ted computation of a three-dimensional Patterson function, $P(uvw)$.

We prepared the vector maps to be expected for all permissible equipoint combinations for four copper and two sulfur atoms in $P6_3/mmc$ and other space groups permitted by the diffraction symbol. None matched the actual Patterson function of high chalcocite, which confirmed that routine distribution of atoms over the equipoints would not lead to a solution.

The Patterson function did, however, have a set of major peaks which could be explained by a set of atoms in close packing. We had supposed that the S atoms would be in close packing with the Cu atoms in disorder in their interstices. We therefore computed structure-factor signs based upon the supposition that these peaks were due to S atoms, and computed a trial electron density, $\rho(xyz)$ based upon these signs. This led to a map on whose interpretation and improvement we spent a great deal of time.

The key to its interpretation was finally provided by implication maps $I_6(x\ y\ \frac{1}{2})$ and $I_3(x\ y\ 0)$, based upon the Harker sections $P(x\ y\ \frac{1}{2})$ and $P(x\ y\ 0)$, respectively. The projection $\rho(x\ y)$ of the electron density had to be consistent with these implication maps, and this permitted us to retain only those possible Cu positions which so fitted the implication. The model we accepted had S in 2 c at $\frac{1}{3}\ \frac{2}{3}\ \frac{1}{4}$ and the copper split into three parts: Cu′ in 2 b at $0\ 0\ \frac{1}{4}$, Cu″ in 4 f at $\frac{1}{3}\ \frac{2}{3}$ 0.578, and Cu‴ in 6 g at $0\ \frac{1}{2}\ 0$.

We refined the resulting structure by least-squares methods, first using isotropic temperature factors; this produced a fair agreement between observed and calculated intensities, but we could not reduce the disagreement factor R below 31 percent for isotropic temperature factors (*7*). A serious difficulty was the strong interaction between the amount of Cu in each of the three sites, and the temperature factors. Section $\rho(0\ y\ z)$ through the Fourier synthesis of our structure, referred to an orthohexagonal cell, is shown in Fig. 1. This section contains all the atoms described.

All structure-factor signs converged during the final adjustments of the disordered copper-atom weights. Electron-density syntheses based on these signs showed that appreciable smears of electron density extended between the three types of Cu sites. The Cu atoms are evidently mobile, and it was clear that, even when anisotropic temperature factors were used, placement of discrete atoms in these sites could merely represent an approximation to the true copper distribution. It remained, therefore, to demonstrate conclusively the correctness of the structure by further improvement of the agreement between the observed and calculated structure factors.

To take into account adequately the continuous nature of the copper distribution, the unit cell was partitioned into volume elements which were represented by a grid whose unit cell is given by the sublattice

$$\mathbf{x}_1 = \tfrac{1}{30}\ (\mathbf{a}_1 + \mathbf{a}_2)$$

$$\mathbf{x}_2 = \tfrac{1}{30}\ (-\mathbf{a}_1 + \mathbf{a}_2)$$

$$\mathbf{x}_3 = \tfrac{1}{30}\ \mathbf{c}.$$

The scattering power of a volume element ΔV located at $x_1x_2x_3$ is the fraction of an electron which the Fourier synthesis shows to be contained in that volume element. This is $w = \rho(xyz)\Delta V$. The amplitude scattered by reflection hkl is therefore

$$F_{hkl} = \sum_{x_1x_2x_3} \sum \sum \sum w_{x_1x_2x_3} \cos 2\pi\ (hx_1 + kx_2 + lx_3).$$

Of the 688 volume elements contained in the asymmetric unit, 350 contained nonzero electron density. Structure-factor calculations based on this distribution of electrons were followed by difference syntheses. Slight reapportionment of the electron-density distribution was made on the basis of these maps. The final electron-density distribution yielded a disagreement factor of 21.5 percent for all data, and a value of 18.0 percent when the unobserved reflections were excluded.

This confirms the correctness of the structure of high chalcocite which we had derived (*7*). While the distribution of Cu atoms is best judged from the map in Fig. 1, it can be said that the four copper atoms are distributed over the sites of 2 b, 4 f, and 6 g in approximately the ratio 1.24:1.63:1.13. These Cu atoms are respectively in three-fold, four-fold, and two-fold coordination. It is apparent that in the high-temperature form of chalcocite the sulfur atoms comprise a substantially fixed structure, with sulfur atoms in hexagonal close packing, while the copper atoms are mobile through the interstices of this structure. The mobility is possible because the copper atoms can assume tetrahedral, trigonal, and linear coordination. In the low-temperature form, the thermal energy is not sufficient to maintain mobility and the structure is doubtless characterized by sulfur atoms in hexagonal close-packed array with copper atoms in at least two of the three coordinations observed in the high-temperature form. The alternation of copper in different coordinations accounts for the large superstructure cell observed for the high-temperature form (*3*). We have a set of three-dimensional diffraction intensities for low chalcocite, and have computed its Patterson function and the Patterson of the substructure. The latter maps indicate that the tetrahedral and trigonal sites are occupied in low chalcocite. Work on the structure of low chalcocite is continuing (*7; 8*).

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Melatonin, a Pineal Substance: Effect on the Rat Ovary

Abstract. *Daily injection of microgram amounts of melatonin in rats decreased the incidence of estrus and reduced ovarian weight. Circulating melatonin was selectively taken up and retained by the ovary and pineal gland; this effect was reduced by exposure of rats to constant light. A single injection of melatonin lowered the incidence of estrus among rats exposed to constant light.*

Many observations have linked the mammalian pineal gland to gonad function. Human males with tumors which destroy the pineal gland have a high incidence of precocious puberty (*1*). Pinealectomy has resulted in an increase in ovarian weight (*2*), while pineal extracts decreased ovarian weight and

Table 1. The effect of melatonin on the rat ovary. The diluent was 0.48 percent KCl and 0.18 percent NaCl. S, serotonin; M, melatonin; i.p., intraperitoneally; s.c., subcutaneously.

Treatment	Dose (mg)	Route	Ovary weight ± S.E. (mg)
Groups of 11 rats (85 to 95 g)			
Control			67.3 ± 3.6
S*	50	i.p.	71.4 ± 4.4
M	20	i.p.	42.6 ± 3.2 ($p \leq 0.001$)
Groups of 10 rats (45 to 55 g)			
Control			49.0 ± 2.8
M	1	i.p.	46.0 ± 2.6
M	10	i.p.	41.3 ± 2.0 ($p \leq 0.05$)
M	1	s.c.	39.1 ± 2.9 ($p \leq 0.05$)

* As serotonin creatinine sulfate.

produced anestrus in aged rats with spontaneous persistent estrus (*3*). When rats were exposed to constant light the ovaries became enlarged and the incidence of estrus increased; both effects are inhibited by the administration of pineal extracts (*4*). Exposure to light produced a decrease in pineal weight, serotonin content, and nucleolar size (*5*). Thus, some of the effects of light on the gonads may be mediated by the pineal gland.

Melatonin (5-methoxy-*N*-acetyltryptamine) is highly localized in the mammalian pineal gland (*6*), and small amounts also occur in peripheral nerves (*6*). An enzyme, hydroxyindole-*O*-methyl transferase, required for the synthesis of melatonin is present exclusively in the pineal gland (*7*). Very small amounts of melatonin produce a lightening effect on amphibian melanocytes (*6*), but melatonin has not been studied in that class. Recently, Baschieri *et al.* have reported that when rats were given large doses (150 μg) of melatonin daily for 10 days, the increments in thyroid-cell height and I^{131} uptake produced by methylthiouracil were diminished (*8*).

Table 2. Effect of melatonin on the estrous cycle of the rat. Animals received 0.2 ml of diluent or 20 μg of melatonin, intraperitoneally. Vaginal smears were taken daily after vaginal opening and 14 to 19 smears were taken from each animal. Results are expressed as the percentage of the total number of smears indicating estrus which were taken from each animal. The incidence of estrus in rats receiving melatonin differed significantly ($p \leq .01$) from that of animals receiving placebo.

Smears indicating estrus (%)	Rats (No.)	
	Control	Melatonin
0–20	0	3
21–40	2	5
41–60	7	3
61–80	2	0
81–100	0	0

Our experiments show that some of the effects of the pineal gland on gonad function might be mediated by melatonin. Immature female rats were given placebo, 50 μg of serotonin, or 1 to 20 μg of melatonin, intraperitoneally or subcutaneously, daily for 28 days. In rats receiving melatonin, there was a delay in spontaneous vaginal opening, and a highly significant decrease in ovarian weight (Table 1) and in the incidence of vaginal estrus (Table 2). As little as 1 μg of melatonin, administered subcutaneously, caused a significant decrease in the weight of the ovary. Larger doses of serotonin, the precursor of melatonin, produced none of the vaginal and ovarian effects but caused a significant increase in adrenal weight. Neither compound altered body or uterine weight.

Tritiated melatonin (*9*), when administered to four cats intravenously, was selectively taken up in endocrine and peripheral nervous tissues, especially in the ovary and the pineal gland. One hour after administration, the concentration of H^3-melatonin in the ovary was 5 to 25 times that in the peripheral tissues, 2 to 3 times that in the thyroid, pituitary, and adrenal glands and the peripheral nerves, and one-third that in the pineal gland.

When 100 rats were kept in constant or normal light for 4 weeks and then given H^3-melatonin intravenously, the uptake by the pineal gland and ovary was significantly reduced. Vaginal smears of these animals showed an 85 percent incidence of estrus, as compared with 50 percent in animals exposed to normal diurnal variation. When estrous rats, exposed to constant light, were given melatonin (10 μg subcutaneously), the incidence of estrus was reduced to 45 percent, while animals given diluent alone showed no change. This effect was observed for only the first day after injection; subsequently, animals that received melatonin returned to a normal estrous cycle. It was not possible to inhibit the onset of light-induced persistent estrus by means of daily injections of 10 μg of melatonin.

Thus, melatonin appears to satisfy the classical criteria for a hormone. (i) It is produced by a specialized glandular structure: only the pineal gland has the enzyme required for its synthesis (*7, 10*). (ii) It is released into the circulation: melatonin is endogenously present in peripheral nerve, a tissue which does not make it but can take it up from the circulation. (iii) It has an effect on a distant target organ: it alters such gross factors as ovarian weight and the estrous cycle. (iv) It is not synthesized by the target organ; and hydroxyindole-*O*-methyl transferase could not be detected in rat or human ovary. (v) It is taken up by the target organ from the circulation. Melatonin is taken up by the brain, and concentrated by the pituitary and ovary; thus, its effects on ovarian weight and function could result from an action at any of these sites.

There is considerable evidence to show that the state of the pineal is related to environmental changes. Variations in day length produced changes in pineal cytology and serotonin content (*11*); exposure to constant light altered pineal size, cellular morphology, and chemical content. It is possible that the physiological disposition and actions of melatonin might be influenced by light (*12*).

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