melatonin did not increase the overall rate of responding during the punished FI (Table 1). Again, however, there was a noticeable increase in responding during the beginning of each interval (Table 1) even though responding was slightly suppressed at the end of each interval.

The behavioral effects of melatonin differ from those of other compounds whose effects on punished and nonpunished behavior have been studied. Several drugs which can produce sleep, including barbiturates and benzodiazepines, are able to increase both nonpunished and punished FI response rates (*8, 11*). It is not inconsistent that melatonin administration, which has been reported to induce sleep, should be followed by increased responding on a nonpunished FI schedule. Since melatonin did not increase the overall rate of responding during the punished FI, it can be distinguished from these sedative drugs. Imipramine, *d*-amphetamine, and phenothiazine derivatives can also increase nonpunished responding, but appear only to enhance the suppressant effect of punishment (*11, 15*).

A comparison of the effects of melatonin on key-pecking behavior with those, previously reported (*9*), of the two serotonin antagonists, methysergide and bromolysergic acid, and those of the serotonin agonist, alpha-methyltryptamine, further indicates the uniqueness of melatonin. The two antagonists increased the overall rate of responding during both punished and nonpunished schedules, while the agonist had only rate-decreasing effects. It would appear that the behavioral effects of melatonin are not simply due to its mimicking or antagonizing the action of serotonin.

The present study demonstrates that the peripheral administration of melatonin produces effects on behavior which vary with the schedule of reinforcement. These results are consistent with the proposal that serotonin derivatives found in the pineal gland can modify central nervous system function and support a role for the pineal in the modulation of behavior.

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Maternally Derived Transferrin in Pigeon Squabs

Abstract. *With the use of genetically marked transferrin, a major portion of circulating transferrin from a newly hatched squab was found to be derived from the mother through the egg. The transfer is not through the parental crop milk. The squab does not accumulate enough transferrin of its own making to be detectable until it is about 8 days old. The maternally derived protein remains detectable until 14 days after hatching. The squab actively synthesizes a portion its own transferrin from hatching onward.*

Transferrin is a nonheme iron-binding protein (*1*) that is widely distributed throughout the chordates from lamprey to man (*2*). One of its principal functions is the transport of iron to reticulocytes for incorporation into hemoglobin (*3*). It may be important in nonspecific resistance to disease, as well as for its iron transport function. Transferrin has been found to be polymorphic in most species, the products of different alleles usually differing in electrophoretic mobility (*4*).

Although the development of transferrin has been studied in several mammalian species, including mouse, rabbit, and man (*5*), I am not aware of any reports on developmental studies of transferrin in birds. In mammals, there is very little transport of transferrin to the developing fetus (*6*); in this, transferrin differs from certain immunoglobulins, which are known to be transported intact to the embryo.

Pigeons have been shown to be polymorphic at a transferrin locus, and have only two reported variants, Tf^A and Tf^B (*7*). The alleles are inherited as autosomal codominants, and in populations so far examined they are present in nearly equal frequency. Transferrin is found in the pigeon blood, egg white, egg yolk, crop milk, and probably in the semen. In all these fluids it is under the control of the same gene. In pigeons, which differ in this respect from chickens (*8*), the electrophoretic mobility of transferrin in all these fluids is identical. In this report, therefore, transferrin derived from egg white will not be distinguished by the specific term ovotransferrin, which is often used.

Pigeons (*Columba livia*) were maintained in the laboratory in mating cages (1 pair per cage) or in large fly-pens. Eggs were checked daily and the day of hatching was designated as day 0.

For adults and squabs over 4 days old, when more than 0.5 ml of blood was to be removed, the birds were bled from the brachial vein by syringe and needle. The blood was allowed to clot and the serum was collected. For younger birds, blood was collected directly onto filter paper for electrophoresis. In order to determine whether this would give consistent serum patterns, several squabs were killed by exsanguination and their serums were collected. The results for collection by filter paper and by syringe were identical.

Transferrin typing was carried out on horizontal starch gels by the use of tris–citric acid buffer (*p*H 7.5) in the gel and borate buffer (*p*H 8.7) as electrolyte (*9*). An ice pack was placed on top of the gel to prevent excessive heating; the gels were stained with Coomassie blue.

Antibodies to pigeon transferrin were

prepared in rabbits. Approximately 5 mg of purified pigeon transferrin in Freund's complete adjuvant (Difco) was injected into toe pads. Four weeks later, 2 mg of this transferrin in 1 ml of saline was injected intraperitoneally; and 3 days after that, 3 mg in 1 ml of saline was injected intravenously. The rabbits were bled 1 week after the last injection. When assayed by immunoelectrophoresis against pigeon normal serum, the rabbit antiserum gave a single band in the transferrin position.

For determination of the synthesis of transferrin by newly hatched squabs, the squabs were injected intraperitoneally with [^{3}H]leucine (specific activity, 38.5 c/mmole; New England Nuclear) and allowed 20 minutes to incorporate the isotope. The squabs were killed; their livers were washed in ice-cold phosphate buffered saline (PBS) (*10*) and homogenized in a Waring Blendor for 2 minutes in 15 ml of PBS. The homogenate was centrifuged 1 hour at 30,000*g* and the pellet was discarded. A portion of the supernatant was precipitated with an equal volume of 10 percent trichloroacetic acid (TCA) and counted in a toluene omnifluor scintillation mixture. One-half milliliter of supernatant was reacted with 0.5 ml of antiserum for 1 hour at room temperature, and then for 2 days at 4°C. The pellets were washed four times in cold PBS, precipitated with TCA, filtered, and counted as above.

The following matings were made to test for maternally derived transferrin: Tf^A/Tf^A ♀ × Tf^B/Tf^B ♂; the reciprocal Tf^B/Tf^B ♀ × Tf^A/Tf^A ♂; and Tf^A/Tf^B ♀ × Tf^A/Tf^A ♂. In the AA ♀ × BB ♂ mating the squab is genotypically AB. However, until 8 days after hatching it appears to have only A type transferrin in its serum, as assayed on starch gels. On the eighth day, the squab's own type becomes detectable. A similar result is obtained in BB ♀ × AA ♂ matings, the offspring showing only B type until 8 days. In AB ♀ × BB ♂ matings, although half the squabs are homozygous BB, all show the heterozygous phenotype until they reach 14 to 16 days of age.

Because transferrin is found in crop milk produced by both the male and the female, protein transfer by this route could affect the results. One pair of squabs of AB genotype was separated at hatching; one squab was put with the A parent, the other with the B parent. Both squabs followed the same pattern; that is, the squabs showed the maternal phenotype regardless of the source of crop milk.

Table 1. Active synthesis of transferrin by squab liver. Phosphate buffered saline (0.5 ml) and 0.5 ml of liver homogenate were centrifuged at 30,000*g*. The supernatant and 0.5 ml of the indicated serums were reacted, pellets were washed and precipitated with TCA as described in the text. The values are given in counts per minute per filter and represent the average of duplicate portions of the same homogenate, subjected to the same treatments.

	Squab No. 1 (0 days, 17 g)	Squab No. 2 (0 days, 17 g)	Squab No. 3 (4 days, 70 g)
Saline	74	30	221
Rabbit normal serum	195	105	204
Rabbit antiserum to human serum	95		
Rabbit antiserum to human serum + human serum	87		
Rabbit antiserum to pigeon serum	3721	4483	1919
Rabbit antiserum to pigeon transferrin	1068	1152	884
Supernatant (0.5/ml)	5688	4027	3038

These experiments demonstrate the transfer of a maternally derived intact protein through the egg, or as a formal possibility, a portion of the maternal protein-synthesizing machinery. Because the homozygous offspring of matings of AB ♀ × AA ♂ show the heterozygous type even though they do not possess the Tf^B gene, the protein's source can only be maternal.

These observations necessitated a study of active synthesis of transferrin by squabs. We tested squabs at various times after hatching for their ability to incorporate [^{3}H]leucine into protein reacting with monospecific antiserums to transferrin. The results are given in Table 1. Antiserum to human serum, alone and with added human serum, served as coprecipitation controls; these controls are not different from the rabbit normal serum or saline controls. The antiserum to transferrin precipitates about 20 percent of the total labeled protein in the supernatant, while the antiserum to pigeon whole serum precipitates almost all of the counts.

With the use of genetically marked transferrin, this study has shown that, in pigeons, newly hatched squabs derive part of their plasma proteins from their mothers. The transferred protein represents the major portion of the young squab's serum transferrin, even though the young bird is actively synthesizing transferrin during this period. This conclusion is reasonable when the growth rate of a young pigeon is considered. The squab, weighing about 16 g at hatching, doubles its weight in the first 2 days, and by 2 weeks it weighs 150 g (*11*). This rapid rate of growth involves a large increase in mass, and extensive synthesis is required even to maintain a constant concentration of transferrin.

All the observations are consistent with the following model. A large amount of transferrin is absorbed intact by the developing embryo, through the egg white or the yolk, or both. Some time prior to the day of hatching, the squab initiates its own synthesis of transferrin. The failure to detect the squab's own type in genotypically heterozygous squabs is a result of inability to detect low levels of transferrin. Unlike mammals, therefore, pigeons provide the major part of their offspring's transferrin supply by maternal transfer, even though the squab is also engaged in active transferrin synthesis.

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