TECHNICAL PAPERS

Isolation of Pituitary Follicle-Stimulating Hormone (FSH)[1]

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A method is herein reported for the isolation of a protein from sheep pituitaries which causes follicular development only in the ovaries of hypophysectomized rats, and which behaves as a single substance in electrophoresis and ultracentrifugation.

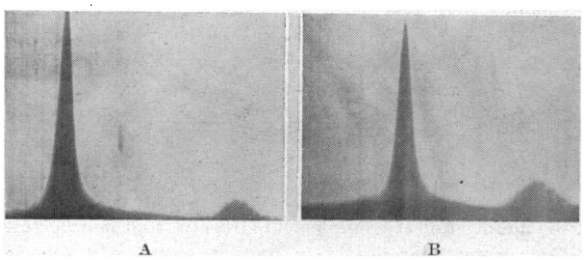

FIG. 1. Electrophoretic patterns of ascending boundaries of two pituitary follicle-stimulating hormone preparations: A—in pH 7.0 phosphate buffer of 0.1 ionic strength, and B—in pH 4.0 acetate buffer of the same ionic strength. The current with a potential gradient of about 6 volts per cm had run for 180 min in A and for 240 min in B. The protein concentration was 1%.

The following procedures were performed at 2° to 3° C:

Ammonium sulfate fractionation: One kg of frozen sheep pituitaries is finely ground and extracted with $Ca(OH)_2$ according to the procedure described previously (*1*). After the removal of the precipitate obtained by adding saturated $(NH_4)_2SO_4$ to half saturation, the supernatant was brought to 0.75 saturation by the addition of solid $(NH_4)_2SO_4$. This precipitate was dissolved in water and dialyzed. A slight precipitate that formed during dialysis was discarded. The clear reddish supernatant solution was adjusted to pH 6.0 and then to pH 4.7. The precipitate formed at either pH was removed by centrifugation. The supernatant was brought to 0.5 saturated $(NH_4)_2SO_4$ by the slow addition of an equal volume of saturated $(NH_4)_2SO_4$ solution at pH 4.7. The precipitate formed was removed and the supernatant again brought to 0.75 saturated $(NH_4)_2SO_4$ by further addition of saturated $(NH_4)_2SO_4$ solution at the same pH. The precipitate was dissolved and dialyzed. The whole procedure was repeated once more, and the final dialyzed solution was frozen and dried in vacuum. This product is called "crude FSH" and may be kept in a desiccator for further use.

Ethanol fractionation: The "crude FSH" powder was next extracted with 0.10 M K_2HPO_4 in 40% ethanol. After removal of the residue, the ethanol concentration in the supernatant was increased to 80% by adding slowly cold 95% ethanol (−5° C). The ethanol precipitate was dissolved in water and dialyzed.

Further $(NH_4)_2SO_4$ fractionation: The dialyzed solution was adjusted to pH 4.7 and centrifuged if precipitation occurred. A pH 4.7 saturated $(NH_4)_2SO_4$ solution was next added until the concentration became 0.55 saturation. The 0.55 saturated $(NH_4)_2SO_4$ precipitate, found to be devoid of FSH activity, was removed by centrifugation. The supernatant was brought to 0.70 saturation with more pH 4.7 saturated $(NH_4)_2SO_4$ solution; the precipitate formed was dissolved in water and dialyzed. This 0.55–0.70 saturated $(NH_4)_2SO_4$ fractionation was repeated twice.

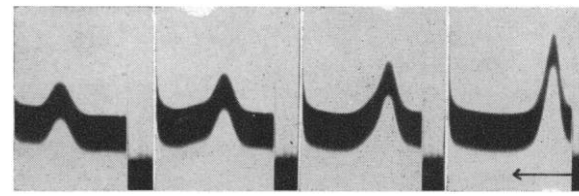

FIG. 2. Four schlieren patterns of a saline solution of the pituitary follicle-stimulating hormone taken at 1920-sec intervals during sedimentation at 165,000× gravity in an ultracentrifuge.

The final 0.55–0.70 saturated $(NH_4)_2SO_4$ precipitate was examined in a Tiselius electrophoresis apparatus using the scanning method of Longsworth and in a Spinco ultracentrifuge.[2] As shown in Figs. 1 and 2, the pattern obtained by either method was characteristic for a single protein.

All biological assays were carried out in female rats hypophysectomized at 27 days of age. Subcutaneous injections were begun about 7 days later, and were given once daily for 3 days. Autopsy was performed 72 hr after the onset of the injections. Histological examination of the ovaries showed that a total dose of 0.05 mg of the hormone initiated follicular development (increase in follicle size beyond that characteristic of controls and beginning antrum development). On the other hand, a total dose of 2.0 mg of the product administered over a 4-day period did not show interstitial-cell-stimulating,

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[2] We are indebted to M. Moskowitz for the ultracentrifugal data.

thyrotropic, adrenocorticotropic, or growth-promoting activities, indicating that at this high level the preparation was free of other pituitary hormones.

Reference

1. Li, C. H., Evans, H. M., and Simpson, M. E. *J. biol. Chem.*, 1945, **159**, 353.

Preliminary Report on the Circumpolar Distribution of *Neoechinorhynchus rutili* (Acanthocephala) in Fresh Water Fishes

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Early attempts on the part of American helminthologists to establish the identity of individuals of the acanthocephalan genus Neoechinorhynchus found in fishes of the Atlantic coast and species found in Europe have all been repudiated. For many years the writers have been accumulating evidences on the continuous dispersal of *Neoechinorhynchus rutili* in fresh water and migratory fishes throughout the circumpolar regions of Europe and North America. Previously unpublished observations, resulting from examinations of fishes of the northern states, the north Pacific area, Alaska and the arctic regions, furnish a chain of evidence on which broad geographical distribution is established. There is no confirmation of the possibility of this species' extending its distribution through the Atlantic fauna.

The most difficult obstacle encountered in the study has been the inadequate morphological description of *N. rutili*, which has been commonly regarded as a distinctively European species. Detailed studies have demonstrated the identity of specimens from Sweden, Finland, and central Europe, and those taken from Wisconsin, Washington, Alaska, and various regions in Canada, including materials from within the Arctic Circle of the Canadian Northwest Territories.

This constitutes the first authentic demonstration of the occurrence of the same species of an acanthocephalan in fresh water fishes of both Europe and North America. Inhabitants of brackish water and migratory fishes are included in the host list for both continents. There is no evidence to indicate that this widely dispersed species has developed any tendency toward the establishment of distinct varieties or subspecies in the various parts of its range or in its adaptation to a highly diversified list of definitive hosts.

A full morphological and taxonomic description of *N. rutili* is possible on the basis of the present study. This, together with a full account of the geographical and host distribution, will be presented in a full account of the investigation which is to be published elsewhere.

A Photographic Technique for the Detection of Presumptive Biochemical Mutants

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A recent article by Wagner (*2*) describing the photographic recording of Petri plate cultures of microorganisms suggested the possibility of a similar technique for detecting biochemical mutants in colonial microorganisms. The layer plate detection technique of Lederberg and Tatum (*1*), as customarily used in this laboratory, involves the laborious hand-marking of individual wild type colonies to enable the later detection of mutant organisms. Cells or spores treated to induce mutation are plated on a minimal agar medium and the wild type colonies allowed to grow up. These colonies are marked by spotting a drop of India ink on the glass surface of the plate directly below the colonies. Thereupon a layer of agar, containing whatever supplements the investigator is interested in, is poured over the agar surface containing the treated cells. Cells requiring the supplements are thus enabled to grow up. The procedure is concluded by picking the unmarked colonies, which develop after the addition of the supplemented media, and testing them further to determine their mutant status. In crowded plates the individual spotting of colonies is very time-consuming and occasional colonies may be overlooked, or the indicator spots rubbed off in handling.

In the photographic method developed here, the treated cells are prepared and plated in the usual way. When the wild type colonies have grown up, the plate is placed upon a piece of high speed, high contrast photographic printing paper with the emulsion side of the paper in contact with the underside of the plate. The plate is momentarily uncovered and exposed to a strong light, and the exposed paper then developed in the usual manner for contact prints. With proper exposure a positive print is obtained in which the white colonies stand out against a dead black background, since the agar layer with colonies acts as a negative. The plate from which the print was made is layered with supplemented media. After an appropriate interval to allow growth of deficient cells the plate is superimposed on the print. The new colonies which grow up are readily detected by comparison with the colonies already visible on the photograph of the original layer, and these putative mutants are picked and tested further. Reference lines and markers to permit easy orientation of plate and print may be drawn on the lower surface of the plate with India ink before photographing. In practice we find Kodabromide F-4 and F-5 to be very satisfactory papers used in conjunction with the light from an enlarger. The exposure time under these conditions is less than 1 sec, and the entire develop-

[1] Standard Brands, Inc. fellow in microbiology.