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INDOLE DERIVATIVES OF PINEAL AND RELATED NEURAL AND RETINAL TISSUES[1]

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I. INTRODUCTION

The pineal organ or epiphysis cerebri of man and other vertebrates is a derivative of the posterior roof of the diencephalon. Most of the cells in this organ in mammals, including man, are structurally unique, and remain metabolically active throughout life (138, 186). These specialized parenchymal cells or pineocytes, as offspring of the primitive ependymal cells lining the early brain roof of vertebrates, are not only embryologically related to neural and retinal parenchyma, but also, as will be shown, have similarities in their contents of indoles.

The function of the pineal organ remains uncertain although it has been studied for over 200 years. Recent ultrastructural and neurophysiological studies of

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distal, eye-like portions of the pineal complex of organs in "lower" vertebrates clearly show photoreceptoral structures and activities. In living "lower" forms, such as lampreys, some amphibians, fish and reptiles, having such eye-like elements of the pineal, the structure is too small and simple to suggest utility in reception of a visual field, but may have potential as a dosimeter of incident radiation (83, 162). In some fossil vertebrates on the other hand the very large parietal foramen may have housed a pineal or third eye of more complex structure and more truly visual significance. In living "higher" vertebrates, and especially in mammals, pineal ultrastructure reveals no recognizable photoreceptors. Rather, the pineocytes or parenchymal cells appear epithelioid and secretory, and have close structural relations with sinusoids and capillaries. Furthermore, they receive innervation rather than give rise to nerve fibers to the brain. Histological and cytological characteristics are thus more in line with an endocrine nature than a sensory one.

Long known is the fact that mammalian pineals contain a material potent in blanching amphibian melanocytes (62, 78, 102). With the isolation and identification of this material as N-acetyl-5-methoxytryptamine, and named melatonin by Lerner and co-workers (95, 96, 97), the story of pineal indole biochemistry and physiology begins. These and subsequent studies suggesting a pineal specificity for melatonin and possibly some of its derivatives have stimulated anew hopes and experiments for demonstrating a pineal function in mammals.

It is not the purpose of this review to evaluate the claims for pineal functional significance. A general review of pineal physiology will be presented elsewhere (142). The primary concern here is the nature and significance of pineal indole derivatives. I believe that scrutiny of these materials in both their biological settings and their chemical and pharmacological characteristics will provide insights and will suggest new approaches for studying their biological significance and potential usefulness.

II. PINEAL INDOLE DERIVATIVES

A. Identification

1. 5-Hydroxy- and 5-methoxyindoles. Melatonin was isolated from beef pineals in 1958 by Lerner *et al.* (97) in search of the pineal factor that lightens amphibian skin by causing aggregation of melanin granules within the melanocytes. The extraction procedure and the characteristics of the isolated material in countercurrent distribution, by paper chromatography, and on staining with Ehrlich's reagent were described. The fluorescence and the ultraviolet absorption were also noted as typical of hydroxyindoles. The structure of the compound as N-acetyl-5-methoxytryptamine was demonstrated in the following year (95). O-Methylation at position 5 was suggested by failure of the compound to migrate as a cation in electrophoresis at pH 11, lack of an acid-base shift of ultraviolet absorption maxima, and increased lightening ability on the skin of *Rana pipiens* of many 5-methoxyindoles over their parent 5-hydroxyindoles. Identity of extracted melatonin was confirmed by comparisons with synthetic (164) N-acetyl-5-methoxytryptamine. The first demonstration of 5-methoxyindole-3-acetic acid (5-

MIAA) in animal tissue was in beef pineals (94) although it had been isolated previously from the urine of rats. Further details concerning the isolation and identification of melatonin and 5-MIAA were provided later (96). In this last report similar data were supplied describing the isolation and identification of 5-hydroxyindole-3-acetic acid (5-HIAA) from beef pineals.

The above well-supported findings along with the contemporaneous knowledge of 5-hydroxytryptamine (5-HT) levels in body fluids and tissues, and the conversion *in vivo* of 5-HT to N-acetylserotonin (105), the most actively utilized substrate for melatonin formation, favored the subsequent repeated demonstrations of 5-HT in mammalian pineals (63, 65, 108, 127, 146). Such demonstrations depended first on bioassay systems used previously with extracts of other tissues, and then on spectrofluorometry coupled with differential extractions. The specificity of these methods varies and will be considered in a section below.

The four above-mentioned compounds with the possible addition recently of 5-hydroxytryptophol and 5-methoxytryptophol are the only 5-hydroxy- and 5-methoxyindoles whose identification within pineal tissue has been documented in publication. Evidence that the pineal contains other indolic compounds including 5-hydroxytryptophol and 5-methoxytryptophol (Fig. 1) has been presented by McIsaac *et al.* (103a, 106). The reports that 5-hydroxytryptophol is a major metabolite of 5-HT in the rat, being excreted in the urine (88), and that platelets can convert 5-hydroxytryptophol to 5-methoxytryptophol *in vitro* (15) are of interest in relation to the possible presence and metabolism of these compounds in the pineal. It seems reasonable to assume that the metabolic intermediates (Fig. 1) 5-hydroxytryptophan (5-HTP), N-acetylserotonin, and 5-hydroxyindole acetaldehyde occur at least transiently in small amounts in mammalian pineals. The levels at least of the first two of these in rat pineals are too low for certain identification by extraction and fluorometry at this time (134). Doubtless the much needed application of more sensitive and specific methods, such as gas chromatography, may establish the presence in the pineal of these intermediates and possibly other hydroxy- and methoxyindoles.

2. Carbolines. Farrell and McIsaac (52) proposed that a mammalian pineal compound affecting aldosterone secretion by the adrenal gland and called by them "adrenoglomerulotropin" is a *beta*-carboline with the structure, 2,3,4,9-tetrahydro-6-methoxy-1-methyl-1H-pyrido[3,4–6]-indole (Fig. 1). The identification of "adrenoglomerulotropin" as this compound, more simply known as 10-methoxy tetrahydroharman, was based upon comparisons of fluorescence, and chromatographic and biological characteristics (52). Subsequent studies by the same group (72) suggest that the structure of "adrenoglomerulotropin" may be different from that which they had previously proposed.

Nevertheless, it was demonstrated by McIsaac (103) that 10-methoxy tetrahydroharman can be formed *in vivo* from 5-methoxytryptamine and acetaldehyde. It was also shown that, at least *in vitro*, a related compound, 10-methoxy harmalan, can be derived from serotonin (5-HT) in three steps (Fig. 1) (104). The recorded occurrence of other *beta*-carbolines in animal materials appears to be limited to a urinary metabolite of *alpha*-ethyltryptamine, with the tetrahydro-

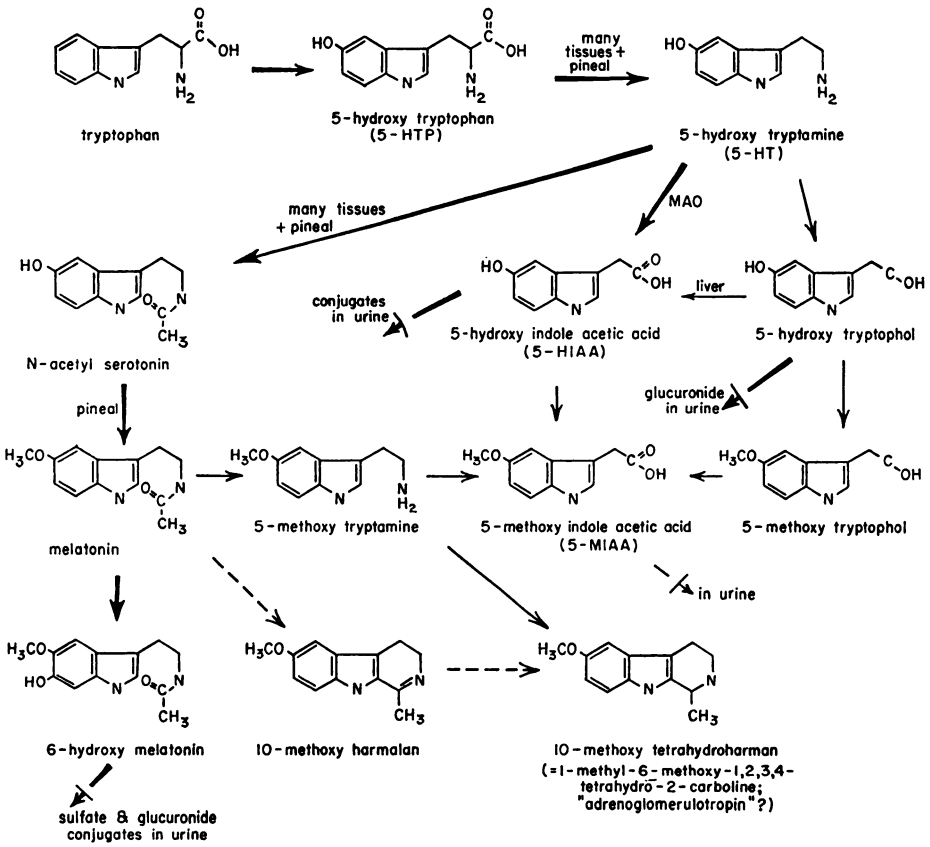

FIG. 1. Known and postulated pineal indole derivatives and their metabolic pathways. Sources and explanations are in the text.

beta-carboline structure, probably formed enzymatically in man by condensation with a formaldehyde precursor (72). The *beta*-carbolines possibly present in pineal and other organs seem especially significant in the light of the biological properties of some of these compounds as serotonin-antagonists, monoamine oxidase inhibitors, and stimulators of hallucinogenic and modified behavioral states [see also the review by Giarman and Freedman (64a)]. An excellent review of the biochemistry and pharmacology of these compounds has been provided by Heinzelman and Szmuszkovicz (72).

The formation and characteristics of *beta*-carbolines as cyclodehydrogenation products of 5-HT and related compounds have significance in pineal research far beyond what might be expected on the basis of the only slight knowledge of the natural occurrence of carbolines *in vivo* in pineal tissue. The conjugation of indoleamines with formaldehyde to produce strongly and distinctively fluorescent carboline products is the basis of the best histochemical method for localization of the parent amines in pineal and other tissues (see below). Furthermore, the

spontaneous formation of biologically active carbolines in solutions of tryptamines may possibly explain some of the discrepancies between bioassays and chemical measurements of 5-HT, and for some of the variable responses to pineal extracts, problems that will be discussed below.

3. Other possibilities. Surveys of indole derivatives and their chemical reactions are available (47, 163). These may sometimes provide clues and hypotheses of biological significance. Rather than pursue speculations along such lines here, I wish merely to comment briefly on two kinds of possible indole derivatives and fates in the pineal. One of these, melanin pigment, is of concern within the pineal as a pineal product but not necessarily an indole product and in the periphery as a possibly affected target of pineal hormonal activity. The occasional partial melanization of mammalian pineals seems to be a peculiarity of individual animals and is restricted to stromal areas without consistency either in exact anatomical distribution or in clearly defined physiological relationships (129, 138, 153, 154). The other kind of indole derivative for comment is that resulting from protein binding and incorporation. The pineal of rats contains electrophoretically distinctive proteins (128). It is hoped that soon we can ascertain by means of isotopically labeled amino acids and indoles whether pineal 5-HT and its natural products are constituents of pineal-specific proteins.

B. Localization

Localization of pineal hydroxyindoles, particularly 5-HT, has been based upon three techniques: fluorescence microscopy after treatment with formaldehyde, radioautography following uptake of isotopes, and electron microscopy of granules and vesicles thought to contain "biogenic amines." Of the available histochemical methods for 5-HT in mammalian tissues, the fluorescence method perfected by Falck (50) is the most sensitive. With the possible exception of a recent report of staining pineal cytoplasmic granules with combined chromaffin and argentaffin reactions (45), the fluorescence method is the only one that has shown by light microscopy "biogenic amines" in pineal organs. It should be noted, however, that histochemistry of 5-HT has had a long history and has been most frequently and productively studied in the enterochromaffin cell system of the gastrointestinal tract and its diverticula. Several reviews of this field are available (49, 61, 119). In the Falck technique, frozen-dried tissues are exposed to formaldehyde gas at 80°, causing the formation of intensely fluorescent products, from catecholamines, 6,7-dihydroxy-3,4-dihydroisoquinolines with green fluorescence, and from 5-HT, 6-hydroxy-3,4-dihydro-*beta*-carboline with yellow fluorescence (18, 33, 50). It is claimed that melatonin and tertiary amines do not give significant fluorescence following this procedure (37, 114). Besides 5-HT, the compounds 5-HTP (5-hydroxytryptophan) and 5-methoxytryptamine are said to possess the molecular requirements for producing the yellow fluorescence with formaldehyde, apparently characteristic of such tryptamine derivatives (115).

Species differences have been reported in pineal localization of yellow fluorescence due to 5-HT and related indoleamines (18, 19, 51, 115). Varying numbers of the parenchymal cells have yellow fluorescent cytoplasm in most of the species

examined. In a few, some fine nerve fibers also fluoresce. It seems probable that these differences are quantitative rather than qualitative, since the pineals of sheep and cattle do contain small amounts of 5-HT even though they show no yellow fluorescence. A number of interesting observations by Owman (115) on details and intrapineal localizations of fluorescent fibers and cells of different mammals merit further studies, to test the constancy of the differences and to see to what extent they may be modified pharmacologically. The distribution of fluorescent monoamines in the rat pineal has been studied also by Csillik (35), who stated that the "grapelike endings" of the pinealocyte processes in intimate contact with small blood vessels showed the most intense fluorescence. Differences in the localizations claimed by Csillik and Owman in the normal as well as the pharmacologically treated rat pineal are probably due to the frequent difficulty of distinguishing pinealocyte processes from the fine nerve fibers in the same regions. Thus, in contrast with Owman (115), Csillik reported complete disappearance of fluorescent material from the perivascular endings of the pinealocyte processes after reserpine. Csillik identified the "grapelike endings" of the pinealocyte processes with the "plurivesicular secretory processes" of De Robertis and Pellegrino de Iraldi (41, 42) as visualized in electron micrographs. The latter authors subsequently retracted the identification of these processes as parts of the parenchymal cells or pinealocytes, and now interpret them as consisting solely of nerves and nerve endings (122, 124).

Published observations on the pineal's fluorescent products of 5-HT do not as yet provide clear evidence of direct involvement of such indole derivatives in any secretory process. On the other hand, localization of synthesis of 5-HT in rat pineal parenchymal cells is suggested by the increase in yellow fluorescence of these cells following combined administration of a monoamine oxidase inhibitor and the serotonin precursor, 5-HTP (18). The yellow fluorescence of the pineal nerves in some species seems most likely to be due to uptake and storage of 5-HT in what are fundamentally noradrenergic, sympathetic, postganglionic fibers (114). This is suggested by the following observations which were largely made by, or modified from, Owman (114): (1) Yellow-fluorescent nerves are normally completely intrapineal. (2) After pinealectomy the remaining extraglandular stubs of the nerves consistently emit only green light, as do the cell bodies within the superior cervical ganglia. (3) Injection of a monoamine oxidase inhibitor and 5-HT produces a yellow fluorescence in the pineal nerves outside the gland as well as an increase in the yellow fluorescence in the intrapineal fibers. (4) Adrenergic nerves growing into pineal tissue transplanted to the anterior chamber of the eye fluoresce a yellow color in those segments lying within yellow-fluorescent transplanted tissue. (5) Treatment with *alpha*-methyl-*m*-tyrosine abolishes the fluorescence in the extraglandular parts of the pineal nerves with little change in the intrapineal parts. *Alpha*-methyl-*m*-tyrosine *in vivo*, as studied with brain tissue, is known to deplete noradrenaline through displacement by its own decarboxylation products, while levels of tissue 5-HT are maintained or are depressed only transiently (25, 34, 59, 74). (6) Two inhibitors (NSD-1015 and Ro 4-4602) of pineal decarboxylase cause the fluorescence in the pineal parenchyma, but not that in

the nerves, to disappear completely within 1 hour. (7) Fading of the labile 5-HT fluorophore during continuous exposure to ultraviolet light reveals the persistence of the less labile green fluorescence of catecholamine derivative in the intrapineal nerves. The extraglandular segments of these nerves remain essentially unchanged in their green fluorescence. (8) During the first two postnatal weeks, when pineal 5-HT is absent or meager, the intrapineal nerves have a green fluorescence.

Localization of radioactivity within rat pineals 30 minutes after slow intravenous infusion of *dl*-noradrenaline-7-H^3 has provided evidence also for differences in activity between parenchyma and nerve fibers. Utilizing thin tissue sections for both electron microscopy and autoradiography, Wolfe *et al.* (185) showed that grain concentrations due to radioactivity occurred in pineals only over nonmyelinated axons which contained granulated vesicles in the immediate vicinity of the grain aggregations. No grain concentrations were found over parenchymal cells, their perivascular processes nor over any other cells in the perivascular spaces. Cells with granules resembling those in chromaffin cells (126) were not seen. The attributing of the autoradiographic grain clusters specifically to H^3-noradrenaline was justified by previous work (182) demonstrating that over 90% of a tissue's radioactivity following injection of H^3-NE is due to that compound, whereas the contribution of the major metabolite, tritiated normetanephrine, is negligible. Descriptions of pineal ultrastructure have repeatedly noted cytoplasmic vesicles and granulated vesicles (2, 42, 109, 121, 185). Early suggestions as to the cellular relations and chemical contents of these were uncertain, lacking the benefits of correlated cytochemical, autoradiographic or fractionation studies. Although treatments with drugs sometimes produce changes in the pineal vesicles of diverse sorts as seen in electron micrographs, the localizations of indoleamines in relation to the vesicles are still obscure (41).

C. Problems of separation and measurement

1. Stability. Evaluation of the accuracy of published measurements of pineal indoleamines is difficult without consideration of the lability of the compounds and the need for adequate tests of their recovery or survival in assay systems. Oxidation of 5-HT by O_2 and other agents under certain conditions can lead to the formation of melanin-like pigments, and various intermediates including a dimer, resulting from dehydrogenative coupling of two 5-HT molecules and identical to a product formed in oxidation of 5-HT by human serum oxidase (46). Stability of 5-HT and related compounds is usually greater in weak acids than in alkaline or strongly acidic solutions. Some kinds of tissue oxidases, such as cytochrome oxidase (77) and monoamine oxidase, may convert the indoleamines to 5-hydroxyindole-3-acetic acid (5-HIAA) or related products *in vivo* and *in vitro* (69). Yields of 5-HT and 5-HTP in such circumstances may be greatly improved by the previous injection or addition of a monoamine oxidase inhibitor. Oxidation of 5-hydroxy- and 5-methoxyindoles in ultraviolet light, as during spectrophotofluorometric analysis, can be lessened by inclusion of ascorbic acid. Spraying of chromatograph plates or papers with a weak ascorbic acid solution in

methanol before runs with these compounds also increases recoveries, as does maintenance of darkness, low temperature and an atmosphere of N_2 (143, 144).

Investigation of indole derivatives in mammalian tissues and body fluids may be complicated by the postmortem formation of degradation products. The converse situation may also impose problems of interpretation in cold-blooded animals, since some of the metabolic intermediates that occur only fleetingly in mammalian tissues, may be present at detectable levels in those of lower vertebrates. One example is 5-HTP, which is normally undetectable in mammalian tissues, including brain and pineal (143, 172), decarboxylating 5-HTP to 5-HT. Nevertheless, it can be measured in lizard brains and is retained at higher levels there when the animals are kept cold (183).

2. Extractions. Many different procedures have been used for extracting indoles from tissues and body fluids. Reviews are available concerning many of these (152, 173). A set of procedures for differential extractions of pineal 5-hydroxy- and 5-methoxyindoles has been presented (134). The specificity of such procedures of course has to be rechecked as we learn of the presence of additional compounds of this group within the pineal and other tissues. The use of acetone in homogenization and extraction of 5-HT has been frequent but can lead to low and variable recoveries (32, 173). Furthermore, the probability of inhibitor substances for bioassay systems being extracted or formed in acetone seems not to have been examined critically.

3. Bioassay. Reviews of bioassay systems for hydroxyindoles have been written (152, 167), but are concerned largely with measurements of 5-HT. The most sensitive and the most frequently used tissues for the bioassay of 5-HT are also stimulated by tryptamine and some related compounds, and may be inhibited by others (168, 173, 180). The system engaging a strip of rat fundic stomach wall was devised by Vane (175) and has been used most frequently for bioassay of pineal 5-HT. However, at least according to some investigators, more potent than 5-HT in causing a response are related compounds including tryptamine and 5-methoxytryptamine (17, 175). Although tryptamine occurs in brain and urine and methods are now available for its measurement (1, 73, 75, 76, 112), it and nonhydroxylated related indoles have not been demonstrated and probably not effectively sought in the pineal.

Of greater importance for pineal research is bioassay for melatonin. In this case, an available bioassay system is more specific as well as more sensitive than spectrophotofluorometry (144). The aggregation of melanin granules in the body melanophores of larval South African toads (*Xenopus laevis*) occurs in the presence of as little as 10^{-10} g of melatonin per ml of aquarium water (24, 144). Of 46 other indole derivatives and related compounds tested in this system, the few that caused any consistent melanophore response did so only in concentrations 10^3 to 10^5 times greater (144). In historical perspective it should be pointed out that assay of pineal extracts by means of amphibian melanophore response has been practiced for many years using different species, ages and body regions of the animals (23, 91, 110). All of these conditions concerning the kind, age and region of the test animals are important for the specificity and sensitivity of the response. In at least one amphibian species (*Hyla arborea*), melatonin as well as

5-HT and epinephrine has no significant effect on the melanophores, although norepinephrine does cause melanophore contraction or skin-lightening (43).

4. Chemical assay. Chemical methods for detecting and measuring indole derivatives in tissue extracts and body fluids have been reviewed (152, 173) and are being presented in ever increasing number, sensitivity and specificity. It is beyond the scope and purpose of this review to consider these. It is important nonetheless, to note some of the characteristics of the most frequently used chemical assay system for 5-hydroxy- and 5-methoxyindoles. This system employs spectrofluorometry and owes its specificity to differential extraction from tissues and to the activation and fluorescence characteristics of the compounds to be measured or of their derivatives. The fluorescence of 5-hydroxy- and 5-methoxyindoles at 540–550 mμ when in 3 N HCl and irradiated with ultraviolet light at 295 mμ is specific to these compounds. This was discovered in relation to 5-HT by Udenfriend *et al.* (171) and later perfected (22) and modified for routine assays (134, 173). Spectrofluorometry of these and other indoles has been reviewed in detail (92, 170). Increased accuracy in fluorometry of 5-HT in extracts can be obtained by measuring the fluorescence of the reaction product formed with ninhydrin (101, 161, 174). While the most frequent complaint with the spectrofluorometry of particular indoles has been specificity, the cogency of this argument is rapidly fading as techniques improve. Difficulties with inhibiting substances continue to appear to be less than in bioassay systems, particularly those bioassay systems employing muscle tissues for responses. Discrepancies in results from bioassay and fluorometry will be noted further in section E below.

D. Changes during development and aging

In rats 1 week old, pineal 5-HT concentration is about half that in adults, and adult levels are attained by 2 weeks of age (146). 5-HT is said to be not demonstrable histochemically, by the fluorescence method, in the rat pineal during the first 2 weeks of postnatal life (114). In a 24-hour-old goat pineal, 0.45 μg/g of 5-HT was measured by bioassay, whereas, in five adult females the average pineal concentration was 3.20 (range: 1.20 to 7.00 μg/g) (125). Further and more detailed examinations of developmental changes in pineal 5-HT would be of value in appraisals of the significance of both adult and earlier levels and in tracing metabolic changes during development.

Information on levels of other pineal indole derivatives and on the enzyme activities concerned in their metabolism is not yet available for embryonic or very young animals. At the other end of the age sequence, in human pineals 5-HT content bears no relation to age in the range 35 to 90 years (65), and the enzymes hydroxyindole O-methyl transferase (HIOMT) and monoamine oxidase are not significantly lower in pineals of aged subjects even when calcification has occurred in the organ (186).

E. Species differences

1. 5-Hydroxytryptamine. When measurements of pineal 5-HT in a species have been obtained both by fluorometry and bioassay, the values from the former are much higher and are probably more accurate. Thus by acetone extraction, incu-

bation with chymotrypsin to "inactivate" polypeptides, and bioassay with the rat stomach strip a mean adult rat pineal 5-HT concentration of 11.1 μg/g was obtained, or 14.5 ng per whole pineal (123). By differential extractions and fluorometry daytime values for the untreated rat pineal 5-HT concentration have been repeatedly shown to be 5 to 10 times greater (19, 54, 133, 146). A range of 80 to 300 ng per whole pineal or 100 to 350 ng/mg which I published earlier (134) was of midday values of pharmacologically treated as well as of untreated rats, and was not corrected for the nonindole base part of the molecule. Values for pineal 5-HT concentration in rhesus monkeys (*Macaca mulatta*) show the same kind and degree of difference, 3.28 (1.20 to 10.00) μg/g being recorded by bioassays (65), and 40.26 μg/g (143) by fluorometry. By bioassay, human pineals are similar to those of rhesus monkeys in their 5-HT concentration, 4.94 ± 1.99 μg/g (= mean ± standard error of the mean; range = 0.36 to 22.82) (65).

5-HT occurs in the pineals of all vertebrates examined for this constituent. Among the species of reptiles, birds and mammals concerned in these determinations, there are as yet no evident phylogenetic or adaptive trends in pineal 5-HT content (19, 115, 125, 141, 143, 145, 147). But, as will be discussed later, diurnal changes and dietary and physiological factors may well mask or obscure such trends in the data at hand. Although fluorescence microscopy fails to show 5-HT in pineals of sheep and cattle (115), it has been detected in them by chemical means [sheep: 5.75 ± 0.66 μg/g (143)] and bioassay [steers: 0.40 μg/g (range: 0.20 to 0.63) (64)].

2. Metabolites of 5-HT. Scant data on the comparative levels of metabolites of 5-HT in pineals have been presented. As others have discovered in mammalian brain, I have found, in both mammalian pineal and brain, 5-HTP to be below detectable levels (134, 143), presumably because it is rapidly decarboxylated to 5-HT. As noted earlier, it is possible that in pineals of poikilotherms 5-HTP may occur in greater concentrations. 5-Hydroxyindole-3-acetic acid (5-HIAA), the main derivative of 5-HT with the most general occurrence in the mammalian body, has been measured in the pineals of cattle (2 μg/g) (96), rats (4 to 18 ng/pineal) (132, 136), kangaroos (0.86 to 8.77 μg/g) (145), and domestic pigeons (4 to 12 ng/pineal) (143). 5-Methoxyindole-3-acetic acid (5-MIAA) has been measured in pineals of cattle (2 μg/g) (96) and rats (0.98 ng/pineal) (136). Melatonin has been measured in pineals of cattle (0.2 μg/g) (14, 96), rats (1 to 5 ng/pineal) (127, 136), and kangaroos (0 to 7.46 μg/g) (145). It has been detected and estimated also (0.3 μg/g) in metastases of a human pinealoma by Wurtman *et al.* (28, 190). Published results from no species as yet show pineal concentrations of melatonin or other derivatives of 5-HT greater than those noted above.

F. Metabolism

The metabolism of 5-HT and related compounds has been surveyed in recent reviews (49, 72, 156, 169). Our focus is on those pathways and enzyme activities that are of especial significance in the metabolism of the known pineal indoles (Fig. 1). Tryptophan is the precursor of the 5-hydroxyindoles in mammals (172), and the hydroxylation of tryptophan to 5-HTP probably occurs to a minor ex-

tent in brain (55, 56, 57, 66, 67) and more significantly in intestinal mucosa (30, 49) and liver (156). It has been reported that notable tryptophan hydroxylase activity is not found in mammalian pineals (30, 63). Perhaps for the pineal, as has been suggested for the brain (56), 5-HTP is supplied in large part from other tissues *via* the circulation. However, there is no evidence for 5-HTP in the blood. In pineal, as in brain, administration of 5-HTP peripherally leads to increases in 5-HT, whereas administration of 5-HT itself in most cases does not (136, 137, 146). The conversion of 5-HTP to 5-HT is catalyzed by aromatic L-amino acid decarboxylase which also catalyzes the decarboxylation of 3,4-dihydroxyphenylalanine, phenylalanine, tyrosine, tryptophan and histidine (98). "5-HTP decarboxylase" is therefore merely one activity of this broadly active enzyme. It has been found in bovine, rat and quail pineals, and, in rats, it is more active in pineal than in liver or small intestine (63, 158).

The widespread occurrence of monoamine oxidase (MAO) and 5-HIAA in brain, and the poor penetration of the brain by systemically injected 5-HIAA have favored the view that MAO is primarily responsible for the metabolism of 5-HT, although the possibility of some 5-HIAA being formed by transamination and oxidation of 5-HTP is not to be ignored (156, 172). The occurrence of MAO (63) and 5-HIAA in pineal suggests that much of the pineal 5-HT may be metabolized by this route.

Greater attention and significance, nevertheless, have been attached to the pineal's N-acetylation and O-methylation of 5-HT to form melatonin. An enzyme which can acetylate 5-HT to N-acetylserotonin in the presence of an acetyl coenzyme A-generating system was found by Weissbach *et al.* (179) in rat liver and pineal, following discovery by McIsaac and Page (105) of an *in vivo* conversion of 5-HT to N-acetylserotonin. The discovery and studies of the enzyme facilitating methylation of N-acetylserotonin were by Axelrod and Weissbach (7, 8, 178) and opened one of the most productive and exciting leads in pineal research. This enzyme, hydroxyindole O-methyl transferase (HIOMT), as far as mammals are concerned, is limited to the pineal organ in its known distribution. It is not found in brain regions outside the immediate vicinity of the pineal, nor in any part of the peripheral nervous system. It is lacking from other organs, including liver, kidney, heart, lung, skin, testis, adenohypophysis, submandibular gland, adrenal, thyroid, and pancreas (5, 7, 8). It has been found in pineal organs of vertebrates from fish to man (6, 10, 141, 186) and in a metastatic parenchymatous human pinealoma (28, 190). N-Acetylserotonin is by far the best substrate for HIOMT, but O-methylation at much lower rates has been detected with bufotenine, 5-HIAA, N-methylserotonin and 5-HT (8). Like other methyl transferases, HIOMT requires adenosylmethionine (AMe) as methyl donor. AMe, or a system generating it, is present in the mammalian pineal but must be added to incubations of purified HIOMT (8). An assay system for tissue AMe has been described using pineal HIOMT (12). The properties and biochemistry of HIOMT have not received as much attention since the studies of Axelrod and Weissbach (8) as would be desirable. The presence of an essential sulfhydryl group in the enzyme was indicated by complete inhibition by 10^{-5} M *p*-chloromercuribenzoate and by slight

activation with high concentrations of glutathione and cysteine. Understanding of the metabolic fates of hydroxyindoles in the pineal would probably be aided by studies of the effects of inhibitors of HIOMT.

The fate and metabolism of melatonin administered to rats and mice were studied by two groups of investigators shortly after its discovery (85, 86, 89, 90). Unlike 5-HT, melatonin readily enters the brain from the blood. It also rapidly enters all other tissues examined, and is rapidly metabolized, none appearing unchanged in the urine (86). Its major pathway (Fig. 1) of metabolism is hydroxylation on position 6 followed by conjugation, primarily with sulfate (70 %) and to a minor extent (6 %) with glucuronic acid. An unidentified product (12 %) still possessing the 5-methoxy group and an acetylethylamine side-chain did not react with Ehrlich's reagent; it was therefore thought to result from either cleavage of the indole nucleus or substitution on position 2 of the indole (86). Less than 0.5 % of the administered melatonin is converted to 5-MIAA (86). 5-Methoxytryptamine has never been definitely demonstrated in the pineal gland, but its presence has been thought likely, especially after inhibition of MAO and through the agency of O-methylation of 5-HT (89). It has been shown that *in vitro* 5-HT with HIOMT and S-adenosylmethionine gives rise to a product with the characteristics of authentic 5-methoxytryptamine, but the rate of O-methylation of 5-HT is only one-tenth of that of N-acetylserotonin (7). The psychopharmacological properties of 5-methoxytryptamine and of other possible melatonin derivatives have provided incentive for investigations, but identifications of products found *in vivo* remain much desired.

A third pathway of metabolism of pineal 5-HT has been suggested by McIsaac *et al.* (106), leading through 5-hydroxytryptophol and 5-methoxytryptophol (Fig. 1). 5-Hydroxytryptophol in the form of a glucuronide is a major urinary metabolite of 5-HT in the rat, and rat liver *in vitro* can oxidize 5-hydroxytryptophol to 5-HIAA as well as conjugate it (88). When 5-methoxytryptophol is administered to rats 91 % is excreted in the urine within 24 hours, mostly as 5-MIAA (40). There is disagreement whether pineal 5-MIAA is more likely to be derived by this route or by O-methylation of 5-HIAA (8, 40).

G. Physiological changes

Fluctuations in mammalian pineal indole derivatives have been experimentally correlated with diet, environmental illumination, estrous cycle and innervation by sympathetic nerves. Nascent from the available data to be noted below is the implication of an interrelationship of the last three of these phenomena in regard to their effects on the pineal. However, it seems premature and potentially misleading to suggest at this time any one of these phenomena or systems for primacy in pineal physiology.

1. Dietary effects. It has been amply demonstrated that a tryptophan-deficient diet (192) or an increased intake of L-phenylalanine (36, 191) decreases the concentration of 5-HT in the brain, and that increased dietary L-tryptophan increases it (176). These and a number of related studies have been most concerned with the central factors and effects of phenylketonuria. I have shown that in

young rats a high tryptophan diet produces a greater increase of 5-HT (250 %) in pineal than in hypothalamus or as reported previously in whole brain (176), and that a high phenylalanine diet produces approximately equivalent depression (30 %) in 5-HT in both organs (135). Suggested mechanisms of the phenylalanine-depression of brain 5-HT, and possibly extensible to pineal 5-HT, include inhibition of the hydroxylation of tryptophan (13, 118), inhibition of the decarboxylation of 5-HTP (39), and competition with 5-HTP transport (107). The last of these seems the most likely from the evidence, although the effects of increased phenylalanine on the free amino acid pools of brain and liver cells are complex and do not appear interpretable on the sole basis of competition (26, 70). Pineal 5-HT concentration in simians was found to be not affected by prior ingestion of a food rich in 5-HT, namely bananas (65).

2. Light and photoperiod. Recently demonstrated yet repeatedly confirmed is the mammalian pineal's specific, but probably indirect, responsiveness to light and photoperiod. Even though no known photoreceptors occur in mammalian pineals, such structures are present and active in pineal organs of some lower vertebrates (83). Whether or not light does affect some activities in the mammalian pineal directly, it can penetrate to the intracranial regions of mammals (58). The pineal changes correlated with, or caused by, light and photoperiod might best be considered separately according to three different kinds of environmental or experimental conditions: (1) those in which over a period of a number of days the length of the daily light- or photoperiod is changed, simulating seasonal change in photoperiod, (2) those in which light or darkness is continuous for several days, and (3) those in which changes within the normal 24-hour, day–night cycle are studied or experimentally altered.

Experimental and quantitative changes in a mammalian pineal caused by photoperiod were first described in 1956 (130). The conclusion in this and subsequent studies that seasonally and experimentally mammalian pineal activity decreases with lengthening daily photoperiods over a period of a number of days is from cytological but still germane evidence, such as size and number of parenchymal nucleoli. On the other hand, experiments with rats in continuous light or darkness have included measurements of pineal indoles and related enzyme activities. Continuous light for either 4½ or 21½ weeks was shown by Quay and Halevy (146) to lower significantly pineal 5-HT as compared with daytime levels in controls in a normal daily light–dark cycle, but 5-HIAA concentration is not affected (132). Pineal 5-HTP decarboxylase activity is twice as great in rats kept in continuous light for 1 to 3 weeks as it is in those in constant darkness (157, 159). Pineal HIOMT activity, contrastingly, is much higher in rats in constant darkness for 6 days than it is in either continuously lighted or normal day–night cycling controls (9, 189). Rat pineal "melatonin-forming activity" measured previously by another, and admittedly less specific method, suggested possible increase following continuous light (132). Chicken pineal HIOMT in contradistinction to that of rat pineals is increased in light and decreased in darkness (10). In both species pineal MAO is not significantly affected by either continuous light or darkness (10, 189). In general, continuous light may be a harsh treatment and

may produce many changes in a mammal, especially in a normally nocturnal one such as the rat. In continuous light, the decrease in rat pineal glycogen, weight, lipid, succinic dehydrogenase activity, and respiration in the absence of exogenous substrates, and associated cytological changes are diagnostic of considerable depression of pineal metabolism (132).

The most profound known physiological changes in the pineal concentrations of 5-hydroxyindoles occur in association with, and in part in response to, the daily light and dark periods. I have shown that in rats, pineal concentration of 5-HT rises during the first part of each day to attain a maximum level 6 to 8 hours after the onset of light; promptly after the start of darkness a precipitous fall begins, reaching in 4 hours a value of only about one-ninth that of the daily maximum (133, 137). The nocturnal fall in rat pineal 5-HT can be delayed or inhibited by delaying the onset of darkness, but the early onset of darkness does not invoke a precocious decline. Beginning the daily light period earlier than usual causes a precocious increase in 5-HT, but the midday level is not modified, and delay in onset of light does not inhibit the morning rise in 5-HT. The fact that daytime levels of rat pineal 5-HT are much greater than nocturnal ones has been confirmed (19, 54, 161). The magnitude of the daily change in rat pineal 5-HT is much greater than that in brain (10, 137) or in cerebral cortical regions (20 to 28 % daily changes) (139); and the association of the pineal changes with illumination is much closer. At least one measurement of pineal composition, the ethanol-soluble fraction (mostly but not entirely lipid), showing a somewhat similar daily cycle, rapidly loses its daily rhythm in continuous light, although the animals' free-running, circadian rhythm in locomotor activity persists (140). This suggests that this 24-hour rhythm in pineal composition is not a result of peripheral activity, and that it is circadian only in terms of the first of Halberg's (71) two original categories of 24-hour periodicities: (1) those that are frequency synchronized with acceptable environmental schedules, and (2) those that are "free-running" from the local time scale, with periods slightly yet consistently different from 24 hours, as in supposedly constant environments.

The daily rhythms in pineal 5-HIAA and melatonin have also been described in the rat (136). 5-HIAA rises during the day from a nocturnal minimum of 4 ng per pineal to an early afternoon maximum of 18 ng per pineal, occurring a little later than the peak in 5-HT. At onset of darkness, pineal 5-HIAA falls abruptly, as does 5-HT, but melatonin rises at this time. These daily rhythms correlated with light and darkness, together with the effects of light and darkness on pineal enzyme activities, and other knowledge reviewed above, suggest that during the early part of the day, and stimulated by light, the pineal parenchymal cells synthesize and store 5-HT. On the beginning of darkness, conversion of 5-HT to 5-HIAA decreases as formation of melatonin increases. Since, however, the melatonin level is never great (1 to 3 ng per pineal) this product must be either rapidly released or rapidly combined with, or metabolized to, other materials (136), if it is indeed synthesized in greater amounts.

3. Neural pathways. Following the discovery of the light-induced and circadian changes in pineal indoles (133, 137, 147) neural pathways for the regulation or

mediation of the changes were sought. Since the best available evidence of both neurohistology and biochemistry suggests that the pineal of rats and probably many other mammals is innervated solely or dominantly from the superior cervical ganglia (3), the pineal nerves, the so-called, and bilateral, nervi conarii, from this source, have been thought to be the most likely final common pathway. Bilateral superior cervical ganglionectomy or sympathectomy has been reported not only to cause increase in 5-HTP decarboxylase activity in the rat pineal (120, 157), but also to block the increase in this enzyme activity following continuous light (159). This last finding contradicts the hypothesis (120) that the nerves are simply inhibitory in their action on pineal 5-HTP decarboxylase. 5-HT of such denervated rat pineals, presumably measured during the day, shows a decrease of over 50%, which has been attributed, with the support of fluorescence microscopy, to loss of 5-HT from the pineal nerve fibers (18, 19, 54, 123). However, when pineal 5-HT levels of denervated and sham-operated rats are compared at night, 5-HT is significantly higher in the former (160). These and other experimental findings on pineal composition and enzyme activities are difficult to interpret without thorough knowledge of the daily rhythms of both control and modified animals. The increase in HIOMT associated with darkness (188) as well as the daily rhythm in 5-HT appears to be lost after denervation (160). Possible mechanisms for these changes may be illuminated further by pharmacological studies.

Rats which had their optic tracts cut 4½ weeks previously had lower pineal concentrations of 5-HT during the daytime and this content in such animals was not significantly affected by continuous light (146). Nevertheless, when pineal 5-HT contents were measured in rats blinded by enucleation, another group of investigators found the maintenance of a day *versus* night difference comparable to that of controls (160). Pineal 5-HTP decarboxylase activity was found to be lower in such enucleated rats and to be unresponsive to continuous light (159). HIOMT activity also was unresponsive to continuous light in bilaterally enucleated animals, but still responded in unilaterally enucleated ones (188). Thus, although incomplete and not entirely consistent, these results do show the eyes and the superior cervical sympathetic pathway to be important factors in the mediation of pineal changes induced by light. The eyes and the superior cervical sympathetic innervation are both necessary for the effect of continuous light to occur. But the difference between day and night levels of pineal 5-HT persists in blinded animals as well as in normal ones in continuous darkness, suggesting an endogenous component in the circadian rhythm in pineal 5-HT (161). This, however, requires the integrity of the superior cervical sympathetic innervation and the latter's connection with the central nervous system (161). Available evidence, therefore, indicates that the mammalian pineal's circadian rhythm in 5-HT depends on basic circadian and resetting mechanisms in the brain itself.

4. Estrous cycle. Because repeatedly a postulated function of the mammalian pineal as an endocrine gland holds the gonads or gonadotropic systems as targets (84), it is well to note any changes in pineal composition associated with phases of the reproductive cycle. Pineals of female rats do seem to have greater nocturnal concentrations of 5-HT and possibly of melatonin and greater early morning

levels of 5-HIAA on the day of proestrus than during the previous day (133, 136). Early morning 5-HT content is lower following the day and night of proestrus than subsequently. Attributing these values to any particular cause-and-effect mechanism related to endocrine or reproductive activities cannot be supported at this time. Nor do these values provide evidence favoring any postulated gonadal effects of pineal activity.

H. Changes induced by drugs

Sympathetic dominance and clues to mechanisms of daily changes in rat pineals were indicated in an earlier study of the effects of drugs and conditions on pineal succinic dehydrogenase activity (SDA) (131). Additional recent information provides further insights but is still not sufficient for a detailed hypothesis on the mechanisms behind the daily cycles in pineal metabolism and the different pharmacologic responses of pineal SDA. The rat pineal's SDA follows a daily rhythm with decrease during the morning light period to a midday minimum, and a subsequent rise to a nocturnal maximum (143). Rats that are chronically stressed by olfactory deprivation (olfactory bulbs transected) or continuous light (31, 132) had lower midday pineal SDA; many conditions, hormone and drug treatments of a nonsympathomimetic nature, failed to modify pineal midday SDA (131). Injection of norepinephrine or sympathomimetic amines in the early morning had different effects on midday SDA a few hours later, depending upon the previous treatment of the animals. Animals that had been recently moved to other cages or that had been stressed had increased pineal midday SDA and those that had been undisturbed for 3 days or more usually had decreased midday SDA following sympathomimetic agents. Administration of a MAO inhibitor at the time of moving the rats to other cages during the evening before the day of sacrifice led to increased pineal SDA, but a sympathomimetic amine given on the following morning then caused decrease. Opposite effects were obtained by administration of Dibenamine in the evening, causing decrease, and by following this with sympathomimetic amines, causing increased pineal SDA. Thus the direction of pineal response to sympathetic agents depended upon the preceding state of the organ in the context of sympathetic activity and mediation of environmental and pharmacological factors.

The study of pineal responses to drugs is of potential importance in the analysis of cause-and-effect relationships in the pineal's normal daily cycles, indolic contents and possible functional activities. But this subject is still in such a fragmentary state that little of a definitive nature can be said at this time, beyond what has been noted above already, in the paragraphs on localization, metabolism and pharmacological changes. Major sources of complexity, and potential confusion, in studies of the pineal's pharmacological responses, which should be considered in the design of experiments, include (a) species differences in most aspects of the daily pineal rhythms (143), (b) effects of history and immediate state of the animal, and (c) multiple possible sites responding to administered compounds and contributing in some way to the mediation of effects on the pineal.

III. COMPARISON OF THE PINEAL WITH BRAIN AND RETINA

Pineal tissue resembles that of the nervous system and retina in its early embryological derivation and in its content and metabolism of 5-hydroxyindoles. The structural and chemical similarities between these tissues are faint in mammals, but are increasingly apparent as one descends the vertebrate series of animals. 5-Hydroxytryptamine occurs in all of these tissues, and, although quantitatively greatest in pineal, it is much greater in brains of many lower vertebrates than it is in brains of mammals (143, 147, 181). Most of the information on retinal indoles and indoles of lower vertebrate brains is scanty and, aside from 5-HT, is of a preliminary or unpublished nature. Nevertheless, the previously considered most pineal-specific feature in indole metabolism, HIOMT, is clearly present in retinas and brains of lower vertebrates as well as in their pineals. Retinal HIOMT occurs in some species of fish, amphibians, reptiles and birds (141), and brain HIOMT occurs at least in some amphibians (6) and probably in some fish as well. Since little is known about the substrate specificities, activity/concentration relationships, and optimal conditions for demonstrating HIOMT in these tissues, it is premature to make many or close comparisons. The results do suggest, however, that at least in submammalian vertebrates consideration of potential sources of melatonin in physiology of melanophore responses, or of other systems, should include brain and retina as well as pineal. In monkeys (*Macaca mulatta*) and rats I have failed to find any retinal HIOMT activity. The rats examined included normal embryos and fetuses as well as pinealectomized adults (143).

IV. PHYSIOLOGICAL SIGNIFICANCE OF PINEAL INDOLES

A. Intramural functions

The possibility of intrapineal or intramural functions for the pineal indole derivatives has received little if any notice. This possibility warrants attention for several reasons. (a) The mammalian pineal's content of indole derivatives, insofar as known, seems low if these materials are hormones and hormone precursors, and if the pineal is compared with proven endocrine glands. For example, the maximum estimated pineal concentrations of "biogenic amines," both catecholamines and indoleamines, are $\frac{1}{100}$ to $\frac{1}{10}$ as great as the total catecholamine content (1500–8000 μg/g) (181a) of the adrenal medulla (142). (b) The demonstrated uptake of 5-HT by intrapineal sympathetic nerve fibers and endings may be a physiologically significant event in the pineal's daily cycles, and may be a necessary link in the normal physiological activity of the pineal 5-HT. (c) The functions of 5-HT in brain and retina are probably intramural also, although at present unclear. In brain, 5-HT is most concentrated within certain midline brainstem neurons that appear, for other reasons as well, to be primitive and serotonergic (37). Localization of retinal 5-HT is unknown. Relation of 5-HT to intraocular pressure has been suggested but is debated as a physiological mechanism (68, 151, 155). (d) Recent electron micrographs of the rat pineal show structures connecting some of the parenchymal cells to each other and resembling

synaptic contacts (77, 184). Although structurally, and presumably functionally highly modified, some of these parenchymal cells may still bear structural and metabolic characteristics, as well as cellular interrelationships, of primitive neuronal and photoreceptive cells. Serotonergic transmission or modulation of transmission between such cells is conceivable.

B. Proposed hormones

Demonstrated and hypothesized pineal indole derivatives that have been suggested as possible hormones include: serotonin (5-HT), melatonin, 5-methoxytryptophol and a carboline-like 10-methoxy tetrahydroharman. The many diverse effects reported following administration of pineal extracts to mammals are too numerous to review here. It seems likely, however, that some of these are nonspecific effects of nonhormonal contents of homogenates and extracts of pineal tissue. 5-HT as a nonhormonal content of the pineal may be suspected in some of these instances (133). 5-HT as a product in mammalian blood, however, has its primary sites of synthesis and storage in the gastrointestinal system, and blood 5-HT, largely bound to platelets, is of this origin (48, 49). A study of serum 5-HT in diseases of the nervous system included two cases of pineal tumors, but significant changes in serum 5-HT were only suggested in certain other patients, those with hypothalamic and limbic lesions (87). Direct effects of 5-HT on the mammalian adrenal cortex have been noted especially by Rosenkrantz and Connors (29, 150). Of particular significance in pineal research is the finding by Jouan *et al.* (31, 80, 82) of stimulation of adrenal aldosterone secretion by 5-HT and that this activity is qualitatively identical to that of pineal extracts.

A material from the pineal region of the beef brain has been proposed by Farrell and associates (53, 165) to have hormonal activity in the regulation of the adrenal zona glomerulosa and its secretion of aldosterone. The proposed hormone, "adrenoglomerulotropin," as remarked earlier, was thought by Farrell to be a carboline resembling 10-methoxy tetrahydroharman. The absence of permanent and specific effects of pineal ablation on aldosterone production (20, 21, 38, 81) and negative results with Farrell's 10-methoxy tetrahydroharman in other laboratories (82, 111) have presented difficulties for this theory. The concurrent substantiation of the importance of the renin-angiotensin system (119a) in regulation of aldosterone secretion also overshadows the "adrenoglomerulotropin" hypothesis. Subsequently the latter, descending deeper within the pineal region of the brain, has been sustained in modified form by evidence that the subcommissural organ may contribute to regulation of aldosterone secretion (116, 117). Indole derivatives have not yet been demonstrated in this organ.

Melatonin's activity in lightening the melanophores of lower vertebrates has been discussed above. A theory of the physiological significance of melatonin as a body-lightening hormone from the pineal in larval amphibians has been proposed and experimentally supported by Bagnara (11). The fact that melatonin-forming ability also resides in the eyes and brains of some amphibians and other lower vertebrates (6, 141) does not negate the possibility of pineal function as a secretor of melatonin in perhaps some species or developmental stages. A direct

humoral regulation of melanophores by the retina's release of melatonin certainly seems, however, a possibility of biological efficiency deserving experimental examination. A recent claim of therapeutic efficacy of melatonin on canine melanosis (148) merits further study. Lack of effect of subcutaneous injections, daily for 1 month to guinea pigs, on skin pigmentation has also been reported (156a).

Running through the fabric of pineal physiology from its earliest to its most recent studies is the recurring thread of claimed antigonadal or antigonadotropic activity (84). Most recently pineal melatonin (27, 187) and 5-methoxytryptophol (106) have been invoked as the significant hormones. Space here permits neither satisfactory review and evaluation of the pineal's possible relation to gonadotropic and gonadal activities, nor a critique of pineal 5-methoxyindoles as the effective hormones. This will be done elsewhere (142). In all fairness it must be recognized that the beautiful and well-supported edifice of hypothalamic and hypophyseal regulation of gonadotropic activities casts a considerable shadow over discourse on possible pineal contributions. Although there have been recent negative reports on the effects both of pinealectomy (79) and melatonin administration (4, 166) on reproductive organs and functions, further quantitative and carefully controlled studies are still appropriate.

The O-methyl and N-acetyl groups of melatonin are required for its effect on skin-lightening, but the presence of the latter group inactivates melatonin, in comparison with many of its congeners, for affecting smooth muscle, blood pressure and gross behavioral signs (60). Nevertheless, recent studies have shown effects with melatonin on brain activities (99, 149). The specificity and significance of these, and reported effects on the thyroid gland (16), are difficult to evaluate at this time.

V. CONCLUSIONS

Resurgence of interest in the mammalian pineal organ and recent successful experimental linking of its activity with the sympathetic system and environmental lighting, have been brought about in part by the pineal's content and metabolism of 5-hydroxy- and 5-methoxyindoles. Methods are being applied for exacting precise information concerning localization and quantification of these compounds and their derivatives within pineal tissue. Much remains to be learned, however, concerning the identity of postulated and unknown, metabolically active pineal indoles and their derivatives. Predilection, perhaps premature, for the hormonal possibilities of these derivatives has led to a neglect of the possibilities of strictly intramural or intrapineal functions for these compounds. The latter seem most promising in the light of recent ultrastructural, histochemical, biochemical and comparative studies. The likelihood of a basic, primitive similarity between pineal, brain and retinal tissue in their metabolites and metabolism of 5-hydroxyindoles also deserves study.

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