berg for stimulating discussions, and to Esso Standard Oil Company for financial support.

(7) Esso Standard Oil Company Fellow, 1955–57.

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RECEIVED MARCH 25, 1958

## ISOLATION OF MELATONIN, THE PINEAL GLAND FACTOR THAT LIGHTENS MELANOCYTES[1]

*Sir:*

During the past forty years investigators have reported that injection of pineal gland extracts into tadpoles, frogs, toads and fish produces lightening of skin color.[2–4] Recently it was found that such extracts, by causing aggregation of melanin granules within the melanocytes of isolated pieces of frog skin, reverse the darkening effect of the melanocyte stimulating hormone (MSH).[5] We wish to report isolation from beef pineal glands of the active factor that can lighten skin color and inhibit MSH. It is suggested that this substance be called *melatonin*.

Fifty grams of powdered lyophilized beef pineal glands[6] was extracted with petroleum ether for two hours in a soxhlet extractor. The defatted powder was mixed with 900 ml. water in a Waring Blendor. After centrifugation at 16,000 × *g* for 30 minutes the supernatant was extracted with 900 ml. ethyl acetate. The ethyl acetate layer was concentrated *in vacuo* at 50° and subjected to distribution in a 30-tube countercurrent apparatus with the solvent system ethyl acetate, heptane, water (1:1:2 v./v.). Tubes 8–15 were combined. The water layer was extracted twice with 80 ml. portions of ethyl acetate. All the organic solvent extracts were combined and evaporated to dryness *in vacuo* at 50°. The residue was sublimed at 80° *in vacuo*. The sublimate was transferred with ethanol to Whatman No. 1 filter paper and chromatographed by descending technique with solvent system benzene, ethyl acetate, water (19:1:20). A test strip on reaction with Ehrlich reagent (*p*-dimethylaminobenzaldehyde) showed a blue spot at $R_f$ 0.38. The unreacted strip was cut into sections and eluted with ethanol. Bioassay was performed using isolated *Rana pipiens* skin darkened with caffeine. The lightening effect of the test substance on the melanocytes was measured photometrically with transmitted light. This revealed that 95% of recoverable biologic activity was present at the position of the blue spot. Spectrophotofluorometric analysis of the active eluate showed a single fluorescent peak at 3380 Å. which was excited maximally at 2950 Å. Ultraviolet absorption analysis showed a maximum at 2725 Å. with inflections at 2950 and 3080 Å. The fluorescence and ultraviolet absorption were characteristic of hydroxyindoles.

The active material was rechromatographed and eluted in three successive solvent systems. The biologic activity, characteristic fluorescence, and blue color with Ehrlich reagent remained exclusively together as a spot on these chromatograms. The solvent systems were isopropyl alcohol, concentrated ammonium hydroxide, water (16:1:3) $R_f$ = 0.83; 1-butanol, acetic acid, water (4:1:5) $R_f$ = 0.87; isopropyl alcohol, concentrated ammonium hydroxide, water (10:1:1) $R_f$ = 0.86.

In preventing darkening of frog skin by MSH, melatonin, the active pineal gland factor, was at least 100 times as active on a weight basis as adrenaline or noradrenaline, 200 times as active as triiodothyronine and 5,000 times as active as serotonin.[5] Melatonin had no adrenaline nor noradrenaline-like activity on rat uterus and no serotonin-like activity on clam heart. No melatonin activity was detected in beef pituitary, hypothalamus, thymus, thyroid, adrenal, ovary, testis or eye.

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## THE STRUCTURE OF BOVINE CORTICOTROPIN[1,2]

*Sir:*

The isolation of bovine corticotropin, a 39 amino-acid polypeptide possessing ACTH activity, has been reported from this laboratory[3]; its amino acid composition is identical with that found for ovine α-corticotropin but different from that of the porcine hormone. It was further demonstrated that the porcine, ovine and bovine hormones possess identical N- and C-terminal residues. We wish to report herein the complete amino acid sequence of bovine corticotropin. It will be noted that there is a difference in certain portions of the amino acid sequence among the hormones of all three species.

By means of the paper-strip modification[4] of the phenyl isothiocyanate method,[5] the N-terminal amino acid sequence Ser.Tyr.Ser.Met.Glu. . . . was established for bovine corticotropin. The rate of release of amino acids from the carboxyl end of the peptide hormone by the carboxypeptidase procedure[6] indicated the sequence . . .Leu.Glu.Phe at the C-terminus.

Chymotryptic digests of the hormone (substrate/enzyme = 100/0.6 (w./w.), *p*H 9.0, 40°, for 24 hours) were fractionated by zone electrophoresis on paper for 7 hours at 200 volts with a collidine–acetic acid buffer of *p*H 7; after elution of each band, the peptide fragments were further purified by paper chromatography in either *n*-BuOH/

(1) This investigation was supported by grants from the American Cancer Society and the United States Public Health Service.

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(3) O. Bors and W. C. Ralston, *Proc. Soc. Exp. Biol.*, **77**, 807 (1951).

(4) J. O. Kitay and M. D. Altschule, "The Pineal Gland," Harvard University Press, Cambridge, Mass., 1954, p. 56.

(5) Y. Takahashi and A. B. Lerner, to be published.

(6) We are grateful to the Armour Laboratories for supplying us with several kilograms of beef pineal glands.

(1) Paper XIV of the corticotropins (ACTH) series; for Paper XIII, see C. H. Li, R. D. Cole, D. Chung and J. Leonis, *J. Biol. Chem.*, **227**, 207 (1957).

(2) This work is supported in part by the U. S. Public Health Service (G-2907) and the Albert and Mary Lasker Foundation.

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(6) J. I. Harris and C. H. Li, *J. Biol. Chem.*, **213**, 499 (1955).