1-1997

Effect of Room Arrangement and Blood Sample Collection Sequence on Serum Thyroid Hormone and Cortisol Concentrations in Cynomolgus Macaques (Macacafascicularis)

Bryan L. Flow
Alcon Laboratories, Inc.

John T. Jaques
Texas Veterinary Medical Diagnostic Laboratory

Recommended Citation

Flow, B. L., & Jaques, J. T. (1997). Effect of Room Arrangement and Blood Sample Collection Sequence on Serum Thyroid Hormone and Cortisol Concentrations in Cynomolgus Macaques (Macacafascicularis). Journal of the American Association for Laboratory Animal Science, 36(1), 65-68.

Effect of Room Arrangement and Blood Sample Collection Sequence on Serum Thyroid Hormone and Cortisol Concentrations in Cynomolgus Macaques (*Macaca fascicularis*)

BRYAN L. FLOW, DVM, PHD,[1] AND JOHN T. JAQUES, MS[2]

Abstract | We evaluated the relationship, in cynomolgus macaques (*Macaca fascicularis*), between rank for order of blood collection with serum concentrations of 3,5,3'-triiodothyronine (T3), thyroxine (T4), free thyroxine (FT4), and serum cortisol. These relationships were determined for males and females that were housed in two room arrangements. For both room arrangements, males and females were housed separately. For room arrangement 1, macaques were housed on both sides of the animal holding room. The sides of the animal holding room were designated as side A or side B. Blood was initially collected from animals on side A, then from animals on side B. Animals on side B were able to visually observe macaques on side A being physically restrained and sedated for blood collection. In room arrangement 2, the macaques were housed on only one side of the animal holding room and could not directly observe other animals being physically restrained and sedated for blood collection. The relationship for serum FT4 concentration with blood sample collection sequence was different for each room arrangement. For room arrangement 1, we observed an inverse relationship between serum FT4 concentration and the rank for order of blood collection. This finding was observed for males and females and was consistent with the lower serum FT4 value observed for animals housed on room side B. We also observed a trend for higher serum T4 concentration on room side A, but the reverse was true for serum cortisol concentration. In contrast, a macaque's rank for blood collection sequence in room arrangement 2 was not predictive of the rank for serum thyroid hormone (T3, T4, FT4) or serum cortisol concentration. These results suggest that the arrangement of cages in a nonhuman primate holding room contributes to the variability for serum FT4 concentrations.

The thyroid gland regulates growth, development, and metabolic rate (1). Thyroid hormones also affect nonendocrine organs such as the heart, kidneys, lungs, gastrointestinal tract, and hematopoietic system (2). Thyroid hormone deficiencies (hypothyroidism) have been associated with a variety of clinical conditions, including skin abnormalities, cold intolerance, hypothermia, impaired cardiovascular function, and reproductive problems (3). Hyperthyroidism has been associated with increased basal metabolic rate, hyperactivity, weight loss, polyphagia, and cardiovascular disease (3).

Serum concentrations of thyroid hormones are commonly used to evaluate thyroid gland function. However, values for these hormones can also be influenced by nonthyroidal factors including hyperadrenocorticism (4, 5). Previous reports indicate that serum thyroid hormone concentrations were decreased in dogs with hyperadrenocorticism (4, 5). However, thyroid hormone concentrations returned to normal after correction of hypercortisolism (5). These findings suggest that an inverse relationship exists between serum concentrations of cortisol and thyroid hormones.

Serum concentration of cortisol can be increased by environmental stress. Previous reports indicate that restraint of macaques for venipuncture increases serum concentration of cortisol (6, 7). This increase may be related directly to the capture and restraint of the individual animal. However, we cannot exclude the possibility that stress/anxiety could also result from observing physical restraint and venipuncture of other macaques.

The United States Department of Agriculture (USDA) revised the animal welfare act to require that research facilities implement programs to promote the psychological well-being of nonhuman primates (8). Environmental enrichment techniques and pair/group housing have been the primary methods described for promoting the psychological well-being of nonhuman primates (9-14). However, pair housing of nonhuman primates may not always be feasible because of social incompatibility or study design restraints. The USDA regulations specifically require that individually housed nonhuman primates be able to see and hear other nonhuman primates of their own or compatible species unless the attending veterinarian determines that it would endanger their health, safety, or well-being (8). Most laboratory animal professionals will likely agree such visual contact promotes the psychological well-being of individually housed nonhuman primates. The arrangement of cages within a room is a primary method for providing visual contact among individually housed nonhuman primates. However, literature contains limited information that describes the effect that room arrangement may have on the physiologic response of the animal.

We describe the effect of two room arrangements on the variability of serum thyroid hormone and serum cortisol concentrations in individually housed cynomolgus macaques (*Macaca fascicularis*). Within each room arrangement, we evaluate the relationships among blood collection sequence and serum concentrations of thyroid hormones and cortisol.

Materials and Methods

We include baseline serum thyroid hormone measurements as part of the routine health monitoring program for our nonhuman primate colony. During the quarantine period, we evaluate serum concentration of 3,5,3'-triiodothyronine (T3), thyroxine (T4) and free thyroxine (FT4). In addition, we recently began to include serum cortisol concentration as part of our baseline health evaluation. The Institutional Animal Care and Use Committee had reviewed and approved standard procedures for the nonhuman primate health monitoring program. The study reported here consisted of an analysis of data that

The Department of Animal Resources, Alcon Laboratories, Inc., Ft. Worth, Texas 76134 and the Department of Endocrinology, Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, 77841

had been collected as part of this health monitoring program.

Environmental conditions: Male and female cynomolgus macaques (age, 2 to 3 years) were housed indoors in two-over-two stainless steel cages that met cage space requirements described in the *Guide for the Care and Use of Laboratory Animals* (15). Each cage contained a perch, foraging tray, and a manipulable item. Room temperature was maintained at 23° ± 2°C. The macaques were fed Certified Primate Chow 5048 (Purina Mills, Inc., Richmond, IN) supplemented with fresh fruit and other treats. Because quarantine and health monitoring had not been completed, animals were being housed individually at the time these data were collected. Males and females were housed in separate rooms. The animal holding rooms were classified as single (24 X 11 ft) or double (24 X 22 ft) module rooms. Because of safety concerns for personnel, the size of the animal holding rooms required different patterns for room arrangement of cages.

Room arrangement 1: Male (N=20) and female (N=28) cynomolgus macaques were maintained in double module animal holding rooms that permitted animals to be housed on both sides of the room (Figure 1). Thus, animals had the opportunity for visual contact with animals housed on the opposite side of the room. The sides of the room were designated as side A or side B.

For measurement of serum thyroid hormone and serum cortisol concentrations, we first collected blood from animals on side A, then on side B (Figure 1). Thus, animals on side B were able to see animals on side A being physically restrained and sedated for blood collection.

Room arrangement 2: Male (N=20) and female (N=20) cynomolgus macaques were housed in a single module animal holding room. For this size room, cages were placed on only one side of the animal holding room (Figure 1). Mirrors were provided so the animals could have visual contact with other animals. However, the mirrors were removed at the time of blood sample collection. Although animals could observe blood being drawn from other animals, the macaques could not directly observe other animals being physically restrained prior to sedation for blood collection.

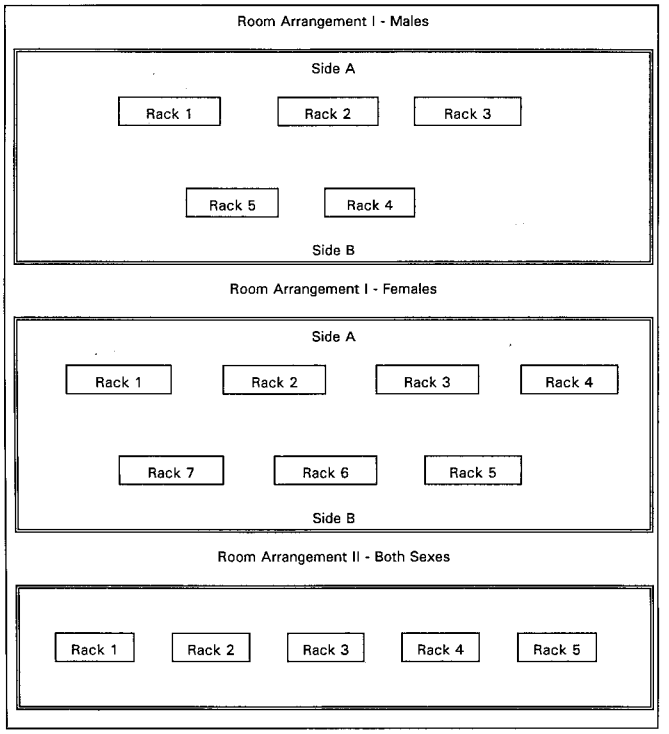

FIG. 1. Location of nonhuman primate cage rack units for males (N=20) in room arrangement 1 (top panel), females (N=28) in room arrangement 1 (middle panel), and males (N=20) and females (N=20) in room arrangement 2 (bottom panel). Each cage rack represents a two-over-two cage housing unit. The number for each cage rack denotes the sequence for blood sample collection within that room arrangement. Within each cage rack, the sequence for sample collection was as follows: Top left, top right, bottom left, bottom right.

Measurement of serum thyroid hormone and serum cortisol concentrations: For blood sample collection procedures, the macaques were initially physically restrained by use of the squeeze back mechanism of the cage. The cynomolgus macaques were then sedated with ketamine (10 mg/kg of body weight, IM), and approximately 5 ml of blood was drawn from the femoral vein. Blood samples were collected within the animal holding rooms. Blood samples were allowed to clot, then were centrifuged, and serum was harvested for measurement of serum T3, T4, FT4, and cortisol concentrations. Serum thyroid hormone (T3, T4, FT4) and serum cortisol concentrations were measured by a radioimmunoassay (RIA) procedure (16). All samples were analyzed in duplicate, using commercially available diagnostic kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). All samples for each room were measured in the same assay. However, the blood sample collection and radioimmunoassay procedures for each room were conducted at different times.

Statistical methods: We evaluated serum thyroid hormone and serum cortisol concentrations by one-way analysis of variance, using the SAS computer software package (17). For room arrangement 1, we compared group means for each side of the room.

For room arrangement 2, we could not compare sides of the room because all animals were housed on the same side of the room. However, we did compare group means for the individual two-over-two cage racks (N=4 macaques/rack).

We used Spearman rank correlations (18) to evaluate the relationship between the individual's rank for sample collection sequence with serum concentrations of T3, T4, FT4, and cortisol. For sample collection sequence, we assigned the first animal a rank of one, the second a rank of two, etc. Rank correlations were determined for each possible combination of sex and room arrangement.

Results

Room arrangement 1: We observed a significant ($P < 0.05$) inverse relationship between the rank for sample collection sequence and that for serum FT4 concentration (Table 1). This inverse relationship was observed in males ($r = -0.54$, $P < 0.01$) and females ($r = -0.42$, $P < 0.05$). We also observed a positive rank correlation ($r = 0.47$, $P < 0.05$) among males for sample collection sequence and serum cortisol concentration, but the correlation among these variables was near zero ($r = 0.05$, NS) among females.

Among males, an individual's rank for serum cortisol concentration was not highly predictive (NS) of his rank for serum T3, serum T4, or serum FT4 ($r = -0.28$, -0.12, and -0.22, respectively).

Table 1. Spearman rank correlations for blood sample collection sequence with serum thyroid hormone and serum cortisol concentrations

Variable correlated with sample collection sequence	Room arrangement 1		Room arrangement 2	
	Males (N=20)	Females (N=28)	Males (N=20)	Females (N=20)
Serum T3	0.41	0.00	0.22	-0.17
Serum T4	-0.32	0.02	0.32	0.09
Serum free T4	-0.54[b]	-0.42[a]	0.05	0.04
Serum cortisol	0.47[a]	0.05	0.23	0.00

[a]$P \leq 0.05$; [b]$P \leq 0.01$

Table 2. Group means[a] for body weight, serum thyroid hormone concentration, and serum cortisol concentrations for room arrangement 1

Room side[b]	Body Weight (kg)	Serum T3 (ng/ml)	Serum T4 (μg/dl)	Serum Free T4 (pmol/L)	Serum Cortisol (μg/dl)
Males					
Side A (N=12)	2.5 ± 0.2	1.6 ± 0.2	5.1 ± 0.9	20.0 ± 2.7	52.5 ± 7.3
Side B (N=8)	2.3 ± 0.2	1.7 ± 0.1	4.5 ± 0.6	17.6 ± 1.6	59.9 ± 10.4
	NS	NS	$P < 0.10$	$P < 0.05$	$P < 0.10$
Females					
Side A (N=16)	2.3 ± 0.2	1.8 ± 0.2	4.1 ± 1.0	17.1 ± 4.4	50.4 ± 8.8
Side B (N=12)	2.4 ± 0.2	1.8 ± 0.3	3.8 ± 0.8	14.1 ± 2.3	52.1 ± 8.0
	NS	NS	NS	$P < 0.05$	NS

[a]Mean ± SD

[b]Blood samples were first collected from animals on side A, then on side B.

We also observed weak rank correlations (NS) among females for serum cortisol with serum T3 ($r = 0.18$), serum T4 ($r = 0.08$), and serum FT4 ($r = 0.17$).

In room arrangement 1, serum FT4 concentration in animals on side A were significantly ($P < 0.05$) greater than those for animals on side B (Table 2). This finding was consistent among males and females. Although differences for serum T4 concentration were not statistically significant at the 0.05 level, we did observe a trend ($P < 0.1$) for higher total serum T4 concentration in males on room side A. In contrast, we observed a trend ($P < 0.1$) for higher serum cortisol values in males on room side B. These trends among males for serum T4, FT4, and cortisol values are consistent with the trend observed among female group mean values for these variables. However, the differences among female group means did not approach statistical significance for T4 and cortisol.

Room arrangement 2: Among males and females, the individual's rank for sample collection sequence was not highly predictive of the rank for serum T3, T4, and cortisol concentrations (Table 1). In contrast to results observed for room arrangement 1, the rank correlation for sample collection sequence with serum FT4 concentration was near zero for males ($r = 0.05$, NS) and females ($r = 0.04$, NS).

The correlation of serum cortisol with serum T3, T4, and FT4 concentrations was not significantly different from zero ($P > 0.05$) for males or females. We did not observe significant differences among cage racks maintained on the same side of the room (results not shown).

Discussion

Table 1 shows a significant inverse relationship between blood sample collection sequence and serum FT4 concentrations for males and females in room arrangement 1. This inverse relationship is consistent with the lower serum FT4 concentration for room side B, compared with side A (Table 2). In contrast, the correlation of sample collection sequence and serum FT4 concentrations was near zero for males and females in room arrangement 2. We believe the difference among these results may be due to anxiety associated with an animal in room arrangement 1 being able to visually observe physical restraint and sedation of other animals for sample collection.

These results do not establish a clear relationship between serum FT4 and serum cortisol concentrations. Table 2 demonstrates a trend toward an inverse relationship between group means for serum cortisol concentration with those for serum concentrations of T4 and FT4. However, the ranks of males and females for serum cortisol concentration were not predictive of the rank for serum FT4 concentration. These data represented serum cortisol concentration measured after physical restraint and sedation. Our data did not permit us to assess the deviation from baseline serum cortisol values, which may have provided a better indicator of the effect of stress on the FT4 concentration.

Within each room arrangement, we observed similar correlations among males and females for sample collection sequence with serum FT4 values (Table 1). In contrast, we generally observed weaker correlations among females, compared with males, for sample collection sequence with serum T3, T4, and cortisol concentrations. These weaker correlations may reflect sex-related differences in response to stress (19). However, previous reports indicate that the stage of the reproductive cycle can influence serum concentrations of T3, T4, and cortisol (20, 21). We cannot exclude the possibility that variability due to female menstrual cycle may have obscured the associations observed among the males.

Current animal welfare regulations require individually housed nonhuman primates be able to see and hear other nonhuman primates of their own or compatible species (8). However, our results suggest that room arrangement and visual contact during physical restraint and sedation can affect serum FT4 concentration. The differences among group means for serum FT4 values (Table 2) may be of limited importance in clinical diagnostic testing for detection of hypo- or hyperthyroidism. However, these differences could have important implications for research studies that are designed to detect differences in thyroid function among experimental groups. Such factors should be considered by researchers when designing studies that involve evaluation of serum thyroid hormone concentrations.

We have described the variability in serum thyroid hormone and serum cortisol concentrations for 2- to 3-year-old male and female cynomolgus macaques. Previous research (22) has indicated differences in behavioral and adrenocortical responses of several macaque species to include rhesus macaques (*Macaca mulatta*), bonnet macaques (*Macaca radiata*), and cynomolgus macaques (*Macaca fascicularis*). Additional studies are required to assess whether these species differences in adrenocortical response could modify the relationship observed in this study for sample collection sequence with serum thyroid hormone and serum cortisol concentrations.

The results of this study expand our understanding of the factors that contribute to variability in serum thyroid hormone concentrations of cynomolgus macaques. Previous reports have described age-related differences for serum T3 and T4 concentrations of cynomolgus macaques (23, 24). The present data suggest that room arrangement can contribute to the variability in serum FT4 concentration. Additional studies are required to determine whether our findings in these 2- 3-year-old animals are predictive of the responses that would be observed in adult cynomolgus macaques or in cynomolgus macaques that have been acclimated to laboratory procedures such as blood sample collection.

Acknowledgements

The authors thank Taryn Robertson and Catherine Smith for their assistance in collection and preparation of the samples and Paula Gadbury for assistance in preparation of the manuscript.

References

1. **Ganong, W. F.** 1993. *In* Review of medical physiology. Lange Medical Publications, Los Altos, Calif. 233.
2. **Guyton, A. C.** 1986. The thyroid metabolic hormones. *In* Textbook of medical physiology. W.B. Saunders Co., Philadelphia.
3. **Merck and Co., Inc.** 1986. The Merck veterinary manual, sixth ed. Merck and Co., Inc., Rahway, N.J.
4. **Ferguson, D. C., and M. E. Peterson.** 1992. Serum free and total iodothyronine concentrations in dogs with hyperadrenocorticism. Am. J. Vet. Res. **53**:1636–1640.
5. **Peterson, M. E., D. C. Ferguson, P. P. Kintzer, *et al.*** 1984. Effects of spontaneous hyperadrenocorticism on serum thyroid hormone concentrations in the dog. Am. J. Vet. Res. **45**:2034–2038.
6. **McGuill, M. W., and A. N. Rowan.** 1989. Biological effects of blood loss: Implications for sampling volumes and techniques. ILAR News. **31**:5–18.
7. **Reinhardt, V.** 1996. Refining the blood collection procedure for macaques. Lab. Anim. **25**:32–35.
8. **United States Department of Agriculture, Animal and Plant Health Inspection Service.** Animal welfare regulations: Final rule, p. 6309–6505. Federal Register vol 56, No. 32, 9 CFR, Part 3, February 15, 1991.
9. **Bayne, K., H. Mainzer, S. Dexter, *et al.*** 1991. The reduction of abnormal behaviors in individually housed rhesus monkeys (*Macaca mulatta*) with a foraging/grooming board. Am. J. Primatol. **22:**23–35.
10. **Bayne, K. A. L., J. K. Hurst, and S. L. Dexter.** 1992. Evaluation of the preference to and behavioral effects of an enriched environment on male rhesus monkeys. Lab. Anim. Sci. **42(1):**38–45.
11. **Crockett, C. M., C. L. Bowers, D. M. Bowden, *et al.*** 1994. Sex differences in compatibility of pair-housed adult longtailed macaques. Am. J. Primatol. **32:**73–94.
12. **Eaton, G. G., S. T. Kelley, M. K. Axthelm, *et al.*** 1994. Psychological well-being in paired adult female rhesus (*Macaca mulatta*). Am. J. Primatol. **33:**89–99.
13. **Reinhardt, V. and S. Hurwitz.** 1993. Evaluation of social enrichment for aged rhesus macaques. An. Tech. **44(1):**53–57.
14. **Schapiro, S. J. and M. A. Bloomsmith.** 1994. Behavioral effects of enrichment on pair-housed juvenile rhesus monkeys. Am. J. Primatol. **32:**159–170.
15. **National Research Council.** 1985. Guide for the care and use of laboratory animals. NIH Publication no. 86-23, Public Health Service, Bethesda, MD.
16. **Refetoff, S.** 1979. Thyroid function tests, p. 387–428. *In* L.J. DeGroot (ed.), Endocrinology: Volume I. Grune and Stratton, Philadelphia, Pa.
17. **SAS Institute Inc.** 1990. SAS procedures guide, version 6, 3rd ed., SAS Institute Inc., Cary, N.C.
18. **Steel, R. G. D., and J. H. Torrie.** 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York.
19. **Mitchell, G.** 1979. Behavioral sex differences in nonhuman primates, p. 415–422. Van Nostrand Reinhold Co., New York.
20. **Reimers, T. J., L. K. Mummery, J. P. McCann, *et al.*** 1984. Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. Biol. Reprod. **31:**148–154.
21. **Buckingham, J. C., K. D. Dohler, and C. A. Wilson.** 1978. Activity of the pituitary-adrenocortical system and thyroid gland during the oestrous cycle of the rat. J. Endocrinol. **78:**359–366.
22. **Clarke, A. S., W. A. Mason, and G. P. Moberg.** 1988. Differential behavioral and adrenocortical responses to stress among three macaque species. Am. J. Primatol. **14:**37–52.
23. **Flow, B. L., J. T. Jaques, and R. L. Thomas.** 1996. Variability of serum thyroid hormone concentrations in subpopulations of male cynomolgus macaques. Contemp. Top. Lab. Anim. Sci. **35:**66–69.
24. **Yoshida, T., M. Sato, K. Ohtoh, *et al.*** 1989. Effects of aging on the in vivo release of thyrotropin (TSH), triiodothyronine, and thyroxine induced by TSH-releasing hormone in the cynomolgus monkey (*Macaca fascicularis*). Endocrinology **124:**1287–1293.