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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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.), pH 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 pH 7; after elution of each band, the peptide fragments were further purified by paper chromatography in either n-BuOH/

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pyridine/HOAc/H_2O (30:20:6:24) or *n*-BuOH/HOAc/H_2O (4:1:5) solvent systems. Each purified fraction was submitted to N-terminal residue analysis,[7,8] to C-terminal residue analysis,[6] quantitative amino acid analysis,[8] and finally to sequential degradation from the N-terminus by the Edman method.[5,9] The following peptide fragments were identified: Gly.Lys.Pro.Val.Gly.Lys; Lys.Arg.Arg.-Pro.Val; Arg.Try; Ser.Met.Glu.His.Phe; Ser.-Tyr; Ala.Phe.Pro.Leu; Lys(Val,Tyr,Pro,Asp_2Gly,-Glu_2,Ala).Ser.Ala.Glu.NH_2; Lys.(Val,Tyr,Pro,Asp_2,-Gly,Glu_3,Ala_3,Ser,Phe) and Glu.Phe.

Tryptic digests of the hormone (substrate/enzyme = 90/1 (w./w.), *p*H 9–9.5, 40° for 6 hours) were submitted to countercurrent distribution for 37 transfers in the *n*-BuOH/20% HOAc system. The material with a partition coefficient (K) of 2 (peptide T2) was isolated and was shown to be homogeneous by N-terminal and amino acid analyses. Sequential degradation[5,9] of this peptide from the N-terminus, together with analysis by the dinitrophenylation method[7,8] gave the structure: Val. Tyr. Pro. Asp (Gly, Glu_4, Ala_3, Asp, Ser, Phe_2, Pro, Leu). Partial acid hydrolysis (3 *M* HCl, 24 hours at 40°) of this material yielded the following peptides: Ala.Glu.Asp; Gly.Glu(Ala_2,Glu,Asp,Ser) and (Val,Tyr,Pro,Asp,Gly,Glu).Ala.

The remainder of the material from the counter current distribution of tryptic digests of bovine corticotropin was isolated and further separated by zone electrophoresis and paper chromatography by means of the techniques employed for the chymotryptic digests. The peptide fragments listed were identified: Ser.(Tyr,Ser,Met,Glu,His,-Phe).Arg; Try.Gly.Lys.Pro.Val.Gly.Lys; Lys.Arg and Arg.Arg.Pro.Val.Lys.

From the above data, an amino acid sequence is proposed for bovine corticotropin

Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val.
1 2 3 4 5 6 7 8 9 10 11 12 13

Gly.Lys.Lys.Arg.Arg.Pro.Val.Lys.Val.Tyr.Pro.Asp.Gly.
14 15 16 17 18 19 20 21 22 23 24 25 26

NH_2 (on Glu 33)

Glu.Ala.Glu.Asp.Ser.Ala.Glu.Ala.Phe.Pro.Leu.Glu.Phe
27 28 29 30 31 32 33 34 35 36 37 38 39

When this structure is compared with the ovine[10] and porcine[11,12,13] corticotropins,[14] it is notable that the amino acid sequences in all three peptide hormones are identical except in the region between amino acid residues 25 and 33, a region rich in acidic amino acids.

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1-METHYL-6-ETHYL-3-AZAPHENANTHRENE, A KEY DEGRADATION PRODUCT OF ATISINE

Sir:

The recent correlation of the aconitum alkaloid atidine[1,2] and the delphinium alkaloid ajaconine[2,3,4] with atisine emphasizes the key position this substance occupies among the diterpene alkaloids of the two genera. Recently structures I and II have been suggested for dihydroatisine[5] and atisine,[6] mainly on the basis of the striking analogy of the chemistry of these substances[7] to that of the garrya alkaloids.[8] Subsequent experimental work has demonstrated the presence of the oxazolidine

moiety[9,10,11] in atisine and isoatisine, the β-aminoethanol group[11,12] in dihydroatisine and the disposition of the D-ring and its substituents.[13] An important piece of evidence bearing on the skeleton of atisine is the structure of the $C_{16}H_{15}N$ base[14] (obtained on selenium dehydrogenation) which contains all but six of the carbon atoms of atisine and relates the heterocyclic ring to the rest of the molecule. We now wish to report the identification of this base as 1-methyl-6-ethyl-3-azaphenanthrene (VII)[15] by an unambiguous synthesis from 7-ethyltetralone-1 (III). This synthesis provides the first evidence fixing the position of the nitrogen atom with respect to the rest of the atisine molecule.

Alkylation of the pyrrolidine enamine of 7-ethyltetralone-1[16] (III) with ethyl α-iodopropionate was effected by the method of Stork[17] to give after

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