can be found only by activities so long as it now appears that activity coefficient for sodium ions in cytoplasm of muscle fibres is considerably different from that of outside chloride solutions.

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[1] Liquori, A. M., Ascoli, F., Botré, C., Crescenzi, V., and Mele. A., *J. Polymer Sci.*, **40**, 169 (1959).
[2] Ascoli, F., Botré, C., Crescenzi, V., Mele, A., and Liquori, A. M., *Nature*, **184**, 1482 (1959).
[3] Ascoli, F., and Botré, C., *J. Mol. Biol.*, **3**, 202 (1961).
[4] Caldwell, P. C., *J. Physiol.*, **126**, 169 (1954).
[5] Hinke, J. A., *Nature*, **184**, 1257 (1959).
[6] Eisenman, G., Rudin, D., and Casby, J., *Science*, **126**, 3278 (1958).
[7] Nicolsky, B. P., Shultz, M. M., and Peshechonova, N. V., *J. Phys. Chem.* (U.S.S.R.), **32**, 262 (1958).
[8] Lev, A. A., and Buzhinsky, E. P., *Cytology* (U.S.S.R.), **3**, 614 (1961).
[9] Nicolsky, B. P., *J. Phys. Chem.* (U.S.S.R.), **10**, 495 (1937).
[10] Nicolsky, N. N., and Vasianin, S., *Biophysica* (U.S.S.R.) (in the press).
[11] Carey, M., and Conway, E. J., *J. Physiol.*, **125**, 232 (1954).
[12] Ulrich, F., *Amer. J. Physiol.*, **197**, 997 (1959).
[13] Naora, H., Naora, H., Jrawa, M., Alfrey, W. C., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **48**, 853 (1962).
[14] Hinke, J. A., *J. Physiol.*, **156**, 314 (1961).

Melatonin Synthesis in the Hen Pineal Gland and its Control by Light

Melatonin (5-methoxy *N*-acetyltryptamine) has been found to be highly localized in the pineal bodies of a number of mammalian species[1,2]. The melatonin synthesizing enzyme (hydroxyindole-*O*-methyl transferase (HIOMT)) has also been found in all mammals examined[3], and is present only in the pineal gland[4]. Evidence has been presented demonstrating that melatonin is secreted by the mammalian pineal and acts on the gonads[5]. This communication describes the enzymatic synthesis of melatonin in a class other than the mammal: birds. It further demonstrates that the synthesis of melatonin is influenced by lighting, which also affects the bird gonads[6].

Pineal glands of hens (single comb white Leghorn) were homogenized in cold water with an all-glass homogenizer, and an aliquot was incubated with *S*-adenosylmethionine-methyl-^{14}C and *N*-actylserotonin, as previously described[4]. A radioactive compound was formed which was extractable into chloroform. The chloroform extract was evaporated to dryness under nitrogen and cochromatographed with authentic melatonin[3]. A single radioactive peak was found, which had the identical R_F value as authentic melatonin. The activity of HIOMT per milligram of pineal in hens was found to be twice as high as that of the monkey, which had the greatest enzyme activity of all mammalian species examined[3], and at least 200 times that of the rat[7]. No detectable melatonin synthesizing enzyme could be demonstrated in any area of the hen brain other than the pineal.

Constant environmental lighting has been shown to increase the incidence of œstrus in rats[8]; this effect of light is partly inhibited by melatonin administration[5]. In addition, exposure of rats to continuous illumination markedly reduces the activity of the melatonin-forming enzyme[7]. Since light also has an effect on avian gonads[6], we examined the action of light on the ability of the bird pineal to synthesize melatonin. Groups of hens were kept in total darkness, continuous lighting, or diurnal lighting (14 h of light a day) for 5 days. They were then killed and the pineal gland was removed and immediately frozen, and afterwards assayed for hydroxyindole-*O*-methyl transferase, as well as monoamine oxidase[9] activity. The weight of the pineal gland in hens kept in light was greater than those left in darkness (Table 1). This was in contrast to the findings in the rat, where exposure to light reduced the pineal weight. There was a highly significant decrease in the activity of the melatonin-forming enzyme when hens were in darkness for 5 days, as compared with controls left in diurnal lighting. In rats, darkness had the opposite effect on hydroxyindole-*O*-methyl transferase activity[7]. In hens kept in continuous light there was a significant increase in HIOMT activity in the whole pineal gland. Darkness or light had no significant effect on monoamine oxidase activity in the pineal.

Table 1. Effects of Light on Melatonin Synthesizing Enzyme in the Hen Pineal Gland

Lighting conditions	Pineal weight (mg)	HIOMT (per pineal)	HIOMT (per mg)	MAO (per pineal)	MAO (per mg)
Diurnal	5·0 ± 0·57	44·4 ± 4·0	8·9 ± 0·8	9·0 ± 0·8	1·8 ± 0·17
Constant light	4·9 ± 0·62	56·9 ± 4·2*	11·4 ± 1·8	9·6 ± 1·5	2·0 ± 0·45
Constant darkness	3·0 ± 0·34*	17·4 ± 3·4†	5·8 ± 0·7†	10·1 ± 3·2	3·3 ± 0·77

Groups of 11 hens were kept in diurnal light (14 h per day), continuous light, or total darkness for 5 days. HIOMT activity is expressed as mμmoles ^{14}C-melatonin formed per hour from ^{14}C-*S*-adenosylmethionine and N-acetylserotonin. Monoamine oxidase (MAO) activity is expressed as mμmoles ^{14}C-indoleacetic acid formed per hour from ^{14}C-tryptamine.
* $P < 0.05$. † $P < 0.001$.

Melatonin has been shown to inhibit ovary growth and the incidence of œstrus in rats[8]. The ability of the pineal glands of fowl to synthesize relatively large amounts of melatonin suggests that this compound may play a physiological part in this class of animals. Since environmental lighting influences both gonad growth and melatonin synthesis, it is possible that some of the effects of lighting on bird gonads could be mediated by alterations in the rate of synthesis of melatonin in the pineal gland.

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[1] Lerner, A. B., Case, J. D., and Heinzelman, R. V., *J. Amer. Chem. Soc.*, **81**, 6084 (1959).
[2] Prop, N., and Kappers, J. A., *Acta Anatomica*, **45**, 90 (1961).
[3] Axelrod, J., and Weissbach, H., *J. Biol. Chem.*, **236**, 3072 (1961).
[4] Axelrod, J., MacLean, P. D., Albers, R. W., and Weissbach, H., in *Regional Neurochemistry*, edit. by Kety, S. S., and Elkes, J., 307 (Pergamon Press, New York, 1961).
[5] Wurtman, R. J., Axelrod, J., and Chu, E. W., *Science*, **141**, 277, (1963).
[6] Wolfson, A., in *Advances in Neuroendocrinology*, edit. by Nalbandov, A. V., 402 (Univ. Illinois Press, Urbana, 1963).
[7] Wurtman, R. J., Axelrod, J., and Phillips, L., *Science*, **142**, 1071 (1963).
[8] Fiske, V. M., *Endocrinol.*, **29**, 187 (1941).
[9] Wurtman, R. J., and Axelrod, J., *Biochem. Pharmacol.*, **12**, 1439 (1963).

Hæmolytic Effects of Steroids

A number of neutral and acidic steroids produce fever and inflammation in man[1]. Inflammation probably reflects the cytotoxicity of these compounds, and since hæmolysis is one expression of cellular toxicity, several steroids were investigated for hæmolytic properties *in vitro*, and their hæmolytic and pyrogenic activities compared.

Steroids were dissolved in 0·5 ml. methanol and added to 2·0 ml. 0·02 M phosphate buffered saline (*p*H 6·6); 0·05 ml. of a 50 per cent suspension of washed human erythrocytes were then added and gently mixed. After incubation at 37·0° C, the suspensions were centrifuged and the hæmoglobin in an aliquot of supernatant determined by conversion to cyanmethæmoglobin with Drabkins reagent and measurement of the optical density at 540 mμ. Because the absolute rate of hæmolysis varied widely with small (0·5°–1·0° C) changes in temperature, incubations were performed with several concentrations of each