

## Plasma Growth Hormone Response to Capture and Venipuncture in Caged Monkeys<sup>1</sup> (38285)

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(Introduced by N. H. Spector)

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Knobil reported some observations in 1966 which suggested that psychological or social factors may influence plasma immunoreactive growth hormone levels in the rhesus monkey (1). Additional evidence was later reported by Meyer and Knobil that exposure of monkeys to such sudden disturbances as telephone ringing, confrontation by unfamiliar persons, or pinching of the abdominal skin was often sensitively reflected in marked plasma growth hormone elevations (2). In studies of conditioned emotional disturbances in monkeys, we have also observed significant mean plasma immunoreactive growth hormone elevations in monkeys exposed to a standardized conditioned avoidance procedure (3). In an effort to make further systematic observations along these lines, a study was made of plasma growth levels before and following placement of monkeys in a restraining chair. Previous studies of 17-hydroxycorticosteroid (17-OHCS) responses have indicated that such factors as the novelty and partial immobilization associated with chair restraint represent potent psychoendocrine stimuli in the monkey (4). During the course of making "control" observations in caged-house monkeys prior to chair restraint, some unexpectedly frequent, rapid, and marked plasma growth hormone responses to the capture, handling, and venipuncture experience were observed and are the main basis of the present report.

*Methods.* Five male rhesus monkeys,<sup>1</sup> weighing between 12 and 16 lb, were used in these studies. Fasting blood samples were obtained at about 9 AM by saphenous venipuncture, using heparin-rinsed syringes. The heparinized plasma was frozen in 13 × 100 mm glass-stoppered, acid-cleaned Pyrex tubes until analysis for growth hormone concentration by radioimmunoassay (3).

The monkeys were housed in individual metabolic cages for 3 months in the same, small sound-resistant cubicles where they were eventually to be placed in the restraining chair. The cage-housed monkeys were captured by net, restrained on a foam rubber mattress, and blood samples were obtained every 2 weeks for a 10-week period prior to onset of chair restraint. Following placement of monkeys in the chair, blood samples were obtained at least twice weekly (Tuesday and Friday) for a total of 8 weeks of chair restraint. In order to minimize extraneous environmental stimuli, the chair restrained monkeys were kept individually in sound-resistant, dimly illuminated, power-ventilated booths which replaced their metabolic cages in the experimental cubicle. In the restraining chair, monkeys have freedom of arms and legs, can feed themselves, and have some limited rotary trunk movement. Other features of the chair apparatus have been described previously in greater detail (5).

*Results and Discussion.* All plasma growth hormone determinations from both cage-housed and chair-restrained periods are shown in Table I. A striking feature of the data is the high incidence of markedly elevated values, greater than 20 ng/ml, in the cage-housing period. There are 18 values during the cage-housing period, com-

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<sup>1</sup> In conducting the research described in his report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

TABLE I. Plasma Immunoreactive Growth Hormone Levels in Monkeys During Cage-Housing and Chair-Restrained Periods.

	Plasma Growth Hormone Levels (ng/ml)													
	Weeks in cage						Weeks in chair							
	-10	-8	-6	-4	-2	0	1	2	3	4	5	6	7	8
840	>20	>20	>20	>20	>20	>20	16	17	13	5	7	3	3	<1
850	> 20	13	> 20	> 20	> 20	> 20	10	15	15	17	5	13	12	> 20
851	> 20	> 20	> 20	> 20	> 20	> 20	12	< 1	18	6	12	7	10	13
149	9	14	15	16	11	10	8	> 20	5	10	7	7	5	5
300	8	4	2	9	4	> 20	2	1	16	5	12	3	> 20	5
Mean														
(n = 5)	15	14	15	17	15	18	10	11	13	9	9	7	10	9
S.E. ±	3	3	3	2	3	2	2	5	2	2	1	2	3	3

pared to only 3 values during chair restraint, that are greater than 20 ng/ml. Statistical evaluation by a standard paired, two-tailed *t* test reveals the values during cage-housing to be significantly higher than those following chair restraint ( $t = 2.80, P < .05$ ). The overall mean value during cage-housing is 16 (SE ± 3) ng/ml as compared to 10 (SE ± 3) ng/ml during chair restraint. In view of the fact that the absolute levels of a large percentage of the cage-housing samples were actually higher than 20 ng/ml, the above evaluation of the contrast between the two periods is probably a rather conservative one.

The most likely explanation for the high values observed during cage-housing would appear to relate to the capture and handling procedure required for obtaining blood samples from the free-ranging monkey. In general, this procedure requires about 5 min, plus or minus 2 min, depending upon such factors as the difficulty of the net capture, movement of the leg during venipuncture, or the condition of the vein and rate of flow of blood into the syringe. Apparently, the plasma growth hormone level can respond with marked elevations at a very rapid rate, in the range of 3–7 min, in the monkey under these conditions. Such rapidity of growth hormone response to psychological stimuli was also indicated in the experiments of Meyer and Knobil (2).

While mean plasma growth hormone values tended to be lower following chair restraint, when net capture and strenuous manual immobilization was no longer necessary, there were still occasional high values when only the leg was handled for venipuncture. While it is an assumption to regard the numerous elevated

growth hormone values during chair restraint as being related primarily to the venipuncture procedure, observations in two normal monkeys with chronic indwelling venous catheters lend some further support to this interpretation. In 12 consecutive measurements in these catheterized monkeys, from whom blood samples were obtained without any handling or confrontation of the animal, fasting 9 AM plasma growth hormone levels averaged 2.5 ng/ml, with no single value higher than 5 ng/ml (3), as compared to the mean of 10 ng/ml observed with saphenous venipuncture in the chair-restrained animals. Knobil has also reported basal plasma growth hormone levels to be quite low in the monkey, about 1 ng/ml, when chronic indwelling catheters are used (1).

The variability of plasma growth hormone values obtained by venipuncture in the monkey would appear to complicate interpretation of possible effects of chair restraint itself upon growth hormone levels. While mean growth hormone levels are generally slightly higher during the first month of restraint as compared to the second month of restraint, these differences are not statistically significant. It may also be possible that an acute growth hormone response of brief duration, less than 24 hr, may have occurred and was not evident because of the time intervals of our sampling schedule. The values following chair restraint, shown in Table I, are mean weekly levels representing at least two measurements in each monkey per week. In several monkeys, values were obtained on each of the first 4 days of restraint, but there was no tendency for levels to be systematically rising or declining during this period. Perhaps the only

conclusion that can be drawn regarding chair-restraint effects from these data is that it does not elicit a marked growth hormone response which is sustained over a period of days. A response of shorter duration cannot, however, be ruled out.

*Summary.* A large percentage of very high plasma immunoreactive growth hormone values was observed in cage-housed rhesus monkeys after net capture and manual restraint for saphenous venipuncture during about a 5-min period. Following chair restraint, mean values were significantly lower, but individual values were still somewhat variable apparently in relation to saphenous venipuncture alone, as judged by lower and less variable values obtained by means of remote blood sampling through chronic indwelling venous catheters. The present findings suggesting marked plasma growth hormone responses in the monkey to net capture, handling, and venipuncture, occurring within about a 5-min period, add emphasis to the need to consider not only the sensitivity of this endocrine system to psychological influences, but also the rapidity with which psychoendocrine responses may be reflected in growth hormone

levels, even within the time of a single venipuncture.

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