THE PINEAL GLAND

The function of this small organ near the center of the mammalian brain has long been a mystery. Recent studies indicate that it is a "biological clock" that regulates the activity of the sex glands

by Richard J. Wurtman and Julius Axelrod

Buried nearly in the center of the brain in any mammal is a small white structure, shaped somewhat like a pinecone, called the pineal body. In man this organ is roughly a quarter of an inch long and weighs about a tenth of a gram. The function of the pineal body has never been clearly understood. Now that the role of the thymus gland in establishing the body's immunological defenses has been demonstrated, the pineal has become perhaps the last great mystery in the physiology of mammalian organs. This mystery may be nearing a solution: studies conducted within the past few years indicate that the pineal is an intricate and sensitive "biological clock," converting cyclic nervous activity generated by light in the environment into endocrine—that is, hormonal—information. It is not yet certain what physiological processes depend on the pineal clock for cues, but the evidence at hand suggests that the pineal participates in some way in the regulation of the gonads, or sex glands.

A Fourth Neuroendocrine Transducer

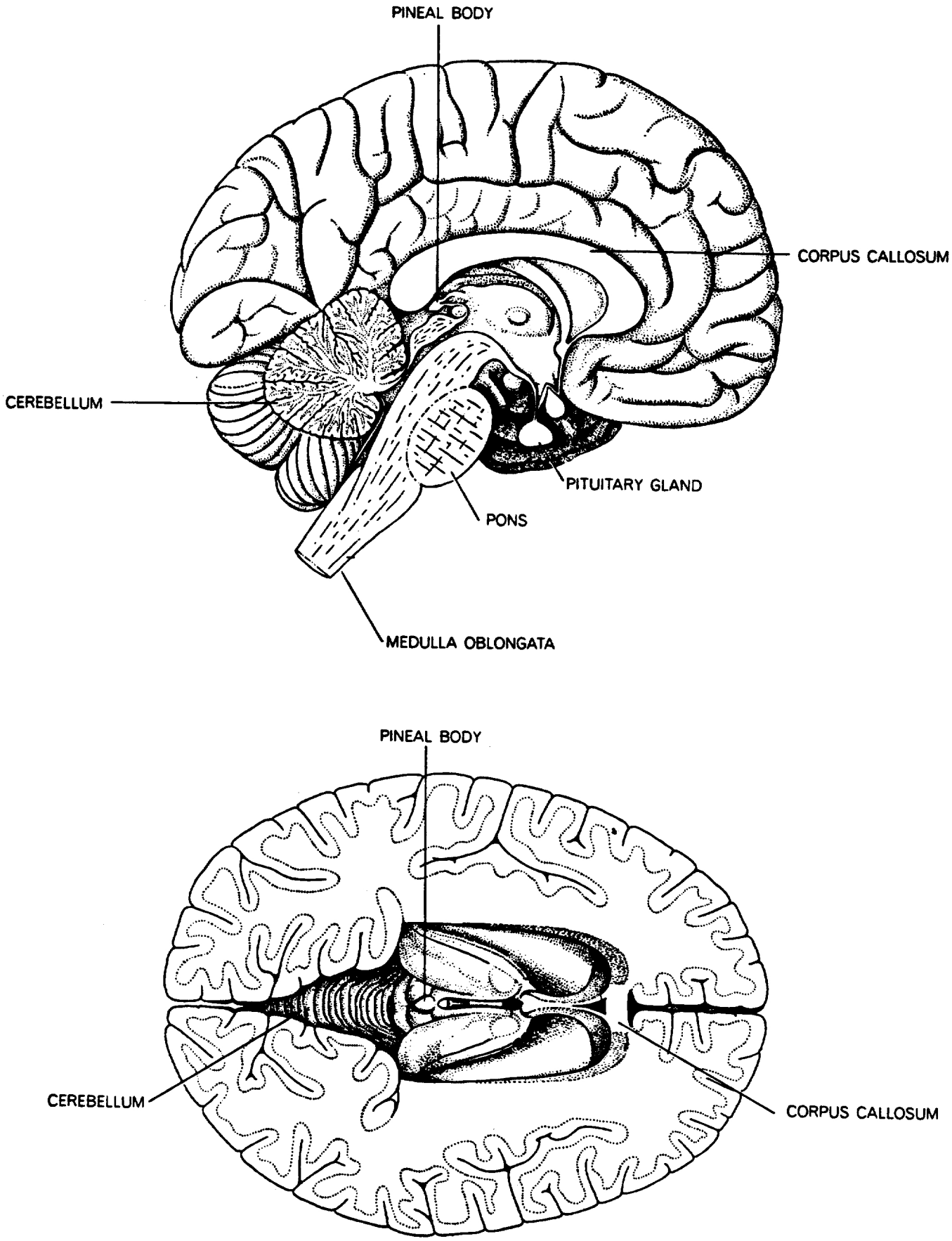
Until quite recently most investigators thought that the mammalian pineal was simply a vestige of a primitive light-sensing organ: the "third eye" found in certain cold-blooded vertebrates such as the frog. Other workers, noting the precocious sexual development of some young boys with pineal tumors, had proposed that in mammals the pineal was a gland. When the standard endocrine tests were applied to determine the possible glandular function of the pineal, however, the results varied so much from experiment to experiment that few positive conclusions seemed justified. Removal of the pineal in young female rats was frequently followed by an enlargement of the ovaries, but the microscopic appearance of the ovaries did not change consistently, and replacement of the extirpated pineal by transplantation seemed to have little or no physiological effect. Most experimental animals could survive the loss of the pineal body with no major change in appearance or function.

In retrospect much of the difficulty early workers had in exploring and defining the glandular function of the pineal arose from limitations in the traditional concept of an endocrine organ. Glands were once thought to be entirely dependent on substances in the bloodstream both for their own control and for their effects on the rest of the body: glands secreted hormones into the blood and were themselves regulated by other hormones, which were delivered to them by the circulation. The secretory activity of a gland was thought to be maintained at a fairly constant level by homeostatic mechanisms: as the level of a particular hormone in the bloodstream rose, the gland invariably responded by decreasing its secretion of that hormone; when the level of the hormone fell, the gland increased its secretion.

In the past two decades this concept of how the endocrine system works has proved inadequate to explain several kinds of glandular response, including changes in hormone secretion brought about by changes in the external environment and also regular cyclic changes in the secretion of certain hormones (for example, the hormones responsible for the menstrual cycle and the steroid hormones that are produced on a daily cycle by the adrenal gland). Out of the realization that these and other endocrine responses must depend in some way on interactions between the glands and the nervous system the new discipline of neuroendocrinology has developed.

In recent years much attention has centered on the problem of locating the nervous structures that participate in the control of glandular function. It has been known for some time that special types of organs would be needed to "transduce" neural information into endocrine information. Nervous tissue is specialized to receive and transmit information directly from cell to cell; according to the traditional view, glands are controlled by substances in the bloodstream and dispatch their messages to target organs by the secretion of hormones into the bloodstream. In order to transmit information from the nervous system to an endocrine organ a hypothetical "neuroendocrine transducer" would require some of the special characteristics of both neural and endocrine tissue. It should respond to substances (called neurohumors) released locally from nerve endings, and it should contain the biochemical machinery necessary for synthesizing a hormone and releasing it into the bloodstream. Three such neurosecretory systems have so far been identified. They are (1) the hypothalamus–posterior-pituitary system, which secretes the antidiuretic hormone and oxytocin, a hormone that causes the uterus to contract during labor; (2) the pituitary-releasing-factor system, also located in the hypothalamus, which secretes polypeptides that control the function of the pituitary gland, and (3) the adrenal medulla, whose cells respond to a nervous input by releasing adrenaline into the bloodstream.

The advent of neuroendocrinology has provided a conceptual framework



TWO VIEWS of the human brain reveal the central position of the pineal body. Section at top is cut in the median sagittal plane and is viewed from the side. Section at bottom is cut in a horizontal plane and is viewed from above; an additional excision has been made in this view to reveal the region immediately surrounding the pineal. In mammals the pineal is the only unpaired midline organ in the brain. The name "pineal" comes from the organ's resemblance to a pinecone, the Latin equivalent of which is *pinea*.

that has been most helpful in characterizing the role of the pineal gland. On the basis of recent studies conducted by the authors and their colleagues at the National Institute of Mental Health, as well as by investigators at other institutions, it now appears that the pineal is not a gland in the traditional sense but is a fourth neuroendocrine transducer; it is a gland that converts a nervous input into a hormonal output.

A Prophetic Formulation

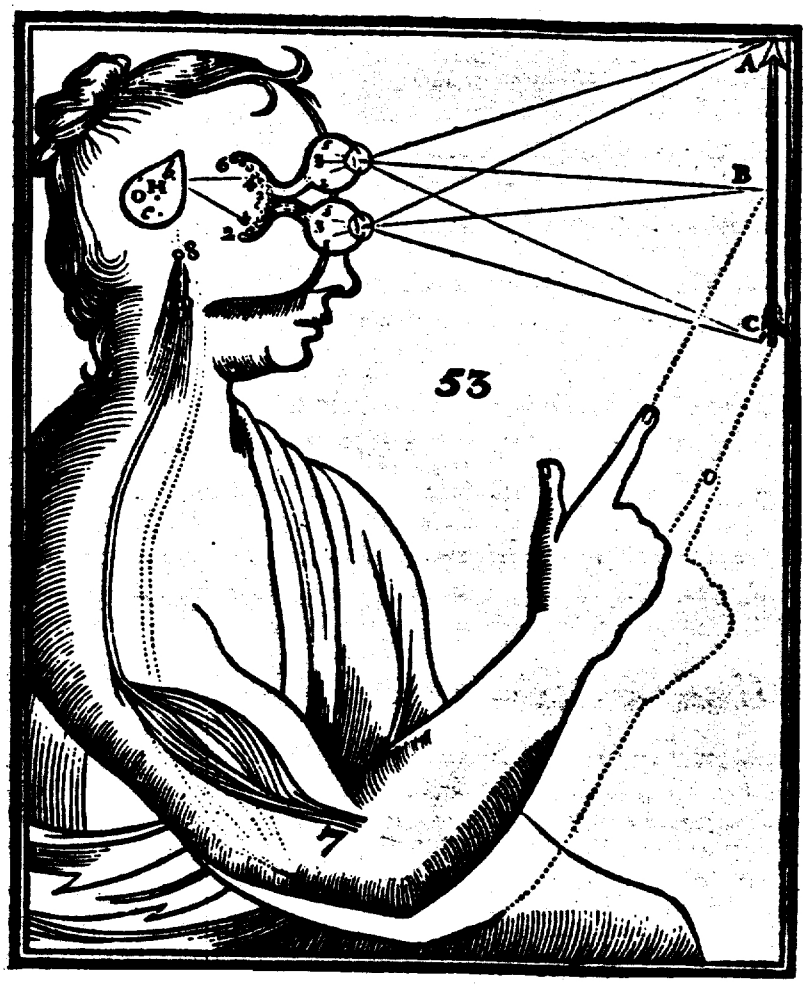
The existence of the pineal body has been known for at least 2,000 years. Galen, writing in the second century A.D., quoted studies of earlier Greek anatomists who were impressed with the fact that the pineal was perched atop the aqueduct of the cerebrum and was a single structure rather than a paired one; he concluded that it served as a valve to regulate the flow of thought out of its "storage bin" in the lateral ventricles of the brain. In the 17th century René Descartes embellished this notion; he believed that the pineal housed the seat of the rational soul. In his formulation the eyes perceived the events of the real world and transmitted what they saw to the pineal by way of "strings" in the brain [*see illustration below*]. The pineal responded by allowing humors to pass down hollow tubes to the muscles, where they produced the appropriate responses. With the hindsight of 300 years of scientific development, we can admire this prophetic formulation of the pineal as a neuroendocrine transducer!

In the late 19th and early 20th centuries the pineal fell from its exalted metaphysical state. In 1898 Otto Heubner, a German physician, published a case report of a young boy who had shown precocious puberty and was also found to have a pineal tumor. In the course of the next 50 years many other children with pineal tumors and precocious sexual development were described, as well as a smaller number of patients whose pineal tumors were associated with delayed sexual development. Inexplicably almost all the cases of precocious puberty were observed in boys.

In a review of the literature on pineal tumors published in 1954 Julian I. Kitay, then a fellow in endocrinology at the Harvard Medical School, found that most of the tumors associated with precocious puberty were not really pineal in origin but either were tumors of supporting tissues or were teratomas (primitive tumors containing many types of cells). The tumors associated with delayed puberty, however, were in most cases true pineal tumors. He concluded that the cases of precocious puberty resulted from reduced pineal function due to disease of the surrounding tissue, whereas delayed sexual development in children with true pineal tumors was a consequence of increased pineal activity.

The association of pineal tumors and sexual malfunction gave rise to hundreds of research projects designed to test the hypothesis that the pineal was a gland whose function was to inhibit the gonads. Little appears to have resulted from these early efforts. Later in 1954 Kitay and Mark D. Altschule, director of internal medicine at McLean Hospital in Waverly, Mass., reviewed the entire world literature on the pineal: some 1,800 references, about half of which dealt with the pineal-gonad question. They concluded that of all the studies published only two or three had used enough experimental animals and adequate controls for their data to be analyzed statistically. These few papers suggested a relation between the pineal and the gonads but did little to characterize it. After puberty the human pineal is hardened by calcification; this change in the appearance of the pineal led many investigators to assume that the organ was without function and further served to discourage research in the field. (Actually calcification appears to be unrelated to the pineal functions we have measured.)

As long ago as 1918 Nils Holmgren, a Swedish anatomist, had examined the pineal region of the frog and the dogfish with a light microscope. He was surprised to find that the pineal contained distinct sensory cells; they bore a marked resemblance to the cone cells of the retina and were in contact with nerve cells. On the basis of these obser-



SEAT OF THE RATIONAL SOUL was the function assigned to the human pineal (*H*) by René Descartes in his mechanistic theory of perception. According to Descartes, the eyes perceived the events of the real world and transmitted what they saw to the pineal by way of "strings" in the brain. The pineal responded by allowing animal humors to pass down hollow tubes to the muscles, where they produced the appropriate responses. The size of the pineal has been exaggerated in this wood engraving, which first appeared in 1677.

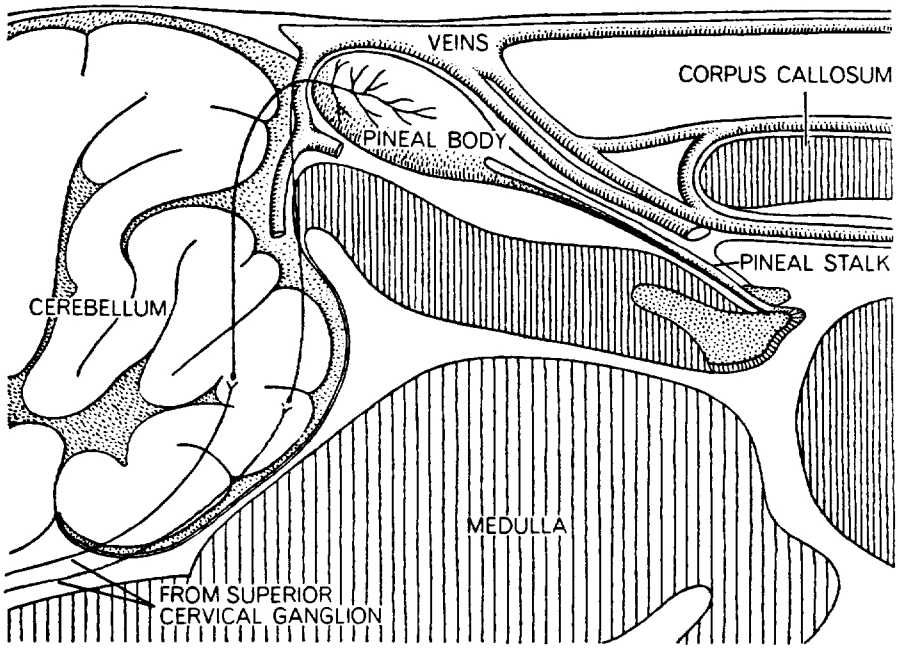
vations he suggested that the pineal might function as a photoreceptor, or "third eye," in cold-blooded vertebrates. In the past five years this hypothesis has finally been confirmed by electrophysiological studies: Eberhardt Dodt and his colleagues in Germany have shown that the frog pineal is a wavelength discriminator: it converts light energy of certain wavelengths into nervous impulses. In 1927 Carey P. McCord and Floyd P. Allen, working at Johns Hopkins University, observed that if they made extracts of cattle pineals and added them to the media in which tadpoles were swimming, the tadpoles' skin blanched, that is, became lighter in color.

Such was the state of knowledge about the pineal as late as five or six years ago. It appeared to be a photoreceptor in the frog, had something to do with sexual function in rats and in humans (at least those with pineal tumors) and contained a factor (at least in cattle) that blanched pigment cells in tadpoles.

The Discovery of Melatonin

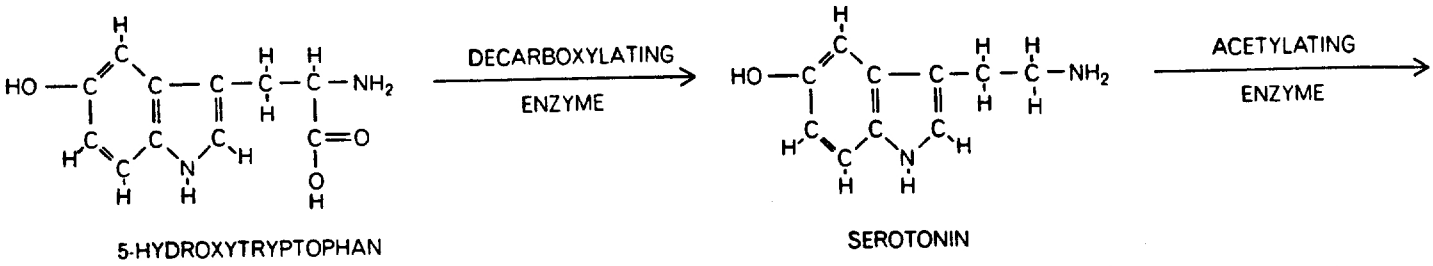
Then in 1958 Aaron B. Lerner and his co-workers at the Yale University School of Medicine identified a unique compound, melatonin, in the pineal gland of cattle [see "Hormones and Skin Color," by Aaron B. Lerner; SCIENTIFIC AMERICAN, July, 1961]. During the next four years at least half a dozen other major discoveries were made about the pineal by investigators representing many different disciplines and institutions. Lerner, a dermatologist and biochemist, was interested in identifying the substance in cattle pineal extracts that blanched frog skin. He and his colleagues prepared and purified extracts from more than 200,000 cattle pineals and tested the ability of the extracts to alter the reflectivity of light by pieces of excised frog skin. After four years of effort they succeeded in isolating and identifying the blanching agent and found that it was a new kind of biological compound: a methoxylated indole, whose biological activity requires a methyl group (CH_3) attached to an oxygen atom [*see illustration on next two pages*].

Methoxylation had been noted previously in mammalian tissue, but the products of this reaction had always appeared to lose their biological activity as a result. The new compound, named melatonin for its effect on cells containing the pigment melanin, appeared to lighten the amphibian skin by causing



INNERVATION OF RAT PINEAL was the subject of a meticulous study by the Dutch neuroanatomist Johannes Ariëns Kappers in 1961. He demonstrated that the pineal of the adult rat is extensively innervated by nerves from the sympathetic nervous system. The sympathetic nerves to the pineal originate in the neck in the superior cervical ganglion, enter the skull along the blood vessels and eventually penetrate the pineal at its blunt end (*top*). Aberrant neurons from the central nervous system sometimes run up the pineal stalk from its base, but these generally turn and run back down the stalk again without synapsing. The pineal is surrounded by a network of great veins, into which its secretions probably pass. According to Ariëns Kappers, the innervation of the human pineal is quite similar.

SYMPATHETIC NERVE terminates directly on a pineal cell, instead of on a blood vessel or smooth muscle cell, in this electron micrograph of a portion of a rat pineal made by David Wolfe of the Harvard Medical School. The nerve ending is characterized by dark vesicles, or sacs, that contain neurohumors. Magnification is about 12,500 diameters.



SYNTHESIS OF MELATONIN in the rat pineal begins with the removal of a carboxyl (COOH) group from the amino acid 5-hydroxytryptophan by the enzyme 5-hydroxytryptophan decarboxylase. Serotonin, the product of this reaction, is then enzymatically

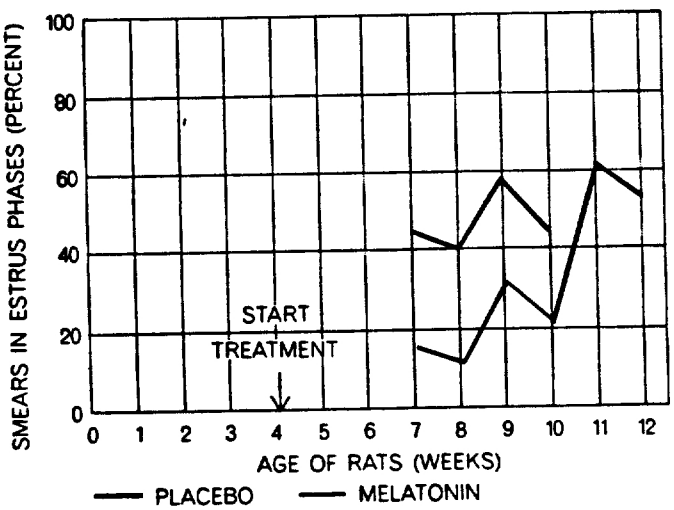
the aggregation of melanin granules within the cells. It was effective in a concentration of only a trillionth of a gram per cubic centimeter of medium. No influence of melatonin could be demonstrated on mammalian pigmentation, nor could the substance actually be identified in amphibians, in which it exerted such a striking effect. It remained a biological enigma that the mammalian pineal should produce a substance that appeared to have no biological activity in mammals but was a potent skin-lightening agent in amphibians, which were unable to produce it!

Both aspects of the foregoing enigma have now been resolved. Subsequent research has shown that melatonin does in fact have a biological effect in mammals and can be produced by amphibians. Spurred by Lerner's discovery of this new indole in the cattle pineal, Nicholas J. Giarman, a pharmacologist at the Yale School of Medicine, analyzed pineal extracts for their content of other biologically active compounds. He found that both cattle and human pineals contained comparatively high levels of serotonin, an amine whose molecular structure is similar to melatonin and whose function in nervous tissue is largely unknown. Studies by other investigators subsequently showed that the rat pineal contains the highest concentration of serotonin yet recorded in any tissue of any species.

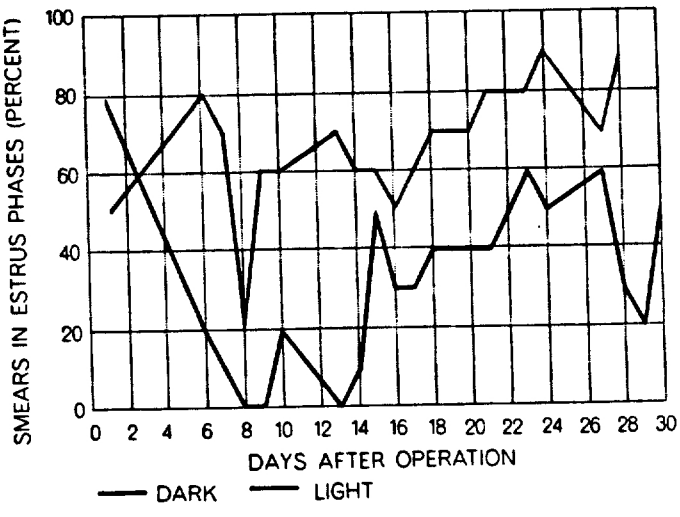
A year before the discovery of melatonin one of the authors (Axelrod) and his co-workers had identified a methoxylating enzyme (catechol-O-methyl transferase) in a number of tissues. This enzyme acted on a variety of catechols (compounds with two adjacent hydroxyl, or OH, groups on a benzene ring) but showed essentially no activity with respect to single-hydroxyl compounds such as serotonin, the most likely precursor of melatonin. In 1959 Axelrod and Herbert Weissbach studied cattle pineal tissue to see if it might have the special enzymatic capacity to methoxylate hydroxyindoles. They incubated N-acetylserotonin (melatonin without the methoxyl group) with pineal tissue and a suitable methyl donor and observed that melatonin was indeed formed. Subsequently they found that all mammalian pineals shared this biochemical property but that no tissue other than pineal could make melatonin. Extensive studies of a variety of mammalian species have confirmed this original observation that only the pineal appears to have the ability to synthesize melatonin. (In amphibians and some birds small amounts of melatonin are also manufactured by the brain and the eye.) Other investigators have found that the pineal contains all the biochemical machinery needed to make melatonin from an amino acid precursor, 5-hydroxytryptophan, which it obtains from the bloodstream. It was also found that circulating melatonin is rapidly metabolized in the liver to form 6-hydroxymelatonin.

Anatomy of the Pineal

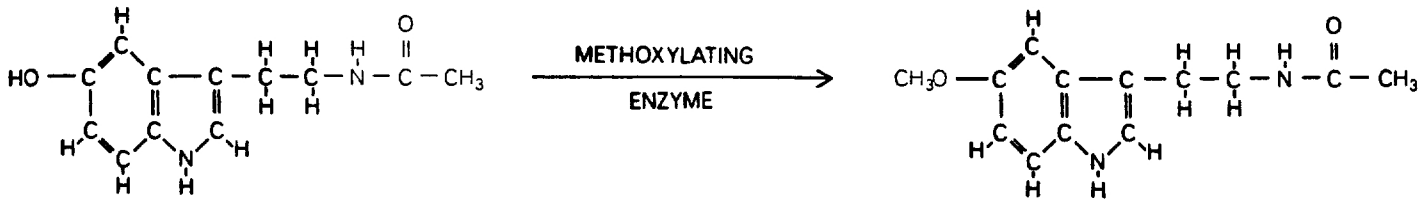
While these investigations of the biochemical properties of the pineal were in progress, important advances were being made in the anatomy of the pineal by the Dutch neuroanatomist Johannes Ariëns Kappers and by several



EFFECT OF MELATONIN on the estrus cycles of female rats is depicted here. Rats that had been given daily injections of melatonin starting in their fourth week of life developed a longer estrus cycle than rats that had been similarly treated with a placebo. When the melatonin-treated animals were 10 weeks old, a placebo was substituted for the melatonin and the estrus cycle returned to normal.



EFFECTS OF LIGHTING on the estrus cycles of three groups of female rats are shown in the graphs on these two pages. The groups, each consisting of about 20 rats, were subjected respectively to a sham operation (*left*), removal of their superior cervical ganglion (*middle*) and removal of their eyes (*right*). Each group was then further subdivided, with about half being placed in constant light



N-ACETYLSEROTONIN

MELATONIN

acetylated to form N-acetylserotonin. This compound in turn is methoxylated by the enzyme hydroxyindole-O-methyl transferase (HIOMT) to yield melatonin. In mammals HIOMT is found only in the pineal. Changes in basic molecule are indicated by color.

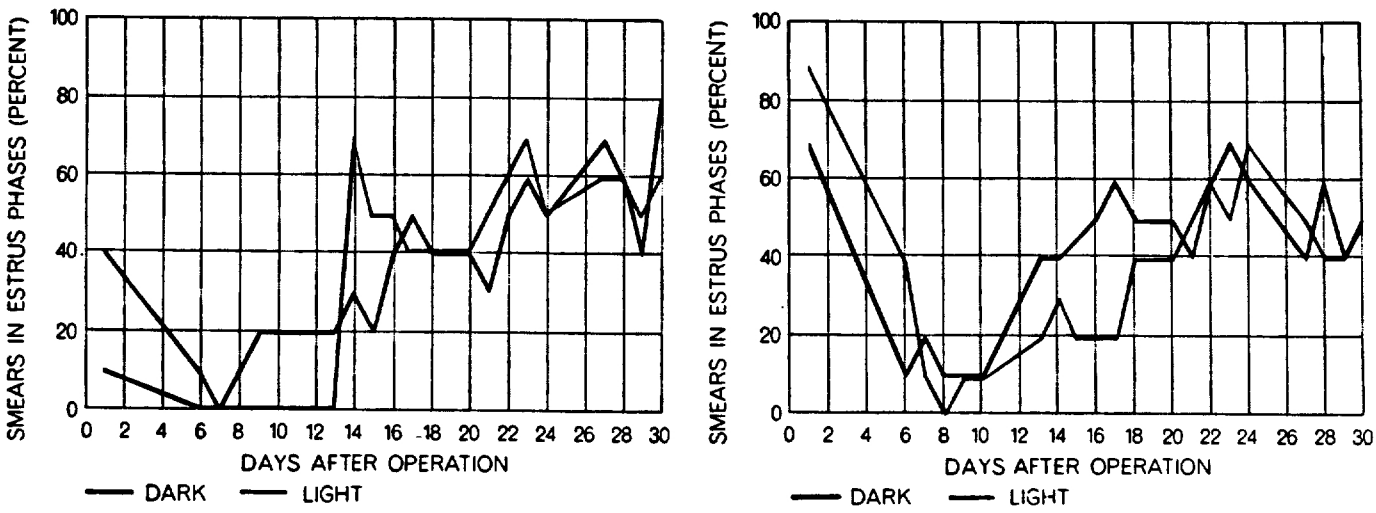
American electron microscopists, including Douglas E. Kelly of the University of Washington, Aaron Milofsky of the Yale School of Medicine and David Wolfe, then at the National Institute of Neurologic Diseases and Blindness. In 1961 Ariëns Kappers published a meticulous study of the nerve connections in the rat pineal. He demonstrated clearly that although this organ originates in the brain in the development of the embryo, it loses all nerve connections with the brain soon after birth. There is thus no anatomical basis for invoking "tracts from the brain" as the pathway by which neural information is delivered to the pineal.

Ariëns Kappers showed that instead the pineal of the adult rat is extensively penetrated by nerves from the sympathetic portion of the autonomic nervous system. The sympathetic nervous system is involuntary and is concerned with adapting to rapid changes in the internal and external environments; the sympathetic nerves to the pineal originate in the superior cervical ganglion in the neck, enter the skull along the blood vessels and eventually penetrate the pineal [*see top illustration on page 53*]. Electron microscope studies later showed that within the pineal many sympathetic nerve endings actually terminate directly on the pineal cells, instead of on blood vessels or smooth-muscle cells, as in most other organs [*see bottom illustration on page 53*]. Among endocrine structures the organization of nerves in the mammalian pineal appeared to be most analogous to that of the adrenal medulla, one of the three demonstrated neuroendocrine transducers.

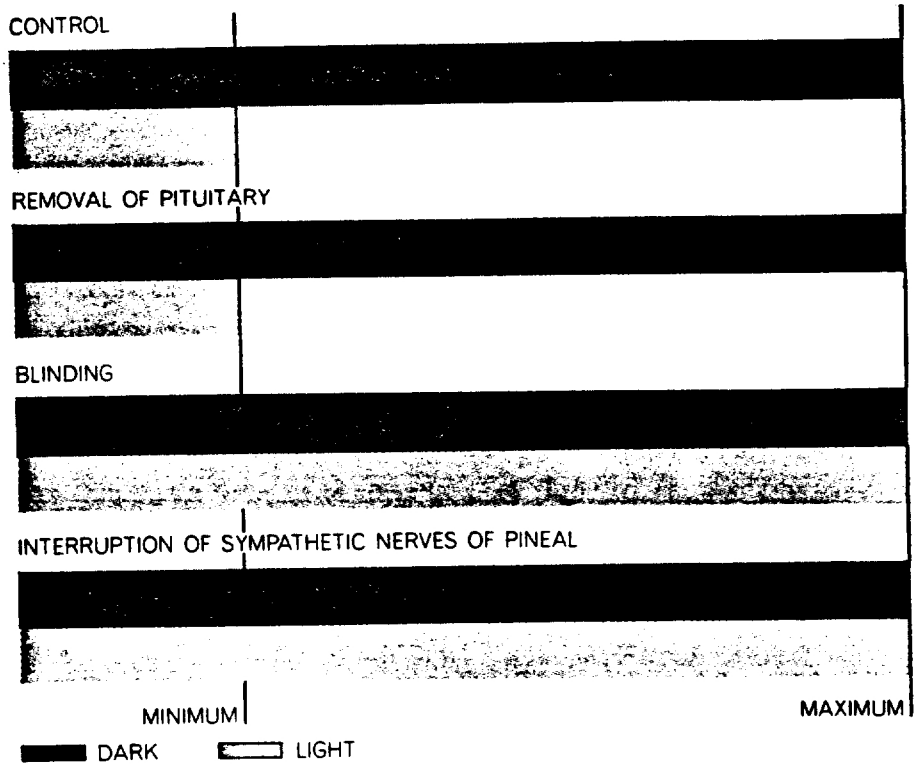
Meanwhile electron microscope studies by other workers on the pineal regions of frogs had confirmed many of Holmgren's speculations. It was found that the pineal cells of amphibians contained light-sensitive elements that were practically indistinguishable from those found in the cone cells of the retina, but that the pineal cells of mammals did not contain such elements. By 1962 it could be stated with some assurance that the mammalian pineal was not simply a vestige of the frog "third eye," since the "vestige" had undergone profound anatomical changes with evolution.

The Melatonin Hypothesis

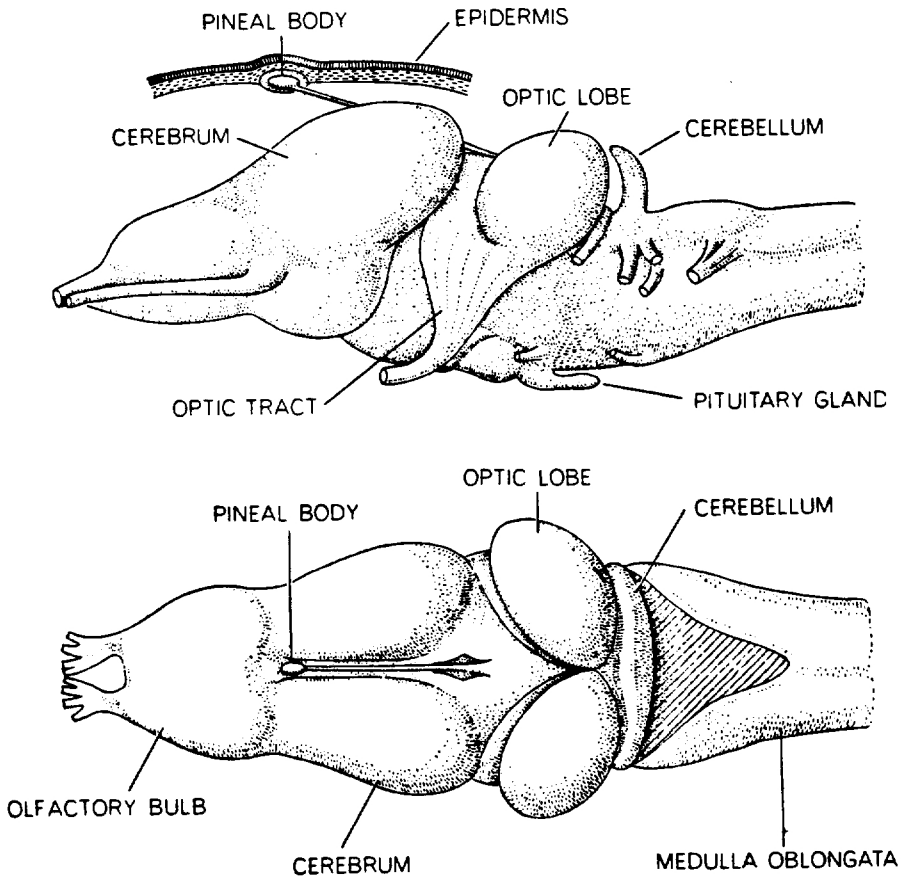
Even though the mammalian pineal no longer seemed to respond directly to light, there now appeared good evidence that its function continued to be related somehow to environmental light. In 1961 Virginia Fiske, working at Wellesley College, reported that the exposure of rats to continuous environmental illumination for several weeks brought about a decrease in the weight of their pineals. She had been interested in studying the mechanisms by which the exposure of rats to light for long periods induces changes in the function of their gonads. (For example, continous light increased the weight of the ovaries and accelerated the estrus cycle). At the same time one of the authors (Wurtman, then at the Harvard Medical School), in collaboration with Altschule and Willard Roth, was studying the conditions under which the administration



and the other half in constant darkness beginning one day after their respective operations. Daily vaginal smears were taken on the first day and on the sixth through the 30th days after the operations. Results were plotted as the percentage of all the smears in a treatment group showing estrus phases each day. In general it was found that interference with the transmission of light information to the pineal gland (either by blinding or by cutting the sympathetic nerves) also abolished most of the gonadal response to light. These findings supported the authors' melatonin hypothesis, which holds that one mechanism whereby light is able to accelerate the estrus cycle in normal animals is by inhibiting the synthesis in the pineal of melatonin, a compound that in turn inhibits estrus.



RESPONSE OF MELATONIN-FORMING ENZYME hydroxyindole-O-methyl transferase (HIOMT) to continuous light or darkness is shown under four different circumstances. In the control, or normal, animal continuous darkness induces an increase in HIOMT activity, whereas exposing the animal to continuous light has the opposite effect. The ability of the pineal gland to respond to environmental lighting is unaffected by the removal of the pituitary gland but is abolished following blinding or sympathetic denervation of the pineal.



PINEAL EYE is a primitive photoreceptive organ found in certain cold-blooded vertebrates such as the frog. Frog's brain is shown from the side (*top*) and from above (*bottom*).

of cattle pineal extracts decreased ovary weight and slowed the estrus cycle.

We soon confirmed Mrs. Fiske's findings, and we were also able to show that the exposure of female rats to continuous light or the removal of their pineals had similar, but not additive, effects on the weight of their ovaries. These experiments suggested that perhaps one way in which light stimulates ovary function in rats is by inhibiting the action of an inhibitor found in pineal extracts. It now became crucial to identify the gonad-inhibiting substance in pineal extracts and to see if its synthesis or its actions were modified by environmental lighting.

In 1962 we began to work together on isolating the anti-gonadal substance present in pineal extracts. Our plan was to subject extracts of cattle pineal glands to successive purification steps and test the purified material for its ability to block the induction by light of an accelerated estrus cycle in the rat. Before undertaking the complicated and time-consuming procedure of isolating the active substance in the pineal glands of cattle, we first tested a mixture of all the constituents that had already been identified in this tissue. The mixture was found to block the effects of light on the estrus cycle.

Next we tested melatonin alone, since it was apparently the only compound produced uniquely by the pineal. To our good fortune we found that when rats were given tiny doses (one to 10 micrograms per day) of melatonin by injection, starting before puberty and continuing for a month thereafter, the estrus cycle was slowed and the ovaries lost weight—just as though the animals had been treated with pineal extracts. In later studies we found that this effect of melatonin was chemically specific: it was simulated by neither N-acetylserotonin, the immediate precursor of melatonin, nor 6-hydroxymelatonin, the major product of its metabolism. Moreover, it was possible to accelerate the estrus cycle by removal of the pineal and to block this response by the injection of melatonin.

On the basis of these studies, performed in collaboration with Elizabeth Chu of the National Cancer Institute, we postulated that melatonin was a mammalian hormone, since it is produced uniquely by a single gland (the pineal), is secreted into the bloodstream and has an effect on a distant target organ (the vagina and possibly also the ovaries). We were not able to identify the precise site of action of melatonin in affecting the gonads. The

slowing of the estrus cycle could be produced by actions at any of several sites in the neuroendocrine apparatus, including the brain, the pituitary, the ovaries or the vagina itself. When melatonin was labeled with radioactive atoms and injected into cats, it was taken up by all these organs and was selectively concentrated by the ovaries.

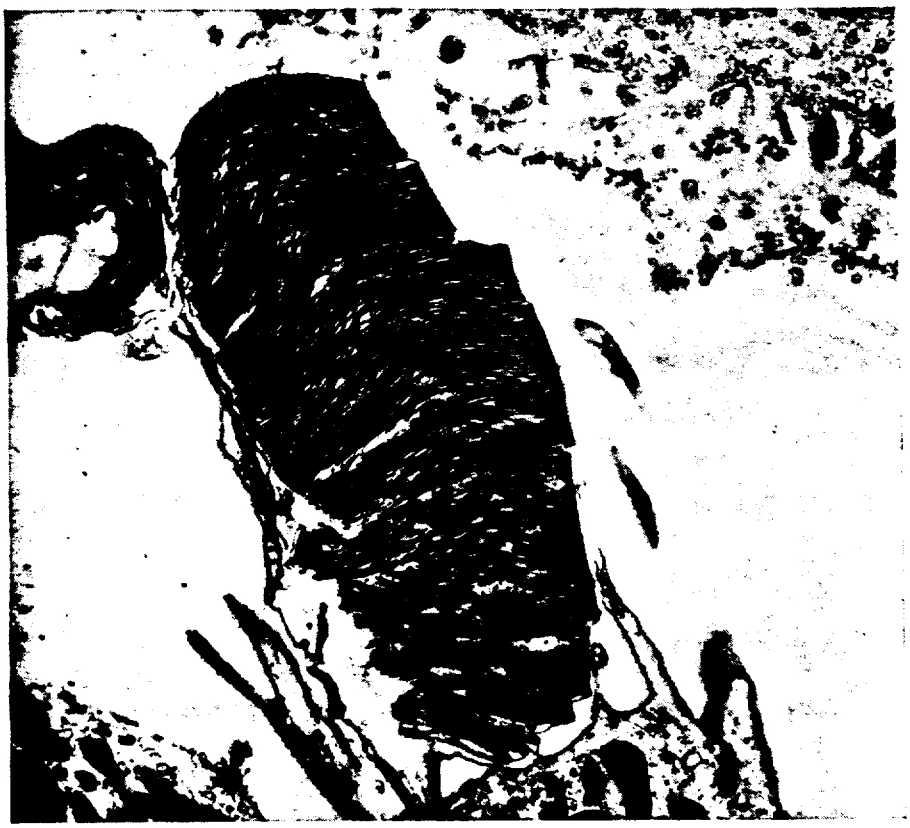
William M. McIsaac and his colleagues at the Cleveland Clinic have confirmed the effects of melatonin on the estrus cycle and have identified another pineal methoxyindole—methoxytryptophol—that has similar effects. It appears likely that pineal extracts contain a family of hormones: the methoxyindoles, all of which have in common the fact that they can be synthesized by the methoxylating enzyme found only in the mammalian pineal.

We next set out to determine whether or not these effects of injected melatonin were physiological. Could the rat pineal synthesize melatonin and, if so, in what quantities? When rat pineal glands were examined for their ability to make melatonin, we were disappointed to find that the activity of the melatonin-forming enzyme (hydroxyindole-O-methyl transferase, or HIOMT) in the rat was much lower than in most other species; the maximum amount of melatonin that the rat could make was probably on the order of one microgram per day. Our disappointment was soon relieved, however, when we realized that the low activity of this enzyme made it likely that it was controlling the rate-limiting step in melatonin synthesis in the intact animal. Knowing that continuous exposure to light decreased pineal weight, as well as the amount of ribonucleic acid (RNA) and protein in the pineal, we next explored what effect illumination might have on HIOMT activity and thus on melatonin synthesis.

Since the rat pineal gland was so small (about a milligram in weight) and had so little enzymatic activity, it was necessary to devise extremely sensitive techniques to measure this activity. When rats were subjected to constant light for as short a period as a day or two, the rate of melatonin synthesis in their pineals fell to as little as a fifth that of animals kept in continuous darkness. Since this effect of illumination or its absence could be blocked by agents that interfered with protein synthesis, it appeared that light was actually influencing the rate of formation of the enzyme protein itself.

How was information about the state of lighting being transmitted to the rat pineal? Three possible routes suggested

RETINAL CONE CELL from the eye of an adult frog is shown in this electron micrograph made by Douglas E. Kelly of the University of Washington. The photoreceptive outer segment of the cell (*top center*) consists of a densely lamellated membrane. Parts of two larger rod photoreceptors can be seen on each side of cone. Magnification is about 13,000 diameters.



PINEAL CONE CELL from the pineal eye of an adult frog is shown in this electron micrograph made by Kelly at approximately the same magnification as the micrograph at top. The lamellated outer segment of the pineal cell is practically indistinguishable from that of the retinal photoreceptor. Part of the membrane has torn away from the cell (*top left*).

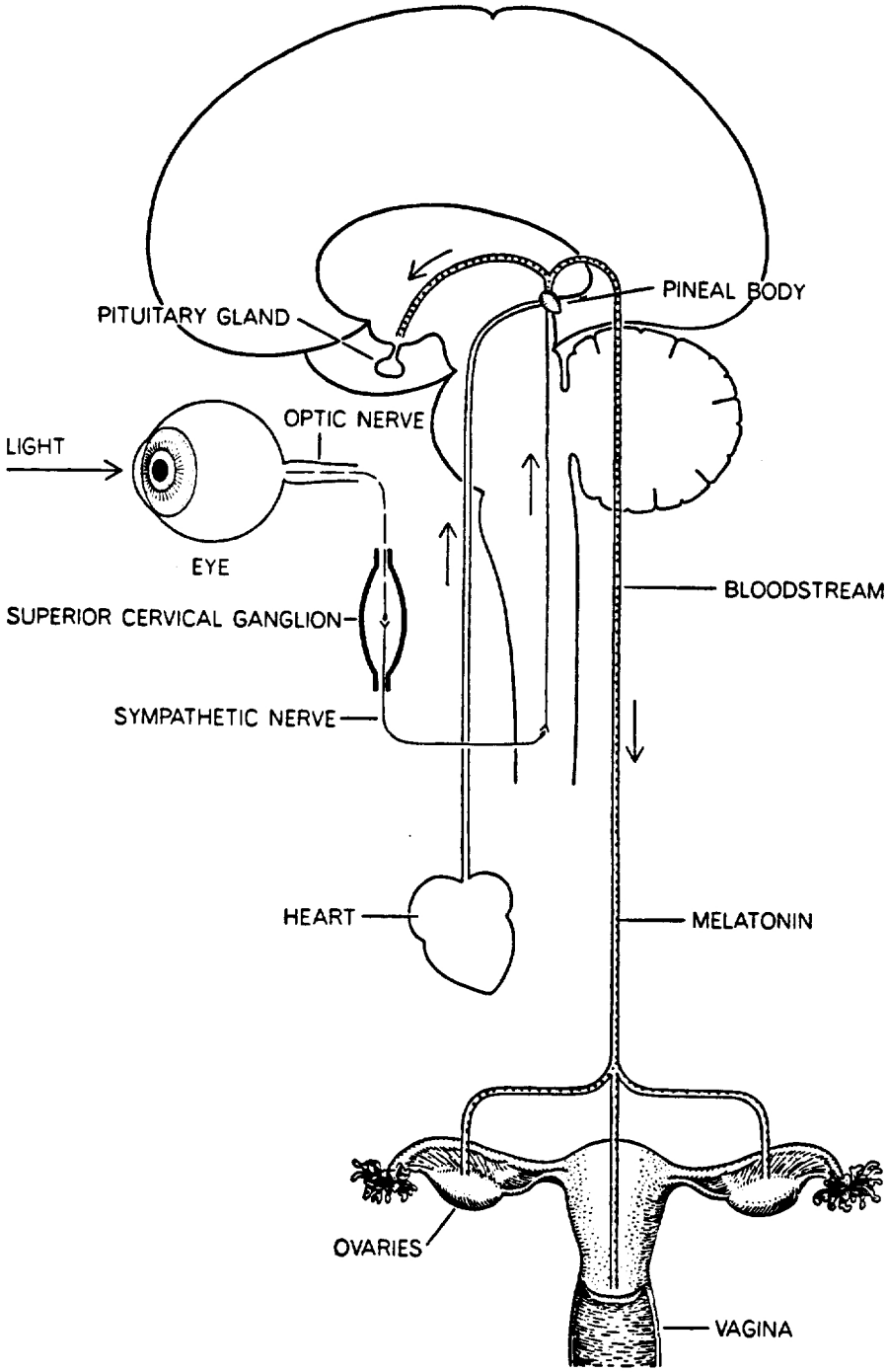
themselves. The first was that light penetrated the skull and acted directly on the pineal; W. F. Ganong and his colleagues at the University of California at Berkeley had already shown that significant quantities of light do penetrate the skulls of mammals. This hypothesis was ruled out, however, by demonstrating that blinded rats completely lost the capacity to respond to light with changed HIOMT activity; hence light had to be perceived first by the retina and was not acting directly on the pineal.

The second possibility was that light altered the level of a circulating hormone, perhaps by affecting the pituitary gland, and that this hormone secondarily influenced enzyme activity in the pineal gland. This hypothesis was also ruled out by demonstrating that the removal of various endocrine organs, including the pituitary and the ovaries, did not interfere with the response of pineal HIOMT to light.

The third possibility was that information about lighting was transmitted to the pineal by nerves. Fortunately Ariëns Kappers had just identified the nerve connections of the rat pineal as coming from the sympathetic nervous system. We found that if the sympathetic pathway to the pineal was interrupted by the removal of the superior cervical ganglion, the ability of melatonin-forming activity to be altered by light was completely lost. Thus it appeared that light was stimulating the retina and then information about this light was being transmitted to the pineal via sympathetic nerves. Within the pineal the sympathetic nerves probably released neurohumors (noradrenaline or serotonin), which acted on pineal cells to induce (or block the induction of) HIOMT; this enzyme in turn regulated the synthesis of melatonin.

Since one way light influences the gonads is by changing the amount of melatonin secreted from the pineal, we reasoned that the effects of light on the gonads might be blocked if the transmission of information about light to the pineal were interrupted. This could be accomplished by cutting the sympathetic nerves to the pineal—a procedure much less traumatic than the removal of the pineal itself. To test this hypothesis we placed groups of rats whose pineals had been denervated along with blinded and untreated animals in continuous light or darkness for a month. Vaginal smears were checked daily for evidence of changes in the estrus cycle, and pineals were tested for melatonin-synthesizing ability at the end of the experiment. It was found that interrupting the transmission of light information to the pineal (by cutting its sympathetic nerves—a procedure that does not interfere with the visual response to light) also abolished most of the gonadal response to light.

Incidentally, the observation that sympathetic nerves control enzyme synthesis in the pineal has provided, and should continue to provide, a useful tool for studies in a number of other biological disciplines. For example, studying the changes in brain enzymes produced by environmental factors offers a useful method for tracing the anatomy



SUGGESTED PATHWAY by which light influences the estrus cycle in the rat is depicted in this schematic diagram. Light stimuli impinge on the retinas and cause a change in the neural output of the superior cervical ganglion by way of an unknown route. This information is then carried by sympathetic nerves to the pineal gland, where it causes a decrease in the activity of HIOMT and in the synthesis and release of melatonin. This decrease in turn lessens the inhibiting effect of the circulating melatonin on the rate of the estrus cycle. The precise site of action of melatonin in influencing the gonads is unknown; the slowing of the estrus cycle could be produced by actions at any one of several sites in the neuroendocrine apparatus, including the brain, the pituitary, the ovaries and the vagina.

of the nerve tracts involved. The observation that the activity of at least one part of the sympathetic nervous system (the superior cervical ganglion) is affected by environmental lighting raises the possibility that other regions of this neural apparatus are affected similarly. If so, physiological studies of the effects of light on other sympathetically innervated structures (for example the kidneys and fat tissue) may be profitable.

We have also found that light influences the serotonin-forming enzyme in the pineal gland but not in other organs. In contrast to HIOMT, the activity of this enzyme increases when rats are kept in constant light and decreases in darkness. When rats are blinded or when the sympathetic nerves to the pineal are cut, the effect of light and darkness on the serotonin-forming enzyme is also extinguished. Furthermore, certain drugs that block the transmission of sympathetic nervous impulses also abolish the effect of illumination on this enzyme. The fact that lighting influences pineal weight and at least two enzyme systems in this organ suggests that it may regulate many additional, undiscovered biochemical events in the pineal, via the sympathetic nervous system.

Diurnal and Circadian Rhythms

The pineal had been shown to respond and function under quite unusual conditions; for example, when an experimental animal was exposed to continuous light or darkness for several days. In nature, of course, animals that live in the temperate and tropical zones are rarely subjected to such conditions. It became important to determine if the pineal could also respond to naturally occurring changes in the environment.

In nature the level of light exposure changes with both diurnal and annual cycles. Except in polar regions every 24-hour day includes a period of sunlight and a period of darkness; the ratio of day to night varies with an annual rhythm that reaches its nadir at the winter solstice and its zenith on the first day of summer. Lighting cycles have been shown to be important in regulating several types of endocrine function: the increase in sunlight during the winter and spring triggers the annual gonadal growth and breeding cycles in many birds and some mammals that breed yearly, and the daily rhythm of day and night synchronizes a variety of roughly daily rhythms in mammals, such as the cycle of adrenal-steroid secretion. Such rhythms are called cir-

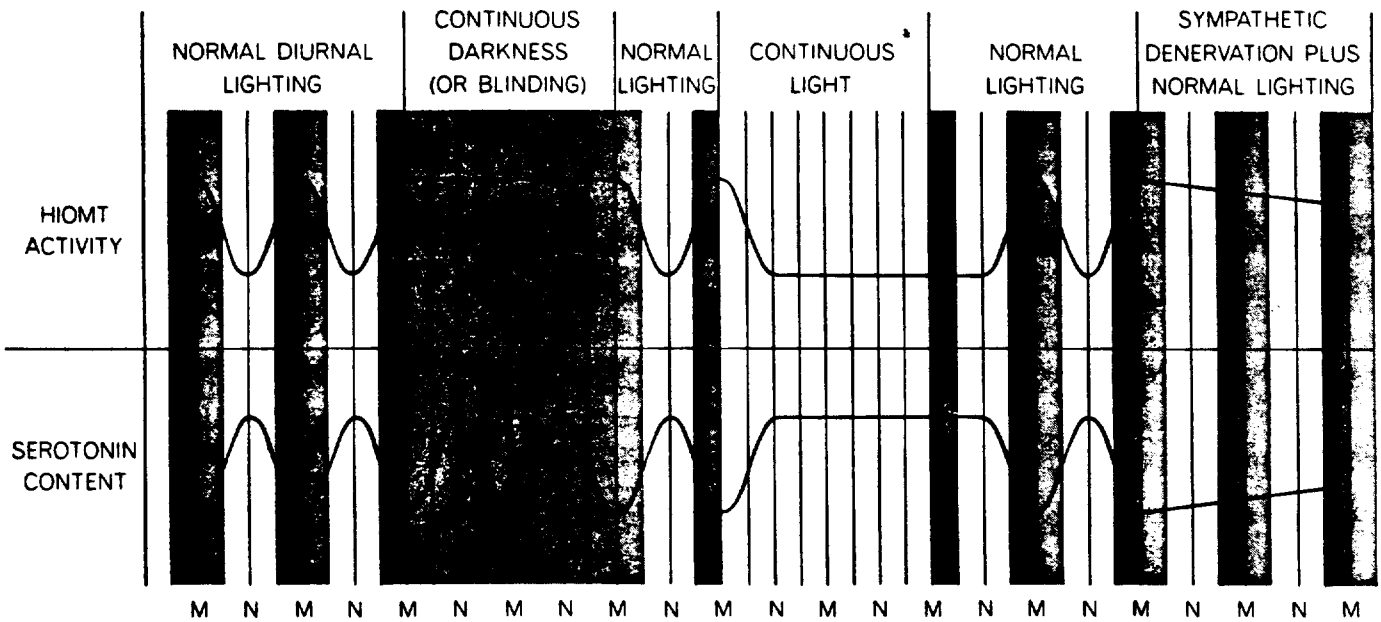
cadian, from the Latin phrase meaning "about one day." Could the pineal respond to natural diurnal lighting shifts? If so, it might function to synchronize the endocrine apparatus with these shifts.

In order to determine if normal lighting rhythms influenced the pineal, we kept a large population of rats under controlled lighting conditions (lights on from 7:00 A.M. to 7:00 P.M.) for several weeks and then tested their pineals for melatonin-forming ability at 6:00 A.M., noon, 6:00 P.M. and midnight. In the five hours after the onset of darkness (that is, by midnight) this enzymatic capacity increased between two and three times. Moreover, pineal weight also changed significantly during this period, again indicating that light was affecting many more compounds in the pineal than the single enzyme we were measuring.

All circadian rhythms studied up to this stage had in common the ability to persist for some weeks after animals were deprived of environmental lighting cues (by blinding or being placed in darkness). These rhythms no longer showed a period of precisely 24 hours, but they did fall in a range between 22 and 26 hours and hence were thought to be regulated by some internal mechanism not dependent on, but usually synchronized with, environmental lighting. Such endogenous, or internally regulated, circadian rhythms in rodents include motor activity and rectal temperature, as well as the rhythm in adrenal-steroid secretion. When we blinded rats or placed them in continuous light or darkness, the pineal rhythm in melatonin-forming activity was rapidly extinguished. If instead of turning off the lights at 7:00 P.M. illumination was continued for an additional five hours and pineals were examined as usual at midnight, the expected rise in melatonin-forming activity was completely blocked. This pineal rhythm in HIOMT activity thus appears to be truly exogenous, or externally regulated, and is entirely dependent on shifts in environmental lighting. Hence this enzyme rhythm may be more important in carrying information about light to the glands than other circadian rhythms that do not depend on light for their existence.

Recently Wilbur Quay of the University of California at Berkeley has found that the content of serotonin in the rat pineal also undergoes marked circadian rhythms. The highest levels of this amine are found in pineals at noon and the lowest levels at midnight. Serotonin content falls rapidly just at the time that melatonin-forming activity is rising. In collaboration with Solomon Snyder we studied the mechanism of the serotonin cycle. When rats are kept in continuous light, the serotonin cycle is extinguished. To our surprise, however, when rats are kept continuously in darkness or blinded, this rhythm persists, unlike the rhythm in the melatonin-forming ezyme. When the sympathetic nerves to the pineal are cut, the serotonin and HIOMT cycles are both suppressed. When the nerves from the central nervous system to the superior cervical ganglion are interrupted, the serotonin rhythm is also abolished [*see illustration below*]. Hence the serotonin rhythm in the pineal gland is similar to most other circadian rhythms (and differs from the HIOMT cycle) in that it is endogenous and depends on environmental light only as an external synchronizer. The mechanism that controls the serotonin rhythm appears to reside within the central nervous system. The pineal gland thus contains at least two distinct biological clocks, one totally dependent on environmental lighting and the other originating within the brain but cued by changes in lighting.

At present little is known about what organs are dependent on the pineal clock for cues. The ability of melatonin to modify gonadal function suggests, but does not prove, that its secretion may have something to do with the timing of the estrus and menstrual cycles—two phenomena about whose mechanisms of control very little is known. One is tempted to argue teleologically that any control mechanism as complicated and sensitive as that found in the mammalian pineal gland must have some place in the economy of the body.



BIOCHEMICAL RHYTHMS in the pineal gland of the rat were recorded under various lighting and other conditions. Normally both the content of serotonin and the activity of the melatonin-forming enzyme (HIOMT) vary with a 24-hour cycle. The serotonin content is greatest at noon (*N*), whereas the HIOMT activity is greatest at midnight (*M*). The HIOMT cycle is completely dependent on environmental lighting conditions: it disappears when animals are kept in continuous light or darkness, or when they are blinded. The serotonin cycle persists in continuous darkness or after blinding but can be abolished by keeping the rats in continuous light. Both cycles are depressed when the sympathetic nerves to the pineal gland are cut (*extreme right*). Gray areas signify darkness.