results is given in M. L. Goff, thesis, Univ. of Virginia, 1965.

8. The effect of variation in intensity has been examined principally under conditions of continuous illumination. According to a recent statement of the "circadian rule," "With increasing intensities of illumination, the circadian period is shortened in diurnal (light-active) animals and lengthened in nocturnal (dark-active) animals." J. Aschoff, *Science* **148**, 1427 (1965). Intensity of illumination has been shown to influence also the phase relation between activity and synchronizing LD cycles of 24-hour or near-24-hour period: J. T. Enright, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 112. Some of our entrained animals exhibited regular phase lead, mostly of 2 to 4 hours, but only with LD cycles of less than 30-hour period.
9. There were seven instances of clear synchronization persisting to the end of such LD 18:18 or LD 24:24 treatment. The intervals (to the nearest hour) between the onset of activity during the last adiurnal cycle and successive activity onsets in continuous darkness were, respectively, 24, 24, 25, 25, 23, 24, 25 (Subject C after 18:18); 26, 23, 23 (Subject C after 24 : 24); 25, 20, 25, 26, 26, 24 (Subject E after 18:18); 36, 22, 21 (Subject E after 24:24); 24, 23, 22, 23, 24, 23, 24 (Subject F after 18:18); 25, 23, 25, 23 (Subject F after 24:24); 25, 23, 24, 23 (Subject L after 24:24).
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Adrenalectomy and Coat Color in Deer Mice

Abstract. *Adrenalectomy in prairie deer mice is followed by a profound darkening of the fur which occurs within 1 to 3 months. The phenomenon is most noticeable on the normally unpigmented ventral surfaces which turn dark gray or black. A possible mechanism for such hyperpigmentation would involve increased release of melanocyte-stimulating hormones.*

Partial dependence of coat color on endocrines is known for a variety of species. Examples are the well-documented effects of some steroids on avian breeding plumage and the effect of melanocyte-stimulating hormones on skin pigmentation in amphibia. Of a much rarer nature is evidence relating color to endocrine function in mammals. In this report we document a relationship between adrenal insufficiency and coat color in prairie deer mice *Peromyscus maniculatus bairdii*.

Colony-born deer mice (12 of each sex) were adrenalectomized at 9 to 10 weeks of age. Postoperative maintenance included 1 percent saline as a drinking solution and a single injection of 80 μg of cortisone acetate (intraperitoneally, in saline) immediately after the operation. An additional six deer mice of each sex (same age) were sham-operated, and another six were left as intact controls. Sham operations consisted of entry into the abdominal cavity only, with no postoperative injections of hormone. Normal coloration of adult *bairdii* is described by Hamilton (*1*) as: fur of the upper parts, brownish-gray mixed with darker hairs; fur of the under parts, gray at their bases with unpigmented tips, giving an all white appearance; and the above two patterns extended to the tail, making it sharply bicolored (hairs completely unpigmented on the ventral surface of the tail). Coat color in the present experiment was graded on an arbitrary 0 to 4 scale with class 0 equivalent to the normal animal just described. Classes 1 to 4 were separated as follows: (i) normal, but with bicolored feature of the tail less distinct and belly slightly more gray; (ii) ventral surfaces, including the tail, definitely gray; (iii) dark-gray ventral surfaces, tail evenly colored top and bottom, normally brown dorsal surfaces noticeably darker (often in blotches); and (iv) animal approaching black on all surfaces, with the exception of the hairs on the ankle region and on the male's scrotum. Classes 0, 2, and 4 are illustrated in Fig. 1.

Darkening of fur became noticeable about 1 month after removal of the adrenals and increased in magnitude for the next 1 to 2 months when a large degree of stabilization occurred. Table 1 shows frequency distributions of the various categories of animals 3 to 6 months after operations. No differences were apparent between intact and sham-operated controls. Adrenalectomy resulted in an obvious but somewhat variable darkening of the coat color (independent of sex). The majority of the adrenalectomized animals turned various shades of gray, a factor most obvious on the normally unpigmented belly and underside of the tail. Two animals turned almost coal black, and two darkened only slightly. No skin pigmentation changes were detected under gross examination. The completeness of adrenalectomy was confirmed in all animals included in Table 1.

Table 1. Frequency distribution of coat color classes of adrenalectomized or control deer mice 3 to 6 months after operations. Sham-operated and intact control groups were pooled.

Treatment	Sex	No.	Color classes				
			0	1	2	3	4
Controls	♂	12	10	2	0	0	0
	♀	12	11	1	0	0	0
Adrenalectomized	♂	8	0	0	4	4	0
	♀	12	0	2	4	4	2

Several possible mechanisms could be postulated to explain the observed changes in hair pigmentation. The most

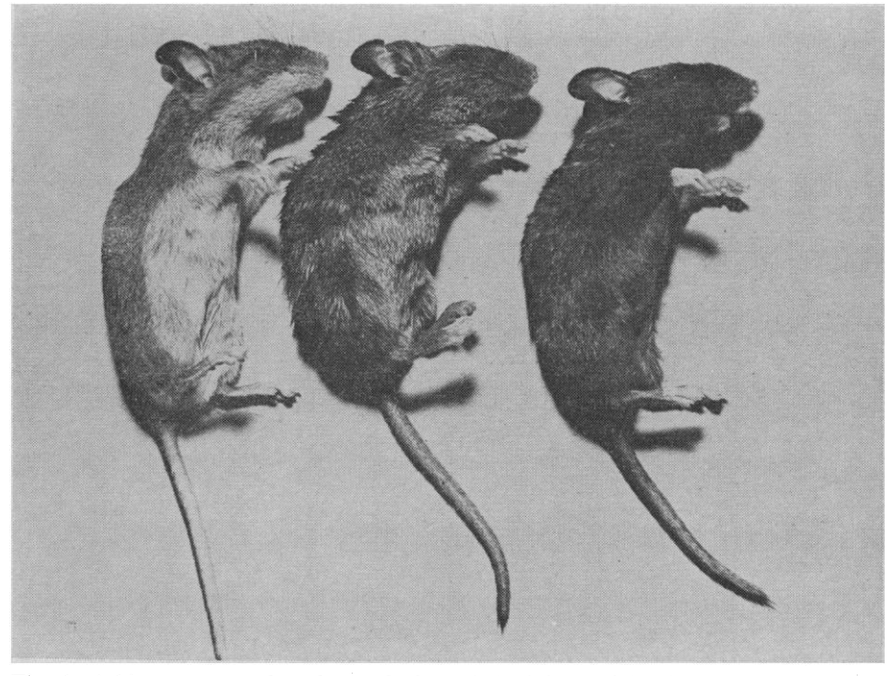

Fig. 1. Arbitrary coat color classes 0, 2, and 4 (left to right) in female deer mice. Class 0 represents a sham-operated control; classes 2 and 4 are adrenalectomized.

probable mechanism, however, would rely on increased synthesis and release of melanocyte-stimulating hormones (MSH) due to the loss of circulating adrenocortical hormones. A fall in circulating levels of corticosteroids would be followed by increased synthesis of corticotropin. A variety of types of MSH have now been found, and their interrelationships with corticotropin have been established (*2*). McGuiness (*3*) found increased concentrations of circulating MSH in the blood of adrenalectomized humans. From a comparative standpoint, in addition to the well-known effects of MSH on amphibia pigmentation, endogenous MSH results in a darkening of human skin color (*4*), and stress results in pigment changes in goldfish (*5*).

Adrenalectomy has been utilized as a common laboratory procedure in a wide variety of laboratory mammals during the past two decades. To the authors' knowledge, there has been no other report of such profound hair darkening following adrenalectomy in a mammal. This would seem to point to the deer mouse as having a relatively unique genetic background in this respect. This species, then, might prove a valuable tool for studying pigmentation-MSH relationships in mammals, particularly with reference to Addison's disease in humans (a result of chronic adrenal insufficiency and probably related to MSH secretion). Another possible facet of this finding is in the role of the adrenal in speciation of *Peromyscus*, one of the most common genera of rodents in North America. Since functional level of the adrenal cortex in rodents is related to population density (*6*) and a host of other environmental factors, differences in pigmentation patterns between populations and among individuals within populations could, in some cases, be due to stress-MSH effects.

F. H. BRONSON
S. H. CLARKE
Jackson Laboratory, Bar Harbor, Maine

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Cell-Free Protein Synthesis: Effects of Age and State of Ribosomal Aggregation

Abstract. *In cell-free extracts derived from* Streptococcus faecalis, *protein synthesis directed by endogenous messenger RNA increases as the culture ages. The increased activity is accompanied by an increase in the percentage of membrane-bound ribosomes and by a decrease in ribosomal monomers and subunits. These changes progress against a background of structural and compositional modifications in the membrane. Membrane modifications possibly related to endogenously directed protein synthesis in cell-free extracts include: (i) decreased specific activity of a membrane-associated polynucleotide phosphorylase capable of polysome degradation, and (ii) increased concentrations of certain phospholipids.*

Cell-free protein synthesis directed by exogenous polyribonucleotides is restricted to ribosomes free of native messenger RNA (*1*). In *Escherichia coli* such a situation obtains in extracts of cells harvested during the early logarithmic (log) phase of growth (*2*). Protein synthesis directed by native messenger RNA is contingent on a high complement of functional polysomes. Maximum activity for endogenously directed protein synthesis occurs in extracts of *Streptococcus faecalis* harvested during the late log phase of growth, just prior to the onset of the stationary phase (*3*). Since the log phase of growth is thought to contain a relatively uniform cell population, in which the rate of protein synthesis is constant and is determined by the ribosomal RNA content (*4*), we investigated the possible alterations in the functional state of messenger RNA in cell-free extracts obtained at various stages of the bacterial growth cycle.

Cells were grown in a phosphate-buffered medium of yeast extract and glucose as described previously (*3*). When cells were to be labeled with P^{32}, the phosphate concentration was decreased from 0.4 to between 0.02 and 0.04 percent, and the medium was supplemented with P^{32} (Squibb, carrier free, 1 μc/ml). Extracts were prepared by the lysozyme procedure (*3*), modified by the addition of 0.5*M* sucrose to the lysing medium. Protoplasts were disrupted by three passages through a large-bore hypodermic syringe. After the extracts had been centrifuged twice at 6000 rev/min to remove intact cells and debris, they were centrifuged at 105,000*g* for 2 hours to give a particulate and a soluble fraction. Fractions containing membranes were obtained from crude extracts sedimented at 30,000*g* for 30 minutes as described previously (*3*). All extracts were fractionated in a buffered medium consisting of $10^{-2}M$ tris-HCl, *p*H 7.6; $10^{-2}M$ magnesium acetate; $10^{-2}M$ β-mercaptoethanol; and 0.9 percent KCl.

Protein synthesis activity in cell-free extracts increases with the age of the culture throughout the log phase of growth and in the subsequent stationary phase (Table 1). The data of Table 1 were obtained with particulate fractions supplemented with supernatant fluid of extracts from cells in late log phase centrifuged at 105,000*g*. If supernatant fluid of extracts from cells in the stationary phase were used, incorporation of amino acids by all extracts was inhibited by over 50 percent.

When extracts of *S. faecalis* are centrifuged in 10 to 35 percent sucrose, three ribosomal fractions are obtained (*3*): (i) ribosomal monomers and subunits (Table 1); (ii) membrane-bound ribosomes containing active polysomes and membrane-bound 50*S* ribosomal subunits; and (iii) aggregates of 30*S* ribosomal subunits held by strands of recently synthesized messenger RNA. The free ribosomes and ribosomal monomers sediment in the upper half of the sucrose gradient; aggregates of 30*S* subunits sediment in a broad peak in the lower half of the gradient. The average sedimentation coefficient of the 30*S* aggregate fraction is 150*S* as estimated with *E. coli* ribosomes as standards. The membrane-bound ribosomes sediment with the pellet and are recovered as such after several washes in solutions containing $10^{-3}M$ Mg^{2+} (*3*).

Throughout the log phase of growth, the content of free ribosomes in the extracts progressively decreases while that of membrane-bound ribosomes increases. During the log phase, the increase in membrane-bound ribosomes parallels the increase of protein synthetic activity in the extracts. This finding agrees with that of a previous re-