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# Plasma 17-Hydroxycorticosteroid Response to ACTH in *M. mulatta*: Dose, Age, Weight, and Sex* (33488)

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The rhesus monkey has been used frequently in studies of the adrenocortical response to various experimental manipulations; however, relatively few data are available concerning the monkey's normal plasma 17-hydroxycorticosteroid (17-OHCS) response to ACTH. Harwood and Mason (1) reported the response of five monkeys injected i.v. with 4 and 16 mg/kg body weight of an ACTH preparation of unspecified activity, and Migeon *et al.* (2), presented data for three monkeys injected i.v. with 0.4 and 4 U of ACTH/kg of body weight. One purpose of the present report is to present data on the 17-OHCS response to ACTH for an extensive series of monkeys. For this purpose, intramuscular depots of ACTH gel were used, both for the convenience of injection and for the longer maintenance of high ACTH concentrations.

As factors of potential importance in the adrenocortical response to ACTH, data were analyzed for changes with respect to age, sex, weight, and rearing conditions. Age was clearly of importance, since previous reports from this laboratory had indicated a plasma 17-OHCS response to ACTH in infant monkeys larger than that in adults (3, 4). It therefore seemed of interest to determine the age range over which this response declined to adult levels, and whether this response changed further in old adults. The possibility of a sex effect in terms of higher responses to ACTH by female monkeys had been suggested by a few observations in this laboratory (unpublished), while weight as an inversely related correlate of adrenocortical response had been suggested from data obtained on the rat (5). Finally, adrenocortical studies of rats subjected to early handling (6) have suggested that early experience is an important factor in adrenocortical function, thus raising the possibility that monkeys reared from birth in laboratory cages might differ in adrenocortical responsivity from monkeys reared in the wild.

*Procedure.* One hundred and two rhesus monkeys, *Macaca mulatta*, ranging in age from 2 days to 15 years were used. To determine ACTH dosages which would elicit maximal plasma 17-OHCS rises, four monkeys, two of each sex at 4–5 years of age, were tested (in 1961) for their response to 4 and 8 units/kg of body weight of Acthar gel (Armour, 40 units/ml) injected intramuscularly. Another four adults, two of each sex at 7–10 years of age, were tested (in 1966) for their response to 2, 4, 8, and 16 USP units/kg of body weight of ACTH gel (Organon, 80 USP units/ml) injected intramuscularly. Both of these groups were restrained in primate

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chairs for several days prior to and during the ACTH tests. The other 94 monkeys, comprising 12 different age groups, were tested for their response to i.m. ACTH while living in their home cages. Two of these age groups, infants and 30-month-old monkeys, were injected with 4 units/kg body weight; all other age groups were injected with 8 U/kg of body weight. Both of these doses produce essentially the same 17-OHCS response in adults for 4 hr following injections. Data for the 2–5-day-old monkeys have been previously published (3, 4).

For the drawing of blood, monkeys not in chairs were netted and held by hand. A control blood sample of 1–2.5 ml was withdrawn from the saphenous vein into a heparinized syringe immediately prior to the intramuscular injection of ACTH. Blood was drawn every hour thereafter for 4–7 hr and the monkeys were returned to their home cages or left undisturbed in their chairs between drawings. Blood was centrifuged immediately and the plasma was stored at −25°. The 17-OHCS were determined by a micro Porter-Silber method which has been demonstrated to measure mainly cortisol in the adult monkey (7).

*Results. Maximal adult response to ACTH.* There was no significant difference in the the plasma 17-OHCS rise over 7 hr to 4 or 8 units/kg of ACTH in the 1961 dose–response study, indicating that both of these doses produced a maximal adrenocortical stimulation. Similarly, the effect of 2, 4, 8, or 16 U/kg of ACTH did not differ in the 1966 dose–response study ($F<1$) when the 17-OHCS response was summed over 5 hr. However, in the 1966 study, there were minor declines in 17-OHCS levels at 5 hr post-ACTH for the 2 and 4 U/kg dosages (dose by hours $F = 3.92$, $df = 15/30$, $p < .01$). In both groups the 17-OHCS response to ACTH was higher in the female monkeys, although this was not statistically significant in a combined analysis of variance ($F = 6.31$, $df = 1, 4$, $p < .10$). On the basis of these analyses, the 1961 data were combined with the 8 and 16 U/kg data of 1966 for both sexes to obtain a curve reflecting the average maximal adult response to ACTH.

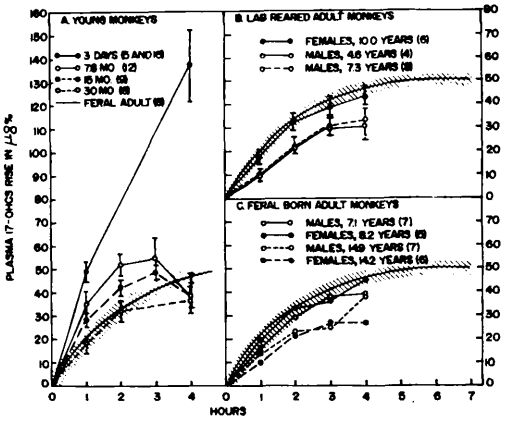


FIG. 1. Plasma 17-OHCS rises above basa 9:00 a.m. values following ACTH administration. Data are group means ± one SEM. The shaded curve represents ± one SEM around the mean response of 8 adult feral monkeys (4 males and 4 females) to maximally stimulating doses of ACH (see text). Numbers of subjects are indicated in parentheses. Where sex of the subjects is not indicated, the data represent both sexes in approximately equal numbers, and statistical analysis has indicated no sex difference. Basal 9:00 a.m. levels of plasma 17-OHCS were as follows for the various groups (μg/100 ml; mean ± one SEM): Panel A, the 1-hr point for 3 days of age (29.5 ± 5.4), the 4-hr point for 3 days of age (48.0 ± 4.4), 7.8 month (29.8 ± 2.3), 15 month (29.0 ± 3.3), 30 month (39.2 ± 5.6 at 8:00 a.m.), feral adult (34.6 ± 2.4); Panel B, 10.0 years (20.6 ± 0.9), 4.6 years (29.8 ± 3.6); 7.3 years (31.2 ± 2.5); Panel C, 7.1 years (33.9 ± 4.0), 8.2 years (25.1 ± 4.4), 14.9 years (23.3 ± 1.4), 14.2 years (26.3 ± 2.2).

The mean curve is shown in each of the three panels of Fig. 1 as a solid line with ± 1 SEM shaded (the SD around this curve was ± 5.4 μg/100 ml). This curve was negatively accelerated, having a 0–1 hr increase of 18 μg/100 ml, a 1–2 hr increase of 14 μg/100 ml, a 2–3 hr increase of 10 μg/100 ml and a 3–4 hr increase of 5 μg/100 ml. By 4 hr, the increase was within 6% of its maximum of 50 μg/100 ml.

*Effect of age.* The plasma 17-OHCS response following ACTH in the 3–5-day-old monkey was about three times higher than in the adult, declined to about twice the adult levels in the 6- and 15-month-old monkey, and was no higher than adult levels in the 30-month old monkey (Fig. 1, Panel A).

These young monkeys were all born and reared in the laboratory. Each group contained about equal numbers of males and females, for which the data were combined since the response to ACTH was not significantly different between sexes in any of these groups. It might also be noted that at 6, 15, and 30 months of age, the 17-OHCS concentration at 4-hr post-ACTH was lower than in the adult.

The 17-OHCS response to ACTH in adult, feral monkeys of about 8 and 15 years of age did not differ according to sex ($F < 1$, $df = 1,21$, $P > .10$), but there was a significant decline with age, as illustrated in Fig. 1, Panel C ($F = 5.80$, $df = 1,21$, $p < .05$). The response in the 15-year-old monkeys was approximately 60% of that of the 8-year-old adults. The response of 3 additional males averaging 4.8 years of age (not shown) was indistinguishable from that of the seven males of 7.1 years of age.

*Effect of laboratory rearing and sex.* The 17-OHCS response to ACTH in laboratory reared, adult females did not differ significantly from that of feral adults. However, two groups of laboratory-reared adult males both exhibited significantly lower responses averaging about 60% of that of the feral adults (Fig. 1, Panel B). Thus, only among adult, laboratory-reared monkeys did there appear to be a sex difference in adrenocortical capacity.

*Effect of body weight.* The sum of the 17-OHCS rises at 1, 2, 3, and 4 hr post-ACTH correlated −.409 (Pearson $r$) with age and −.288 with weight for 29 feral adults from 4.5 to 15 years of age, whereas weight and age correlated +.445 (the data for 25 of these monkeys is that averaged in Panel C of Fig. 1; the other data were taken from the three males averaging 4.8 years old, described above, and from an 11-year-old male whose age barred his data from inclusion in any of the groups). From these values, the partial correlation of adrenocortical responsivity with age (holding weight constant) was −.322 ($p < .05$, one-tailed) while partial correlation with weight (holding age constant) was −.056 (not significant). This analysis tended to corroborate the above noted decline in responsivity with increasing age of feral adults, and further clearly indicated that body weight was not a factor in this effect.

*Basal* 9:00 a.m. 17-OHCS *levels.* The legend of Fig. 1 lists the 9:00 a.m. 17-OHCS levels for all groups just preceding ACTH injection. These values did not appear to decline significantly with age, although the values at 14.2 and 14.9 years of age were among the several lowest observed across all these groups.

*Discussion.* The plasma 17-OHCS response of the 2.5–8-year old feral monkey was similar to that previously reported by others (1, 2) in terms of an average first hour rise of about 18 μg/100 ml. However, this response was found to be higher for younger monkeys and lower for older monkeys. There was no convincing evidence for a sex difference in this reponse in feral monkeys, since a tendency for the male response to be lower in the ACTH dose–response experiments was only significant at the 10% level and did not appear in the other feral groups tested. It was interesting, therefore, that the adrenocortical responsivity of laboratory-reared males appeared lessened, although that of the laboratory-reared females did not. The possible reasons for this have not been investigated, but the effect does suggest that the adrenocortical response of the monkey, like that of the rat, may be alterable by early experience.

The significant decline in adrenocortical responsivity with advancing age of feral monkeys is in line with data reported for aged cattle (8), for aged goats (9), and for the aged human (10). The functional significance of this has not been considered, nor do we yet have aged, laboratory-reared animals in which to investigate this effect.

The basal plasma 17-OHCS concentrations observed at 9:00 a.m. did not differ among monkeys as a function of age, suggesting stability of this aspect of pituitary–adrenocortical function. Considering the changes in *in vivo* adrenocortical responsivity over the life span, the stability of the basal levels was probably maintained by appropriate shifts in plasma ACTH titer.

The very large 17-OHCS response of infant monkeys to ACTH does not represent a

failure of cortisol metabolism, since these animals metabolized injected trace doses of cortisol-4-$^{14}$C at the normal adult rate (3). In considering the maximal response of infants and adults, the 17-OHCS rise at 4 hr was respectively about 2.8 and 2.7 times the rise seen at 1 hr. However, this relationship did not hold for the 7, 15, and 30-month- old monkeys, in which the maximal rise noted was less than twice as great as that noted at 1 hr. This seems likely to be related to the decline in 17-OHCS at 4 hr noted in these young monkeys, and might reflect differences in ACTH absorption or metabolism or an onset of a refractory stage of the adrenal cortex to ACTH. Clarification of this point must await determinations of dose response curves to ACTH at this age range.

*Summary.* Plasma 17-OHCS response to ACTH in *M. mulatta* of 2.5–8years of age has been found to average 18 μg/100 ml in the first hour and to increase more slowly subsequently, achieving a maximum rise of about 50 μg/100 ml in 4 or more hr. The response was 3-fold higher in infants, 2-fold higher in monkeys of 6 and 15 months of age, and only 60% as high in 15-year-old monkeys. There was no significant correlation of the response with weight, nor was there any significant sex difference except perhaps in male laboratory-reared monkeys in which the response was only 60% that of adult feral-reared monkeys or female laboratory-reared monkeys.

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