mobility ranges may be due to minor variations in folding corresponding to little or no energy differences, but yielding slight variations in the surface charge distributions which in turn result in corresponding mobility variations.

Preparations of the hormone in different degrees of purity were also studied in the ultracentrifuge. The apparatus used was of the air turbine drive type described by J. Bauer and E. Pickels.[9] The sedimentation rate was followed by the scale method of Lamm and the "schlieren" method of Philpot as modified by Svensson. Both methods gave identical results. The solvent commonly used was an aqueous solution of 1 per cent. or 0.5 per cent. sodium chloride. In one case the solvent was a cacodylate solution (pH = 6.2) which was used in the electrophoretic studies. Seven runs were made on preparations of different degrees of purity. The least pure preparation, in which three components were detected in electrophoretic studies, showed four well-separated boundaries. Two of the impurities sedimented at a faster rate than the biologically active main component (the fastest at three times the rate), whereas the third impurity sedimented at one half the rate of the hormone. A "schlieren" diagram of the sedimentation of the pure hormone can be seen in Fig. 2. The diagram represents the positions of the boundary moving from right to left at half hour intervals under a mean field of 2.37×10^8 cm/sec.[2]

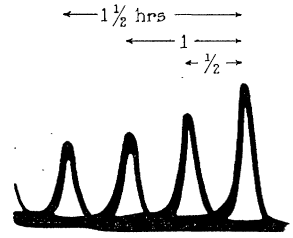

FIG. 2. Sedimentation patterns of the hormone after final purification.

(57600 r.p.m.). The symmetry of the curves and the absence of any displacement of the base line with time indicate the purity of the hormone in agreement with electrophoretic patterns and solubility tests.[10] The average sedimentation constant in a 1 per cent. sodium chloride solution was $S_{20} = 5.39 \times 10^{-13}$, the maximum deviation from this average value being ± 1 per cent. (An abnormally high value of $S_{20} = 6.65 \times 10^{-13}$ was found for the cacodylate solution.) The diffusion constant determined in the cacodylate medium was found to be $D^{20} \cong 5.9 \times 10^{-7}$, this value decreasing with time. From these data the molecular weight may be estimated to be approximately 90,000, assuming the value 0.749 for the specific volume of the hormone.

An example of the results of a study of the solubility of the hormone is shown in Fig. 3. The solvent

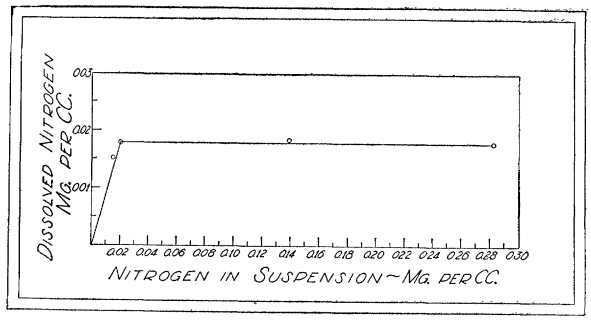

FIG. 3. Solubility of the hormone in 0.25M acetate buffer, pH = 4.35, containing 21.05 per cent. Na_2SO_4.

used was 0.25 M acetate buffer, pH = 4.35, containing 21.05 per cent. sodium sulfate. The solution remained water clear after the first addition of hormone (0.015 mg N per cc), but became turbid when 0.020 mg of N was present in each cc of solvent. The solubility then and thereafter was constant (0.018 mg N per cc), although the amount of hormone suspended in the solvent was nearly sixteen times the saturating concentration. Therefore, within the described limits, only

[9] T. Svedberg, "The Ultracentrifuge," Oxford, 1939.

[10] Close inspection of the plate revealed a faint residual trace of the impurity sedimenting slightly faster than the hormone.

one component was demonstrated, since the solubility of this component was constant whether the solution was just turbid or whether a great excess of hormone was in suspension. These results, agreeing with the studies made with the Tiselius electrophoresis apparatus and the ultracentrifuge, furnish proof of the purity of the hormone by the only other available physical chemical test.

The results of analysis of one specimen of pure hormone were: carbon, 49.37 per cent.; hydrogen, 6.83 per cent.; nitrogen, 14.93 per cent.; ash, 0.93 per cent.

All the assays were performed in rats hypophysectomized at an age of twenty-one days. Injections were begun two days later and were given subcutaneously once daily for four days.[11] At necropsy, twenty-four hours after the last injection, the appropriate organs were weighed and then fixed in Bouin's fluid. Typical results are shown in Table 1. Owing

TABLE 1

Dose micrograms of nitrogen	Number of rats	Mean and S.E. of wts.		
		Body gm	Ant. lobe prostate mg	Testes mg
–	8	48.5 ± 2.90	8.49 ± 0.549	130.7 ± 5.25
1	9	52.4 ± 2.49	11.01 ± 0.695	142.0 ± 7.35
2	6	49.3 ± 3.62	16.97 ± 0.624	160.8 ± 12.10
10	5	44.9 ± 1.18	34.58 ± 2.130	209.6 ± 7.91

to stimulation of the testicular interstitial cells, 1 microgram of hormone nitrogen caused a significant increase in the fresh weight of the anterior lobe of the prostate ($P = < 0.05$ by Fisher's method of paired comparisons). A dose of 2 micrograms of hormone nitrogen was required to produce an increase of testicular weight ($P = < 0.05$). This testicular hypertrophy was probably caused chiefly by the action of secreted androgen on the germinal epithelium. In hypophysectomized, immature female rats, the hormone maintained the interstitial cells. However, if it was administered after follicle-growth had been stimulated by follicle-stimulating hormone, it caused the formation of corpora lutea perhaps preceded by ovulation. Our results indicated that the secretion of oestrogen did not occur following the injection of follicle-stimulating hormone into hypophysectomized rats, although the growth of histologically normal follicles was clearly present. If, however, interstitial cell-stimulating hormone was also administered, all the morphological phenomena of oestrus were provoked.

SUMMARY

Interstitial cell-stimulating (luteinizing) hormone was isolated from swine pituitary glands. This protein hormone, having a molecular weight of about 90,000 and an isoelectric point of pH 7.45, was shown to be pure by tests in the electrophoresis apparatus of Tiselius and in the ultracentrifuge as well as by its constant solubility. The hormone stimulated the interstitial tissue of the testis or ovary and caused the formation of corpora lutea provided that maturing follicles were present. Under the conditions described, its minimal effective total dose in hypophysectomized immature male rats was about 1 microgram of nitrogen or 6.7 micrograms of hormone. Extracts of swine pituitary with purely follicle-stimulating effects did not cause oestrus in immature hypophysectomized female rats unless luteinizing hormone was also administered.

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SYNTHESIS OF THE PYRIDINE ANALOG OF VITAMIN B_1

THE concept of isosterism as proposed by Grimm[1] and extended by Erlenmeyer[2] has recently attracted attention by its possible application with respect to the substitution of a pyridine nucleus for the thiazole component of the thiamine (vitamin B_1) molecule. As part of a general study of various pyridine derivatives in this laboratory, one of us[3] recently described the preparation of 2-(β-hydroxyethyl)-3-methylpyridine and the condensation of this substance with the pyrimidine component of thiamine. Meanwhile, others have reported the synthesis of what was assumed to be 2-methyl-3-(β-hydroxyethyl)-pyridine, and from this the preparation of a substance presumably isosteric with thiamine by condensation with the appropriate pyrimidine. Schmelkes[4] described an isosteric vitamin as well as the substances formed by condensation of what he believed to be 2-methyl-5-(β-hydroxyethyl)-pyridine with the thiamine pyrimidine. He reported anti-polyneuritic activity for the former. Baumgarten and Dornow[5] arrived at Schmelkes' anti-polyneuritic substance by a different route and reported its activity as being about one-twenty-sixth that of thiamine. However, in a later paper, Dor-

[11] Results of other investigators indicate that intraperitoneal injection would have provoked a greater response.

[1] Grimm, *Naturwiss.*, 17: 535, 557, 1929.

[2] Erlenmeyer *et al.*, *Helv. Chim. Acta*, 16: 733, 1381 (1933); 20: 1388 (1937) inter alia.

[3] Finkelstein and Elderfield, *Jour. Org. Chem.*, 4: 365, 1939.

[4] Schmelkes, SCIENCE, 90: 113, 1939; Schmelkes and Joiner, *Jour. Am. Chem. Soc.*, 61: 2562, 1939.

[5] Baumgarten and Dornow, *Ber.*, 73: 44, 1940.