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Received February 1, 1965. P.S.E.B.M., 1965, v119.

Plasma 17-OHCS Response of the Infant Rhesus Monkey to a Noninjurious, Noxious Stimulus.* (30118)

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In a previous study(1), the 2-day-old rhesus monkey was found to have a resting plasma 17-hydroxycorticosteroid (17-OHCS) level of the same magnitude as adult animals. In addition, the 4-hour plasma 17-OHCS response to ACTH was 2-fold that of the adult, while the rate of cortisol metabolism was the same as the adult. In view of these data, which indicate that the infant monkey has a functional adrenal cortex, the present study was initiated to determine if all the other components of the central nervous system (CNS)—hypophyseal—adrenocortical axis also are functional in the 2-day-old monkey. The approach selected for this purpose was to determine if the newborn monkey would respond to a noninjurious but noxious stimulus. Such a stimulus could be assumed to be effective *via* some CNS mechanism acting to release ACTH, rather than by acting directly on the hypophysis or adrenal cortex.

The noxious, noninjurious stimulus chosen was that of bodily restraint and rotation. This choice was based upon our observation that such a stimulus induces a maximal rate of plasma 17-OHCS elevation in the adult monkey.

Methods. Eight two-day-old rhesus monkeys (*M. mulatta*) were restrained by being wrapped securely in cloth diapers and then manually rotated head over heels for one hour at approximately 3 revolutions per minute. This rotation rate produced no observable evidence of nausea or other vestibular disturbance. Blood samples were obtained from the saphenous vein immediately before and after one hour of restraint and rotation and always within 20 minutes of the hours of 9:00 and 10:00 a. m. As a control group, 6 additional 2-day-old rhesus monkeys were subjected to the blood drawing procedure only.

All blood samples were centrifuged within 30 minutes after withdrawal and the plasma frozen at −15°C. Plasma concentrations of 17-OHCS were determined by a micromodification of the Eik-Nes method(2).

Since the initial 3 of the eight rotated infants showed marked elevations of plasma 17-OHCS following rotation, it seemed apparent that the infant monkey was exhibiting a "stress" response. To assess the degree of this response relative to the maximal response of which the infant adrenal cortex was capable, studies on the remaining 5 of the rotated infants included a measurement of adrenocortical capacity as determined by the one-hour response to an intramuscular injection of 4 units/kg of ACTH (Acthar Gel, Armour). This dosage had previously been found to produce a maximal rate of plasma 17-OHCS elevation in the adult. The ACTH tests on the infants were conducted 2 days following rotation when the animals were 4 days of age. The response to ACTH at 4 days was assumed to be the same as that at 2 days of age since it was previously ob-

* Supported by a research grant from Nat. Inst. Health (FR-0167).

served that adrenocortical capacity in the infant monkey did not change significantly between 2 and 9 days of age(1).

Results and discussion. Both groups of infant monkeys had 9:00 a. m. plasma 17-OHCS levels in the normal adult range. The 8 rotated infants had a concentration of 30.8 ± 4.0 (S.E.) μg% prior to rotation and the 6 control infants, a level of 42.2 ± 5.4 μg%. These levels did not differ significantly ($t = 1.70$, $df = 12$, $p > .05$ by a 2-tailed test), because of variability among animals. Despite this variability, the rotated infants had a higher 10:00 a. m. 17-OHCS level, 56.6 ± 5.3 (S.E.) μg% than the 10:00 a. m. steroid level of the control monkeys of 42.4 ± 6.6 (S.E.) μg% ($t = 1.71$, $df = 12$, $p \cong .06$ by 1-tailed test), suggesting in terms of absolute plasma 17-OHCS concentrations a significant effect of restraint and rotation.

In view of the differences in 9:00 a. m. pretreatment 17-OHCS levels between rotated and control infants, it is probably more accurate to assess the magnitude of the effects of restraint and rotation, blood drawing and ACTH by using as a measure the one-hour change (from 9:00 a. m. to 10:00 a. m.) in plasma 17-OHCS. In these terms, all 8 of the restrained and rotated infants exhibited one-hour rises ranging from 10 to 30 μg% with a mean rise of 25.8 ± 3.3 (S.E.) μg%. In contrast, the 6 control infants subjected only to the procedure of blood withdrawal showed a mean change of + 0.2 ± 3.5 (S.E.) μg% in corticoid level. This difference between the elevation following restraint and rotation and the lack of elevation following blood withdrawal is clearly significant by t-test. Thus, by all measures, the 17-OHCS elevations were the result of the restraint and rotation and could not be ascribed to any trauma associated with the blood drawing procedure itself.

To assess the maximal one-hour 17-OHCS elevation ACTH was administered to 5 of the previously rotated infants and resulted in a mean 17-OHCS rise of 49 ± 4.6 (S.E.) μg%. This value was in keeping with the previously observed 4-hour elevation of 132 μg% in infants given ACTH(1), suggesting that the 17-OHCS response of infants to ACTH follows the same type of negatively accelerated rise over 4 hours which we have observed in the adult (unpublished). The elevation in 17-OHCS of 49 μg% one hour after ACTH administration was significantly greater than the mean rise of 21.6 ± 3.6 μg% after one hour of restraint and rotation in the same 5 infants ($F = 42.0$, $df = 1,4$, $p < .01$). That is, in these 5 infants the rise after the restraint and rotation was 44 ± 6 (S.E.) per cent as great as the rise following ACTH.

Two possible explanations can be offered to account for this less than maximal response by the infant to the stress of restraint and rotation: 1) restraint and rotation may not be a sufficiently strong stimulus to the infant to induce a maximal "mobilization" of the CNS-hypophyseal components which stimulate 17-OHCS production, or 2) the infant CNS-hypophyseal components, even when maximally mobilized, may not be sufficiently developed to fully stimulate the adrenal cortex.

In favor of the first possibility, some of the infants appeared to sleep at times during the restraint and rotation, in a manner suggesting minimal disturbance. This behavior was unlike the disturbed behavior exhibited by adult monkeys during restraint and rotation, and suggests that the infants may not be induced by the restraint and rotation to generate maximal CNS stimulation of the hypophysis and adrenal cortex. However, this is indirect evidence, and does not eliminate the possibility that the second explanation is true. Further studies are needed.

Finally, it should be noted that the mean one-hour elevation in 17-OHCS of 25.8 μg% following restraint and rotation in the infant is as large or larger than the maximal 17-OHCS rises after ACTH administration (approximately 10-30 μg%) observed by us (unpublished) and others(3 and 4) in adult animals. It seems reasonable therefore in view of its magnitude and reliable elicitation that the 17-OHCS elevation which the infants exhibited under restraint and rotation is evidence for a physiologically and psychologically meaningful stress response of infant monkeys to noninjurious but noxious stimulation.

Summary. Two-day-old rhesus monkeys, restrained and rotated for one hour, exhibited elevations of 25.8 ± 3.3 (S.E.) μg% in plasma nonconjugated 17-OHCS. This sizable and consistent increase was 44% as great as the one hour increase in plasma nonconjugated 17-OHCS following ACTH, suggesting either that the restraint and rotation was not sufficiently stressful or that the infant CNS-hypophyseal axis was not sufficiently developed to stimulate fully the adrenal cortex.

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Received February 1, 1965. P.S.E.B.M., 1965, v119.

Studies of Connective Tissue Permeability in Pregnant Rats.* (30119)

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Skin "permeability" appears to be increased in patients afflicted with rheumatoid arthritis(1). Since remissions of this disease are known to occur during pregnancy, seemingly related to altered steroid concentrations(2), it appeared interesting to examine the effect of pregnancy on dermal connective tissue permeability. Previous studies on hypophysectomized rats and adrenalectomized rats have already indicated that connective tissue permeability is in some way dependent or regulated by pituitary and adrenal hormones(3).

The experiments were performed on white rats of the CFN strain. The animals were 2 months old and impregnated on the same day. For measurements of connective tissue permeability a technique based on the intradermal diffusion of Evans blue dye was used (4,5). Since earlier studies showed that the permeability enhancing effect of L-ascorbic acid was inhibited in hypophysectomized rats as compared to animals with glands intact, the effect of this vitamin was tested on pregnant and nonpregnant (control) rats.

As in previous experiments(4,5,6), 0.05 ml of a 4% solution of Evans blue dye dissolved in saline (pH 7.3), with and without ascorbic acid (1 mg/ml), was injected intradermally into the abdomen of the animals. Three injections were made for dye alone (control) and 3 for ascorbic acid. For each animal, the areas of the spots produced were traced onto semi-transparent paper 30, 60, 120 and 180 minutes after injection. For pregnant animals, the permeability was measured on the 6th, 12th, and 18th day of gestation. The animals were then sacrificed and pregnancy was verified.

The results are given in Table I. The areas of the spots are expressed in mm^2. They represent the average values obtained on each group of animals at different periods. Since 3 spots were obtained on each animal with dye alone and 3 others with ascorbic acid, the results are thrice the number of animals used for the test.

The results show no difference in the rate of spread between control and pregnant animals (Table I). For dye alone, the areas of spots were the same in all groups. The permeability enhancing effect of ascorbic acid was also the same in both pregnant and control groups. Ascorbic acid as well as the dye alone showed no significant change in spread area with respect to time in both pregnant and control animals.

In conclusion, dermal connective tissue permeability, when tested by the spreading method, is not modified in pregnancy.

Summary. Connective tissue permeability was studied in pregnant and non-pregnant

* The work was supported by the U.S.P.H.S. Nat. Inst. for Arthritis and Metab. Dis., Grant A-4619, and The John Polachek Foundation for Medical Research.