lar in histology to human thymomas, which are often associated with various autoimmune disorders (*11*). One action of MC may be to interfere with the thymic regulatory effect.

Of equal interest is the rising occurrence of autoimmune thyroiditis in the Buffalo strain rat with increasing age. It suggests diminishing thymic control during aging. Many human autoimmune disorders such as chronic thyroiditis seem to increase with advancing age. On the other hand, immunological potential generally is thought to decline with time. In a separate study, MC was found to depress the delayed hypersensitivity response to Bacillus Calmette-Guérin in Buffalo strain rats, although it did not significantly affect the immunoglobulin M or immunoglobulin G antibody response to sheep red blood cells. It would be useful to titrate thymus-dependent and thymus-independent immune reactivities during aging.

Another animal in which autoimmune disease occurs frequently, NZB mice, has also been reported to show an increased evidence of disease following neonatal thymectomy (*12*). However, the major autoimmune disorders of NZB mice, such as hemolytic anemia and immune complex renal disease, are the types most frequently associated with the products of B cells, that is, antibodies. In the rat, autoimmune thyroiditis has been transferred between histocompatible animals by viable lymph node cells but not by serum (*13*). It must presently be associated, therefore, with the cell-mediated immunological responses. The results presented in this report may require a reassessment of the roles of T and B cells in autoimmune tissue damage.

DAVID A. SILVERMAN*
NOEL R. ROSE†
Center for Immunology and
Department of Microbiology,
State University of New York at Buffalo, Buffalo 14214

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* Present address: Department of Tumor Biology, Karolinska Institute, Stockholm 60, Sweden.
† Present address: Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201.
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Pineal Gland: 24-Hour Rhythm in Norepinephrine Turnover

Abstract. *There is a 24-hour rhythm in the turnover of norepinephrine in sympathetic nerves innervating the pineal gland. This rhythm persists in blinded animals but is suppressed in normal rats by light. The rhythm in norepinephrine turnover generates the rhythms in pineal indoleamines and* N*-acetyltransferase.*

There are 24-hour cycles in the concentrations of serotonin (*1*), *N*-acetylserotonin (NAS) (*2*), and melatonin (*3*) in the pineal gland of the rat. The levels of NAS, the immediate precursor of melatonin, and of melatonin itself are higher at night than during the day, while the level of serotonin is higher during the day than at night. The reciprocal relation between the levels of serotonin and NAS is the consequence of a 24-hour cycle in the activity of serotonin *N*-acetyltransferase (NAT) (*4*), the enzyme that converts serotonin to NAS. At night, when the activity of this enzyme is 25 to 60 times higher than it is by day, serotonin is rapidly converted to NAS; thus, the former falls and the latter increases (*5, 6*). The cycles in NAT and serotonin are endogenous in that they persist in animals placed in constant darkness and in blinded animals (*4, 7*), although the phase of the cycles gradually shifts in such animals (*8*).

Sympathetic (norepinephrine-containing) nerves that arise in the superior cervical ganglia provide the major, if not the sole, source of innervation to the pineal gland (*9*). Denervation of the pineal gland by removal of the superior cervical ganglia abolishes the serotonin (*10*) and NAT (*11*) rhythms. Physiological or pharmacological stimulation (*12–14*) of the pineal β-adrenergic receptors during the day markedly increases the level of NAT. Conversely, the nighttime rise of NAT and NAS and the fall of serotonin are prevented or reversed by the administration of the β-adrenergic receptor blocking agent, propranolol (*5, 13*). These observations indicate that norepinephrine released from the sympathetic nerve terminals regulates circadian rhythms of indoleamines by stimulating β-adrenergic receptors on pineal cells. A 24-hour rhythm in norepinephrine in the rat pineal has been reported (*15*). Paradoxically, the norepinephrine rhythm in the pineal was not endogenous since it was abolished by blinding rats whereas the serotonin and NAT cycles were not (*16*). To provide a link between release of the sympathetic neurotransmitter and circadian rhythms, we studied norepinephrine turnover in the pineal gland at night and during the day in normal and blinded rats.

Osborne-Mendel rats (NIH strain) weighing 180 to 200 g were housed under diurnal lighting conditions (lights on from 6 a.m. to 6 p.m. and off from

Table 1. Daily rhythm in norepinephrine turnover in the rat pineal in normal and blinded animals. Half-lives ($t_{1/2}$) were calculated by multiplying the slope of the regression line by $\log_{10} 2$. The rates of flux were obtained by multiplying the slopes of the regression line by the steady-state level of norepinephrine. Data for norepinephrine content are means ± S.E.M.

Group	$t_{1/2}$ (min)	Norepinephrine per pineal (pg)	Norepinephrine efflux (pg/min)
Day	111	2450 ± 610	15.3
Day, blinded animals	117*		
Night	43	2660 ± 270	42.9
Night (lights on)	115		
Night, blinded animals (lights on)	43		

* This $t_{1/2}$ value in blinded animals during the day agrees well with the value of 113 minutes determined by measuring the rate of fall of norepinephrine after treatment with α-methyl-*p*-tyrosine (*22*).

6 p.m. to 6 a.m.). Bilateral orbital enucleations were performed under ether anesthesia. Blinded animals were used 3 to 7 days after surgery; during this period a marked (25-fold) difference between day and night NAT values was still present. Animals were killed by decapitation. Norepinephrine turnover was determined by measuring the rate of disappearance of intravenously administered [^{3}H]norepinephrine from the pineal gland. Denervated pineals take up only 5 percent as much norepinephrine as do intact glands, which indicates that the exogenous compound is taken up by sympathetic nerves (*17*). Rats were placed in a restraining cage, and 75 μc of [^{3}H]norepinephrine (6.48 c/mmole) in 2 ml of normal saline were injected into the tail vein of each animal. The animals were injected between 9 a.m. and 11 a.m. or between 9 p.m. and 11 p.m. Rats injected in the dark were removed from a lighttight enclosure with the aid of a dim red light and placed in a restraining cage, which was inserted into a black opaque fabric bag; the mouth of the bag was held closed about the tail of the animal with a purse string. The [^{3}H]norepinephrine was then injected with the aid of a small white light. At varying times after the injection, animals were killed and their pineals were removed and homogenized in 6 ml of 0.4*N* perchloric acid to which 50 μg of norepinephrine had been added as carrier. The perchloric acid homogenates were centrifuged, and the extracts were passed through alumina columns from which norepinephrine was eluted with 0.2*N* acetic acid (*18*).

The rate of disappearance of [^{3}H]-norepinephrine from the pineal during the day differed markedly from that at night (Fig. 1B). The half-life for [^{3}H]norepinephrine turnover in the pineal gland was more than twice as long during the day as at night. The amount of [^{3}H]norepinephrine taken up from the blood by nerve endings in the pineal gland during the day was not significantly different from that at night (Fig. 1B).

The nighttime fall in serotonin can be prevented by keeping the lights on at night (*1, 7*). Furthermore, NAT activity in the pineal falls precipitously when rats are placed in a light room at night (*13, 19*). However, when blinded rats are placed in a light room at night, NAT does not change from its nighttime value (*19*). To establish whether the daily rhythm in norepinephrine turnover is endogenous, we removed normal and blinded rats from their dark quarters at 9 p.m. and placed them in a light room. After 15 minutes, the rats received intravenous injections of [^{3}H]norepinephrine, and the rate of disappearance of the radioactive compound was determined. The rates of turnover of norepinephrine in blinded animals at night and during the day were identical to those in normal animals. Thus, the rhythms in pineal indole levels and NAT activity appear to persist in blinded animals because of an endogenous rhythm in norepinephrine turnover. The decline of [^{3}H]norepinephrine in glands of blinded rats placed in a light room at night was more than 2.5 times as rapid as that observed in normal animals treated in an identical manner (Fig. 1A). [^{3}H]Norepinephrine disappeared from the glands of normal animals exposed to light at night at a rate identical to that measured in normal and in blinded animals during the day (Table 1). Consequently, light slows the rate of turnover of norepinephrine in pineal sympathetic nerve endings; this reduction of turnover is associated with a rapid fall in pineal NAT activity and an elevation of serotonin level.

Norepinephrine and dopamine contents were measured at 6 a.m., 10 a.m., 6 p.m., and 10 p.m. in each of six pineal glands by a sensitive enzymatic-isotopic method (*20*). The norepinephrine contents (mean $\pm$ standard error of mean) at 6 a.m., 10 a.m., 6 p.m., and 10 p.m. were 3420 $\pm$ 390, 2450 $\pm$ 610, 1620 $\pm$ 215, and 2660 $\pm$ 270 pg per pineal. The 6 a.m. and 6 p.m. values were significantly different from one another ($P < .01$), a result confirming earlier reports of a 24-hour rhythm in norepinephrine (*15*). The pineal contained approximately one-fourth to one-sixth as much dopamine as norepinephrine. Because of the small amount of dopamine in the pineal, our determina-

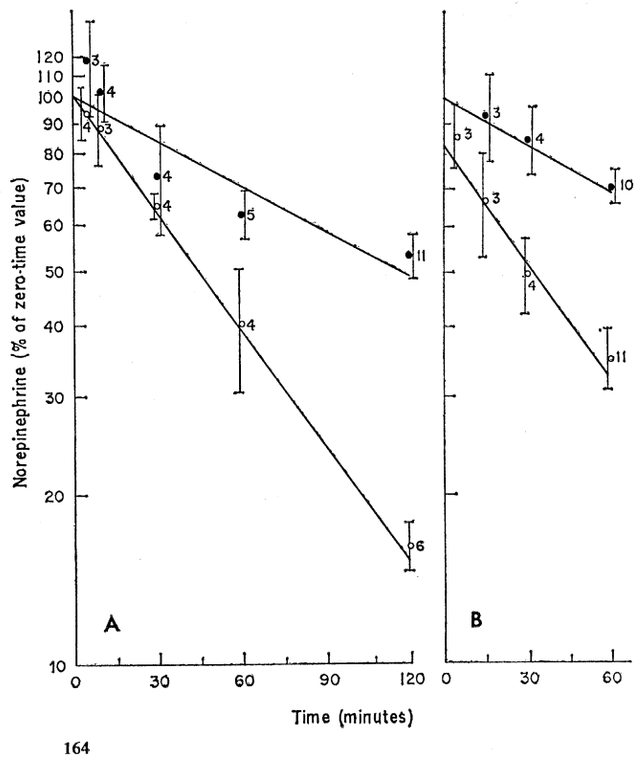

Fig. 1. Rates of disappearance of [^{3}H]norepinephrine from the rat pineal gland after intravenous injection of DL-[^{3}H]norepinephrine (75 μc). Each point is the mean for the number of determinations shown beside it. After logarithmic transformation of the original data, the following were calculated: linearity of regression, standard error of the regression coefficients, and significance of differences between regression coefficients. (A) Normal (●) and blinded (○) animals at night in a light room. The zero-time value corresponds to an initial [^{3}H]norepinephrine uptake of 8205 disintegrations per minute (0.576 pmole). The intercepts of the two lines were not significantly different but the two slopes differed significantly ($P < .05$, *t*-test). (B) Animals during the day (●) and at night (○). The zero-time value corresponds to an initial [^{3}H]norepinephrine uptake of 8580 disintegrations per minute (0.602 pmole). Comparison of the intercepts and slopes of the two lines by *t*-tests showed no significant difference between the former and a significant difference ($P < .05$) between the latter.

tions are not precise enough to ascertain that there is a day-night rhythm for this catecholamine.

A 24-hour rhythm in norepinephrine turnover in nerves innervating the pineal gland probably reflects diurnal variations in the release of the neurotransmitter. The daily rhythm in stimulation by norepinephrine of β-adrenergic receptors on pineal cells appears to be responsible for the circadian cycle in pineal indoleamine metabolism. That the rhythmic changes in pineal indoleamines persist in blinded rats but can be abolished by interrupting nerve impulses from the brain to the superior cervical ganglia suggests the presence of a "clock" in the central nervous system of the rat. Recent work (*21*) suggests that this clock resides in the suprachiasmatic nucleus of the hypothalamus.

MICHAEL BROWNSTEIN
JULIUS AXELROD

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014

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Erythrocyte Deformation in Human Muscular Dystrophy

Abstract. *Erythrocytes from patients with congenital muscular dystrophy exhibit dramatic surface deformation when observed with a scanning electron microscope. A similar alteration, but one affecting a smaller proportion of cells, occurs in the case of female carriers of the sex-linked Duchenne dystrophic condition. These observed changes in the erythrocyte surface may reflect a systemic defect in membrane properties.*

The fundamental lesion underlying congenital muscular dystrophy has been variously suggested to reflect a purely myopathic (*1*), neuronal (*2*), vascular (*3*), or autoimmune (*4*) mechanism. Investigations of the congenital disease in the laboratory mouse have provided evidence that effects of the lesion are systemic and are, moreover, discernible in altered membrane properties, including permeability to cations by liver mitochondria (*5*) and structural irregularities in the surface of erythrocytes (*6*). It is possible to question the comparability of the mouse disease, which is autosomal, to human Duchenne muscular dystrophy, which is sex-linked (*7*); it is therefore desirable to examine the human disease, as well, for signs of possible membrane alteration. We report here the results of a scanning electron microscopic examination of erythrocytes from patients suffering from several categories of human muscular dystrophy as well as erythrocytes obtained from carriers of the sex-linked (Duchenne) form.

Samples of blood were donated at the muscular dystrophy clinic at the Milton S. Hershey Medical Center by patients, their normal siblings and parents, and laboratory personnel. Blood was obtained by a finger stab and drawn into a heparinized capillary tube. A 10-μl portion was diluted tenfold in cold 0.9 percent NaCl and centrifuged at 900*g* for 3 minutes. Sedimented cells were resuspended in 100 μl of the same medium and centrifuged again. The sedimented cells were suspended in 3 percent glutaraldehyde containing 10 m*M* sodium cacodylate buffer (*p*H 7.4) and incubated for 2 hours at 22°C. Cells were then centrifuged, washed in 0.9 percent NaCl, and dehydrated in 70 percent ethanol and, after 5 minutes, 95 percent ethanol. Cells were finally diluted with 95 percent ethanol, spread

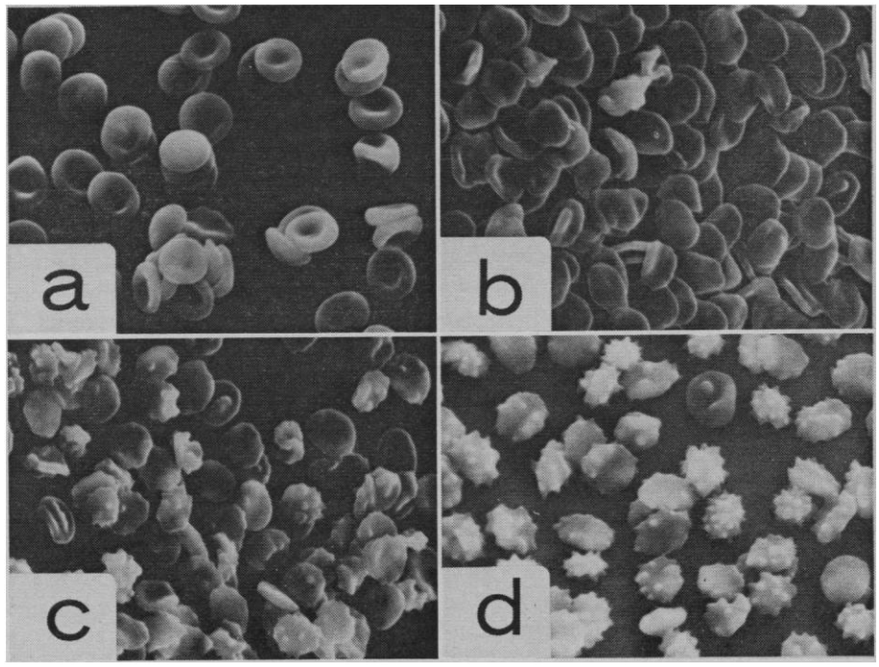

Fig. 1. Scanning electron micrographs of erythrocytes from normal subjects, patients with Duchenne muscular dystrophy, and probable carriers of Duchenne muscular dystrophy (×1500). Preparation of cells is described in the text. (a) Cells from normal subject; no saline wash. (b) Cells from normal subject; twice washed. (c) Cells from probable Duchenne carrier; twice washed. (d) Cells from Duchenne patient; twice washed.