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Received Oct. 8, 1968. P.S.E.B.M., 1969, Vol. 130.

# Melanocyte-Stimulating Activity Following Adrenalectomy in Deermice* (35597)

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Melanin deposition is inhibited to some degree by feeding black or brown rats a diet free of vitamin B; an effect that is reversible by adrenalectomy but not when adrenalectomized animals are provided with desoxycorticosterone acetate (1–3). A profound darkening of coat color follows adrenalectomy in prairie deermice (*Peromyscus maniculatus bairdii*) without prior dietary deficiency (4). This species of deermouse, whose normal adult coloration may be described as a brownish back and a white to light gray belly, turns dark gray or black within 3–10 weeks after removal of the adrenal glands. The purposes of the present experiments were twofold: (a) to determine the effect of hypophysectomy with or without adrenalectomy on coat color, and (b) to study the development of melanocyte-stimulating (M-S) activity in the plasma of adrenalectomized animals.

*Materials and Methods.* Adult male deermice were either adrenalectomized, hypophysectomized, or subjected to both types of operations. A single dose of 80 μg cortisone acetate (i.p., in saline) was given immediately following adrenalectomy and such animals were maintained thereafter on 1% NaCl drinking water. Hypophysectomized and hypophysectomized–adrenalectomized animals were maintained on a solution of 1% NaCl and 5% glucose. Reflectometer readings were obtained as indices of belly fur color for all animals that survived 7 weeks or longer (20 Adx, 6 Hypox, and 5 Adx-Hypox) and compared to similar readings obtained from 20 sham-operated and 80 intact males of the same approximate age. Reflectometer observations were made as averages of 3 readings on the lower half of the belly of conscious animals. The reflectometer (model 610, Photovolt Corporation) utilized a tristimulus filter and its meter was set to read 80 against a gray cardboard working standard (which, in turn showed a reading of 58 when the meter was adjusted to 100 against the white enamel standard provided with this machine).

* Supported by Public Health Research Grants No. HD-00767 from the Institute of Child Health and Human Development and No. AM-11195 from the National Institute of Arthritis and Metabolic Diseases. The principles of laboratory animal care as promulgated by the Council of the American Physiological Society are observed in this Laboratory.

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Another group of adult males was adrenalectomized, maintained as described above, killed by decapitation, and their plasma was collected at various times after the operations in order to study plasma M-S activity. A total of 4, 5, and 5 plasma pools (each pool consisted of plasma from 5 animals) was obtained at 12, 24, and 48 days postoperation, respectively. Control males were sham-operated and their plasma was collected and pooled in a like manner (5, 5, and 6 pools obtained at 12, 24, and 48 days, respectively). The M-S activity in the plasma was estimated following the general procedures of Shizume *et al.* (5) as modified to utilize smaller amounts (6). Three-point assays, usually utilizing 0.1, 1, and 10 $\mu$g volumes, were made on each pool of plasma and compared to a five-point standard curve based on a purified $\beta$-MSH generously supplied by Dr. I. I. Geschwind (assay potency 2000 U/mg). A lambda of 0.11 was obtained for the standard curve.

*Results.* Adrenalectomy was followed by a variable, but often profound, darkening of coat color while the fur lightened in color in males which were either hypophysectomized or adrenalectomized and hypophysectomized (Table I). Reflectometer readings for control animals (either sham-operated or intact males) ranged from 36 through 68 with an average of 52. Such readings correspond to a light gray through a dull white. Adrenalectomized males, on the other hand, had readings as low as 12 while hypophysectomized and adrenalectomized–hypophysectomized males each had readings as high as 85. The magnitude of these changes may be illustrated by comparison with average readings for two strains of laboratory mice: approximately 100 for albino SJL/J mice and 10–15 for black mice of the C57BL/6J strain.

The M-S activity was not consistently detectable in any pools of plasma from sham-operated animals. The M-S activity in the plasma of adrenalectomized males averaged 176 $\pm$ 17, 229 $\pm$ 35, and 243 $\pm$ 11 U/ml ($\pm$ SE) at 12, 24, and 48 days post-operation, respectively. Analysis of variance showed the differences in mean M-S activity at each period following adrenalectomy to be nonsignificant.

*Discussion.* Probably a combination of three factors prevented the consistent detection of M-S activity in the plasma of sham-operated controls: the small concentrations normally found in circulation, the low dilutions used (dilutions which were, in turn, necessary to avoid a maximum response to plasma from adrenalectomized animals), and nonspecific interference from plasma (6, 7). For comparison, however, M-S activity in the plasma of intact mice and dogs is usually less than 6 U/ml (6, 8). The M-S activity increased markedly in deermice following adrenalectomy. Individual pools of plasma yielded assay potencies of 132–341 U/ml. Such circulating levels are easily within the range necessary to cause fur darkening in at least one other species, a laboratory mouse strain also carrying the agouti gene. Geschwind (9) reported hair darkening in $C3HXAF_1$ hybrids following a series of $\alpha$-MSH injections that yielded plasma M-S

TABLE I. Frequency Distributions of Reflectometer Readings Taken on the Belly Fur of Adrenalectomized, Hypophysectomized, or Adrenalectomized and Hypophysectomized Male Deermice (7–8 weeks postoperation).

| | | ← Black : White → | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Type of operation | No. ♂♂ | 11–20 | 21–30 | 31–40 | 41–50 | 51–60 | 61–70 | 71–80 | 81–90 |
| Control[a] | 100 | | | 3 | 37 | 50 | 10 | | |
| Adrenalectomy | 29 | 6 | 10 | 8 | 5 | | | | |
| Hypophysectomy | 6 | | | | | 1 | 2 | 2 | 1 |
| Adrenalectomy and hypophysectomy | 5 | | | | | 1 | 2 | 1 | 1 |

[a] Values pooled from 20 sham-operated and 80 intact males since there was no effect of sham-operations on coat color.

activities of 47–640 U/ml depending upon time after injection. Another study in this strain utilized tumors that apparently secrete primarily α-MSH and correlated fur darkening with plasma M-S activities as low as 20–25 U/ml (7). The fur darkening following adrenalectomy in deermice, therefore, is pituitary-mediated and is correlated with a sufficiently high level of M-S activity in the plasma to result in the observed changes in melanin deposition. The time duration between adrenalectomy and darkening of coat color would then be a function of the time at which the next hair cycle began. The lightening of the belly fur following hypophysectomy (with or without adrenalectomy) may also be indicative of the role of the pituitary in normal melanin deposition in deermice although this change in reflection seemed to be, in large part, due to increased hair growth.

Two interesting questions arise because of the findings in deermice: (a) the identity of the pituitary hormone(s) responsible for darkening, and (b) why the deermouse should darken following adrenalectomy while other species apparently do not. Whether the pituitary factor resulting in fur darkening is an MSH or ACTH cannot adequately be answered short of isolation and identification studies since it is well documented that even synthetic ACTH has an intrinsic melanocyte-stimulating activity. With respect to the second question, the deermouse is not completely unique among mammals with respect to fur darkening following adrenalectomy since earlier studies showed darkening in black or brown rats in which the fur had first been lightened by feeding diets deficient in vitamin B (1–3) or copper (10). The relevancy of these findings to the phenomenon observed in the deermouse may be questioned, however, since the darkening effect of adrenalectomy, at least in copper-deficient rats, was duplicated by hypophysectomy. Rust (11) reported darkening of the hair of hypophysectomized short-tailed weasels (*Mustela erminea bangsi*) following injections of MSH and, to a lesser extent ACTH, thus implicating these hormones in the normal, seasonal color changes in this species. The effect in deermice is nevertheless outstanding considering the number of mammalian species that have been subjected to adrenalectomy. It was thought originally, on the basis of the above cited studies on weasels and agouti mice (9, 11) that the presence of hair follicles capable of different degrees of melanin deposition might be a prime factor. Adrenalectomy of adequate numbers of wild *Mus* or laboratory mice carrying either the agouti or dilute genes (CBA/J and DBA/J strains) resulted in no effect on coat color. It is apparent, then, that the unique effect in deermice is probably traceable to a somewhat different relationship between regulation of the pituitary principle by corticosteroids or to the nature of the pituitary hormone itself.

*Summary.* Adrenalectomy is followed by a somewhat variable darkening of fur color in deermice. The effect is so extreme in some cases (about 20%) that the normally brown-backed, light gray to white-bellied animals take on the same general appearance as black laboratory mice. The darkening effect does not occur in the absence of the pituitary and is correlated with an elevated M-S activity in the plasma.

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Received June 3, 1968. P.S.E.B.M., 1969, Vol. 130.