tions but deviates when the concentration is above 10 g/100 ml; this is expected since Huggins' equation is valid only at low concentration.

It is very important to remove all cell debris from solutions of RBC contents before attempting to measure the flow properties of the solutions. Solutions prepared with cell debris in them were non-Newtonian and showed apparent viscosities (at 20 per second shear rate) 50 to 100 times that of the debris-free solutions of the same hemoglobin concentration. In some cases, solutions containing debris showed rheopectic flow behavior due to the flow-induced aggregation of debris with time.

These experiments were performed with a saline solution as the continuous medium rather than with plasma. Erythrocytes suspended in plasma aggregate at low shear rates. Except for cases where the hematocrit is very close to zero or 100 percent, this causes the apparent viscosity of human blood at a given shear rate to be much larger than the apparent viscosity of the same RBC's suspended in isotonic saline at the same hematocrits (*7*, *11*). Also, experiments performed with crystallized human hemoglobin (Nutritional Biochemicals Corporation) dissolved in plasma showed such solutions to be Newtonian with relative viscosities close to the relative viscosities of the hemoglobin solutions described here (*11*). Consequently, at low shear rates, the ratio of the apparent viscosity of blood at a given shear rate to the viscosity of a solution of hemoglobin in plasma will be equal to or larger than (depending on hematocrit) that shown for the same fluids with isotonic saline as the continuous phase. The discrepancy will be largest at lower shear rates.

It should also be noted that the use of smooth viscometer walls probably results in the RBC suspensions with very high hematocrits showing lower shear stresses at a given shear rate than is correct (*12*). If true, this would only reinforce the main conclusion of this work.

With these findings, one cannot tell if the heart work is lower with the hemoglobin in RBC's than it would be if the hemoglobin were dispersed in solution. While our data indicate that the contribution to the heart workload from blood flow in the larger blood vessels is lower when the hemoglobin is in solution, this cannot be said about the contribution from flow in the smaller vessels. The continuum model for blood flow fails in the smallest vessels, but the size of the vessels where the failure is first noticeable is not known. Since the major drop in pressure in the circulatory system occurs in such small vessels, further discussion on this question must await data on blood flow in vessels ranging in size from 5 to 100 μm.

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Brain Serotonin Concentration: Elevation Following Intraperitoneal Administration of Melatonin

Abstract. *The intraperitoneal administration of melatonin to rats caused an increase in brain serotonin concentration, especially in the midbrain. This effect could be demonstrated within 20 minutes of melatonin administration and was not associated with changes in norepinephrine concentration.*

Melatonin (5-methoxy-*N*-acetyltryptamine) is produced in mammals only in the pineal gland (*1*, *2*). The synthesis of this compound is inhibited by environmental lighting and stimulated in darkness (*3*); information about the state of lighting is transmitted to the pineal by a special pathway involving the eyes, the inferior accessory optic tracts, the superior cervical ganglia, and the sympathetic innervation of pineal parenchymal cells (*2*, *4*). Because the rate of melatonin synthesis varies with a 24-hour rhythm in response to the light-dark cycle (*5*), it has been suggested that the pineal functions in mammals as a "biologic clock" or timing apparatus which emits an endocrine signal whose amplitude varies as a function of time of day (*6*).

The administration of melatonin to experimental animals produces both endocrine and neural effects (*2*). Melatonin injections cause a rapid fall in the concentration of the melanocyte-stimulating hormone (MSH) in the pituitary gland (*7*); they have also been reported to modify pituitary and serum gonadotropin levels (*8*) and the size and functional activity of rat gonads (*9*). Effects of melatonin administration on neural function include (i) the induction of sleep and of electroencephalographic changes in cats and (ii) the potentiation of hexobarbital sleeping time in mice (*10*). Since circulating melatonin enters the brain with little difficulty (*11*) and melatonin implants in the midbrain or median eminence also modify pituitary-gonadal function (*12*), it has been suggested that neuroendocrine centers in the brain constitute loci at which melatonin acts to produce its endocrine effects (*11*, *12*). We now report that intraperitoneal injections of melatonin are followed by a rapid rise in the concentration of brain serotonin. The greatest effects of melatonin administration may be seen in the midbrain, that portion of the brain which contains most of the cell bodies of the central serotoninergic neurons (*13*).

Female Sprague-Dawley rats (180 g) received intraperitoneal injections of melatonin dissolved in dilute (2 percent) ethanol in a final volume of 0.1 ml. Control animals received only the ethanol solution. Animals were caged individually and kept under standard laboratory lighting conditions (lights on from 6:00 a.m. to 6:00 p.m.; light provided by cool white fluorescent bulbs yielding approximately 27 to 54 mphot at the level of the animals); they were

Table 1. Changes in serotonin content of various brain regions after melatonin administration. Rats received 150 μg of melatonin intraperitoneally. Data are given as the number of micrograms of serotonin per gram of tissue ± standard error of the mean.

Region	Serotonin in tissue after melatonin administration (μg/g)			
	0 min	20 min	60 min	180 min
Midbrain	0.56 ± .01	0.65 ± .09	0.70 ± .05*	0.78 ± .07†
Hypothalamus	1.67 ± .14	1.87 ± .13	2.35 ± .27*	1.41 ± .05
Cerebral cortex	0.99 ± .03	0.85 ± .03‡	0.84 ± .03‡	0.97 ± .05

* $P < .05$. † $P < .02$. ‡ $P < .01$.

given free access to Purina Chow and water. Melatonin was administered to rats between 10:00 a.m. and noon, and groups of five animals were killed at intervals. The brains were quickly removed without the pineal, dissected (*14*), and frozen on dry ice until they could be assayed fluorometrically for serotonin (*15*).

In one experiment, rats received 500 μg of melatonin and were killed 1 hour later. Brains of treated animals contained 0.53 ± .027 μg of serotonin per gram of tissue, but those of control rats contained only 0.40 ± .031 μg per gram of tissue ($P < .01$, *t*-test). Studies in vitro in which various amounts of melatonin were added to brain extracts demonstrated that this rise in brain serotonin concentration was not an artifact caused by fluorescence of melatonin retained in the brain tissue. Melatonin treatment had no effect on concentration of norepinephrine in the brain; brains of treated rats contained 0.38 ± .04 μg per gram of tissue while those of control rats contained 0.39 ± .04 μg per gram of tissue.

Serotonin is widely distributed in the rat brain (*16*). In most of the brain the amine is located within nerve endings; however, much of the serotonin in the midbrain is present in cell bodies of serotoninergic neurons (*13*). To determine whether melatonin acted primarily on the serotonin in cell bodies or in nerve endings, we examined the effects of administered melatonin in the midbrain and in several other regions of the brain. Midbrains of rats killed 90 minutes after receiving 150 μg of melatonin contained 63 ± 13 percent more serotonin than those of control animals ($P < .001$, *t*-test); the concentration of the amine within the hypothalamus also rose, but not significantly. Concentration of serotonin in the cerebral cortex and the olfactory bulb and tubercle remained essentially unchanged.

The temporal sequence with which serotonin concentrations changed in various brain regions was examined in rats treated with 150 μg of melatonin and killed 20, 60, or 180 minutes later (Table 1). The earliest effect was in the cerebral cortex; 20 minutes after administration of the methoxyindole, serotonin content had declined by 14 percent. After 60 minutes, the cerebral serotonin concentration was still depressed, but the concentration of the amine in the midbrain and the hypothalamus had risen significantly. After 180 minutes, cortical and hypothalamic serotonin concentrations had returned to normal, but that of the amine in the midbrain was still rising.

The mechanism by which melatonin increases the serotonin content in the midbrain is not clear. Possibly melatonin has more than one effect on serotonin-containing neurons. For example, it might initially cause the serotonin concentration in nerve endings to decline (by liberating the amine or by facilitating its metabolism within the nerve) and subsequently stimulate serotonin synthesis in the region of the cell body. Alternatively, melatonin might elevate brain serotonin concentration by inhibiting the release of the amine, enhancing its reuptake, or inhibiting its intraneural or extraneural metabolism.

The effect of melatonin injections on brain serotonin levels is not the same for all brain regions. Other factors influencing this effect may include the time of day at which melatonin is administered, and the interval between injection of the indole and the killing of the animal. The dose of melatonin and its route of administration may also be important; very large intraperitoneal doses (500 μg) appear to be less effective in raising brain serotonin concentrations than smaller doses. The intravenous injection of as little as 5 μg of the indole increases the serotonin in the midbrain.

In the biosynthesis of melatonin and other methoxyindoles, the *O*-methylation of serotonin derivatives by the pineal allows them to cross into the brain from the circulation (*11*). Our data indicate that melatonin acts within the brain to modify the metabolism of its structural analog serotonin. The loci at which melatonin produces its greatest endocrine effects (the midbrain and the hypothalamus) are also the sites at which circulating ^{3}H-melatonin is most highly concentrated in the brain (*17, 18*).

Serotoninergic axons arising from cell bodies in the midbrain provide a major input to the medial forebrain bundle (*13*) and thus might be expected to modify the activity of neuroendocrine centers in the medial hypothalamus. Both the neural and the endocrine effects of administered melatonin may be related to the changes that this compound produces in the functional activity of the central serotonin-containing neurons.

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