er hand, when 16-day mammary epithelium was associated with salivary mesenchyme, salivary glandlike development was observed. The branching pattern was dichotomous rather than monopodial, and closely grouped complexes of adenomeres resembling salivary gland structure were found (Figs. 3 and 4). These observations are consistent with the finding of Kratochwil (*7*) that the morphogenetic pattern of mammary gland epithelium in vitro is controlled by the type of mesenchymal tissue with which it interacts. During pregnancy and lactation, remarkable lobuloalveolar development occurred in these grafts. Many new end buds and alveoli appeared along elongated ducts. Proliferation and enlargement of alveoli with increase in lumen sizes occurred during lactation, and dilated alveoli filled with milk were observed (Fig. 5).

Biochemical analysis of recombinants of mammary epithelium with salivary mesenchyme revealed significant quantities of the B protein of lactose synthetase in the grafts, relative to quantities found in lactating mammary glands in situ (Table 2). While values for two of the four recombinant samples were only about one-tenth those for normal lactating gland, the level in one recombinant was more than 60 percent of that in the normal lactating gland, and in another it was well above that in the normal lactating gland. Turkington *et al.* (*10*) have used the B protein of lactose synthetase as an indicator of lactational function, and by this criterion recombinants of the salivary mesenchyme with mammary epithelium clearly functioned as mammary glands, given the proper hormonal stimuli. It is probable that some of the variability among the recombinants can be attributed to the fact that they lacked proper outlets for their secretions, and hence lactocoeles developed. Depending on the extent to which these may have inhibited secretion at the time of sampling, variability in enzyme levels might be expected. The degree of success in achieving good recombination of epithelial and mesenchymal components is undoubtedly another factor affecting the uniformity of results.

These findings reinforce previous observations (*2*, *4*, *5*) showing that mesenchymal factors largely govern the form acquired by glands during morphogenetic processes, while biosynthetic function is qualitatively predetermined by the developmental history (that is, the organ type of origin) of the epithelial component. They do not exclude the possibility that qualitative or quantitative biochemical abnormalities may result from heterotypic recombinations of epithelium and mesenchyme. As yet there is no evidence available as to whether estrogen or prolactin receptors (or both) in mammary epithelium combined with heterotypic mesenchyme may differ qualitatively or quantitatively from hormone receptors of normal mammary glands. Whether or not heterotypic mesenchyme can affect the response of mammary epithelium to mammary tumor virus is also unknown. The work reported here was undertaken with questions such as these in mind, and with the object of developing further experimental methods to analyze the influence of stroma on the development of epithelial neoplasms in endocrine target organs.

Table 2. Lactose synthetase B protein activity in glands developing from 16-day mammary epithelium combined with 14-day salivary mesenchyme under the kidney capsule, and in mammary glands in lactating host mice.

Case No.	B protein activity (pmole/mg wet tissue/30 min)	
	Mammary glands in situ	Mammary epithelium plus salivary mesenchyme grafts
1	1918	290
2	2370	213
3	1716	2024
4	1176	752

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Melatonin: Effects on the Circadian Locomotor Rhythm of Sparrows

Abstract. *The continuous administration of low levels of melatonin via intraperitoneally placed Silastic capsules either (i) shortened the free-running period of activity or (ii) induced continuous activity in house sparrows (*Passer domesticus*) maintained in constant darkness. After the melatonin-filled capsules were removed, the period of the circadian rhythm of activity lengthened in rhythmic birds and normal rhythmicity was restored in continuously active birds. The results suggest that melatonin is involved in the physiological control of circadian rhythmicity in sparrows.*

There is now abundant evidence indicating that the pineal gland is involved in the control of circadian rhythmicity in house sparrows (*1*, *2*). Removal of the pineal gland abolishes the free-running circadian rhythm of activity in constant darkness, and transplantation of a pineal gland to the anterior chamber of the eye restores rhythmicity to pinealectomized arrhythmic birds. This latter result, and the fact that denervation of the pineal in situ does not abolish the free-running cir-

Table 1. Effect of melatonin-filled capsules on the circadian rhythm of locomotor activity in house sparrows. The period of the activity rhythm was considered to have shortened if it decreased by at least 5 minutes.

Melatonin capsule (mm)	Birds tested (No.)	No effect	Period shortened	Continuous activity
Five (empty)	7	7	0	0
Two (filled)	8	2	3	3
Five (filled)	19	2	6	11
Ten (filled)	3	0	1	2

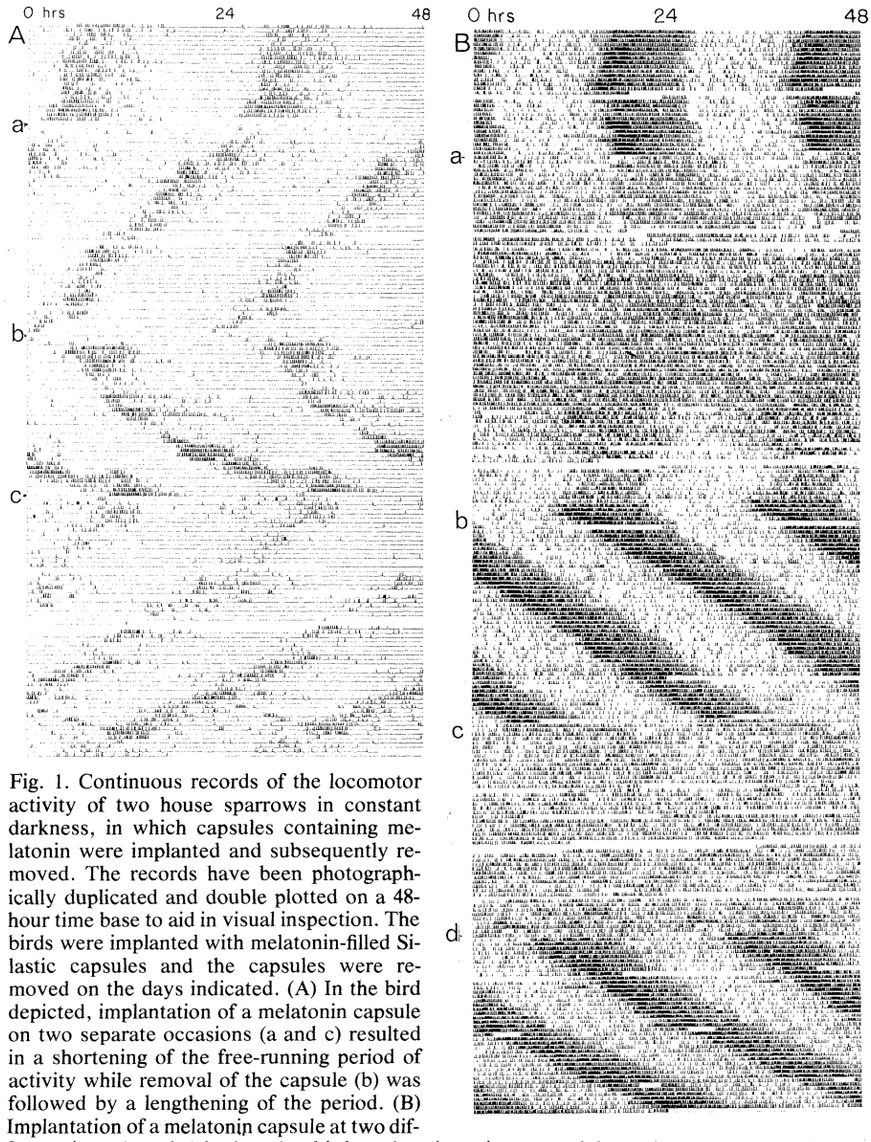

Fig. 1. Continuous records of the locomotor activity of two house sparrows in constant darkness, in which capsules containing melatonin were implanted and subsequently removed. The records have been photographically duplicated and double plotted on a 48-hour time base to aid in visual inspection. The birds were implanted with melatonin-filled Silastic capsules and the capsules were removed on the days indicated. (A) In the bird depicted, implantation of a melatonin capsule on two separate occasions (a and c) resulted in a shortening of the free-running period of activity while removal of the capsule (b) was followed by a lengthening of the period. (B) Implantation of a melatonin capsule at two different times (a and c) in the other bird produced continuous activity while removal (b and d) of the capsule restored normal rhythmicity. In both birds the free-running period in the absence of melatonin lengthens during the course of the experiment. This is a normal property of the behavior of the circadian system of untreated house sparrows in constant darkness which has been extensively described (*12*).

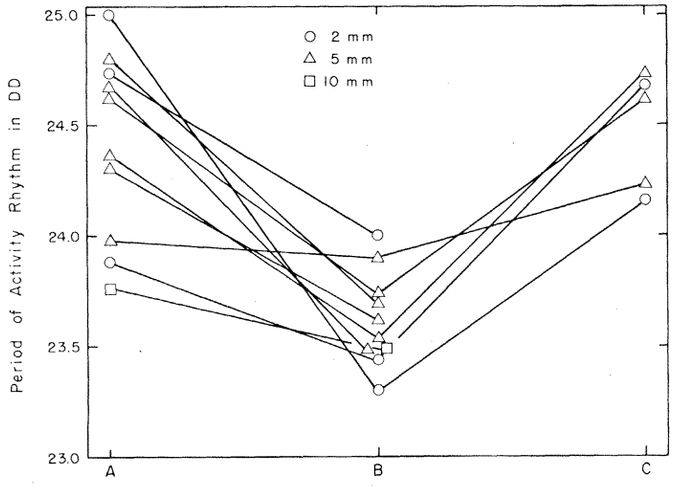

Fig. 2. Changes in the period of the circadian rhythm of activity in sparrows receiving melatonin-filled capsules that shortened the period of the rhythm. A, period (in hours) before intraperitoneal placement of melatonin-filled capsule; B, period while capsule was in place; C, period 10 days after the capsule was removed (*13*).

cadian rhythm (*2*), indicates that the pineal organ is coupled with other components of the circadian system hormonally rather than neurally. The search for a chemical agent of pineal origin that might be involved in the regulation of circadian rhythmicity led us to investigate the effects of exogenously administered melatonin on the free-running period of locomotor activity. Melatonin is an indoleamine synthesized in the pineal gland, and melatonin found in the blood and in the brain is thought to be of pineal origin (*3*). Our results demonstrate that exogenous melatonin has profound effects on the circadian rhythm of activity, and suggest that endogenously produced pineal melatonin may play an important role in the regulation or integration (or both) of the components of the circadian system.

House sparrows (*Passer domesticus*) were captured near Austin, Texas, moved indoors, and held individually in light-tight boxes. Locomotor (perch-hopping) activity was recorded as described (*4*), and the period of the activity rhythm was determined (by measuring the time interval between the onset of activity on successive days) throughout the study for each bird. Sparrows were initially maintained on a light dark (LD) 12:12 cycle (12 hours of light per day) for 3 to 4 weeks before being transferred to constant darkness (DD) for the duration of the experiment. After at least 3 weeks in DD, sparrows were implanted intraperitoneally with either empty or melatonin-filled Silastic capsules ranging in length from 2 to 10 mm. The amount of melatonin released from each capsule was determined by weighing the capsules before intraperitoneal placement and at the conclusion of the experiment (*5*). Melatonin was released from Silastic capsules placed in the peritoneal cavity at a constant rate for prolonged periods of time. The rate of release was directly proportional to capsule length [about 4 μg of melatonin per day per 5 mm of capsule length (*6*)].

The implantation of melatonin-filled capsules that were 2, 5, or 10 mm long had pronounced effects on the circadian activity rhythm of sparrows in DD (Table 1). Melatonin-filled capsules either shortened the period of the activity rhythm (Fig. 1A), or induced continuous activity (Fig. 1B) in 26 of 30 birds tested (87 percent). In contrast, implantation of empty capsules had no effect on the rhythms of any of the seven birds receiving them.

The effects of melatonin-filled capsules on the circadian rhythm of locomo-

tor activity were reversible. Figure 2 summarizes the data on period change in the ten birds which showed shortening of the period in response to the administration of melatonin. When melatonin-filled capsules were removed from five of these birds, the free-running period lengthened (Figs. 1A, and 2). When melatonin-filled capsules were removed from continuously active birds, normal rhythmicity was apparent soon after removal (Fig. 1B). Removal of empty capsules did not alter the rhythm of activity.

In chickens, Ralph *et al.* (*7*) observed circadian rhythms in the concentrations of melatonin in the pineal gland and the serum. Both rhythms had the same constant phase relationship with the locomotor activity rhythm. Serum and pineal melatonin concentrations were high during the period of locomotor inactivity (subjective night); melatonin concentrations were low when the birds were active (subjective day). Exposure to constant bright light appeared to abolish the circadian rhythm of melatonin found in the pineal of chickens (*8*) as well as the circadian rhythm of locomotor activity in sparrows (*4*). These correlations between melatonin concentrations and locomotor behavior do not necessarily imply casual relationships. Indeed, although such correlations have been obtained (*7, 9*) and the pineal gland is known to be involved in the regulation of circadian rhythmicity in birds, data from our study offer the first evidence that a particular pineal product can have consistent effects on circadian rhythms.

We have deliberately refrained from referring to the "continuous activity" produced most frequently by the larger implants, as arrhythmicity or aperiodicity. Power spectrum analyses of these data are not yet complete, and we have the (somewhat subjective) impression that we can detect periodicities within the records of continuously active birds. This distinction is important at this stage in our understanding in order to avoid a possibly erroneous equating of the effects of the larger doses of melatonin with those of either pinealectomy or constant bright light. Constant bright light produces true arrhythmicity in sparrows; constant dim light does not do so, but instead produces a shortening of the free-running period (*10*). Our data indicate that exogeneous melatonin either mimics the effect of constant dim light (shortening of free-running period) or produces an effect that resembles, but may not be identical with, the effect of bright constant light (continuous activity).

While our results suggest that melatonin normally plays a physiological role in the regulation of avian circadian rhythms, they do not allow us to distinguish among the several possible ways in which it might be involved. Melatonin, rhythmically synthesized or released (or both) by the pineal could be the hormonal signal whereby the pineal synchronizes other rhythmic centers. In contrast, it could function solely as a regulator, within the pineal, of other secretory events that have the above function. Alternatively, it might function in both capacities.

The complexities inherent in the experimental situation reported here make it premature to engage in detailed speculation about the precise role normally played by melatonin. The birds in our study retained their own pineals, which may or may not have continued rhythmic secretion of melatonin while the implants were in place. As we have not yet measured concentrations of circulating melatonin in either normal, pinealectomized, or implanted birds, we do not know how the implant affects those concentrations. We are ignorant of the site of action of melatonin and of its possible interaction with other agents, notably light, which affect the circadian system. The lack of this information is particularly serious in view of the fact that, in a previous study on the reproductive effects of melatonin in hamsters (*5*), it was shown that the direction of these effects depends both on dose and photoperiodic conditions. Nonetheless, we are encouraged by having available for the first time, a naturally occurring substance with which we can produce major effects on the central parameter of the circadian system, its free-running period (*11*).

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