ABSTRACT. A standard colony weaning procedure to reduce distress and consequent diarrhea provides a nurse monkey to groups of four infants after separation from their mothers. The present study was designed to develop methods for evaluating this husbandry method. Four infants were separated from their mothers at 17-to-20 weeks, and caged with an unfamiliar adult female monkey (nurse) during one month of a two month study period. Weekly blood sampling for cortisol began one week before maternal separation. Infant behavior was scored from video tapes. In three infants, cortisol rose at separation from both the mother and the nurse. One infant had a high level of depressive behavior, which was significantly correlated with cortisol. It also lost 14% in body weight over the study period. Two other infants gained substantial weight, while a third had little weight change. The infant exhibiting the most locomotion and play showed neither cortisol rise nor depression. The data revealed marked between-infant variability in growth, behavior, cortisol, and behavior-cortisol associations following social separation. The results also suggest that infants can become attached to the nurse, with an adverse stress response occurring when they are separated from her. It was concluded that valid characterization of individual reactions to social loss for husbandry, psychological well-being, or scientific purposes requires both clinical symptom, physiological, and behavioral assessment.

Key words: Maternal Separation, Depression, Cortisol, Weaning, Adult Female Nurse, Husbandry Practices, M. fascicularis

INTRODUCTION

Husbandry procedures at the Tsukuba Primate Center for Medical Science (TPC) include a program for captive breeding and rearing of Macaca fascicularis (Honjo 1985). Female
breeders live in individual cages during pregnancy, parturition, and a nursing period. Weaning involves separating the infant from the mother and housing it with three other weanlings when it achieves various developmental criteria at about 15 postnatal weeks. Many infants exhibit depressed behavior and have diarrhea and serious body weight loss during this process. To counteract these adverse effects, a program was instituted in which a nurse female lived with each infant group for four weeks after maternal separation. This treatment reduced the diarrhea incidence from 48% without a nurse to 16% with a nurse (Hanari et al. 1987).

The success of the TPC nurse program seems to be predicted by the results of many maternal separation studies in a number of macaque species. Macaque separation effects are characterized by an initial period of agitation and hyperactivity—termed protest behavior—which lasts for 24–72 hours. This is followed in some infants by hyperactivity, a hunched-over collapsed posture, little play or exploration, and reduced food and water intake—a reaction termed depression (Mineka & Suomi 1978, Capitanio 1986). In addition to behavioral effects, maternal separation can produce changes in diurnal cycles of heart rate and temperature and in sleep brain wave patterns (Reite et al. 1978, 1981, Reite & Capitanio 1985). A consistent effect of maternal separation is a rise in serum cortisol, lasting for 1–7 days depending on the species (Kaplan 1986). Cortisol even rises in bonnet macaque infants, who show almost no behavioral effects unless completely isolated from social contacts (Kaufman & Stynes 1978). Given these findings, it seems reasonable to propose that the provision of a nurse after maternal separation reduces these behavioral and physiological disruptions.

The TPC weaning process consists of four phases: (i) separation from the mother, (ii) moving to a new cage, (iii) meeting unfamiliar agemates, and (iv) meeting an unfamiliar adult female. Each phase could make a unique contribution to the disruptions caused by weaning and to the beneficial effects of the nurse. Prior TPC work used only growth and clinical symptoms to evaluate this total process. The present study was designed to develop a broader range of measures to evaluate husbandry practices in terms of both physiological and psychological well-being.

We expected that the nurse would attenuate the initial adverse responses after maternal separation, and that the infants would show adequate attachment to her to the extent that her removal would not produce serious separation symptom. This study describes growth, clinical symptoms, and behavior and cortisol changes during the nurse-assisted weaning process. Cortisol was chosen as a physiological index based on its consistent response in prior studies and its theoretical relationship to psychological distress.

PROCEDURE

Preweaning

The four subjects (two males: S1, S2, two females: S3, S4) were healthy and normal in birth weight and gestational age. The mothers were born in the wild except one (S3’s), but
all have well nursed their own infants at least once prior to this study. The subjects were all well nursed by their mothers in a 43 × 60 × 60 cm cage, from which they could see and hear but not touch other mother-infant pairs. Body weight was examined weekly and other health indices were observed each day. Each infant began the weaning process when it reached the following criteria: over 90 days old, weight more than 700 g, eruption of a deciduous molar, eating solid foods. The subjects were unfamiliar to each other and to the non-kin adult female nurse who lived with them. The adult female had nurses weanling groups 7 times prior to this one. The nurse did not lactate, but provided physical contact and grooming.

**Weaning Procedure**

One week before weaning each infant was briefly separated from its mother, and a baseline blood sample was drawn within a minute of capturing the mother. Each infant received a distinctive facial tattoo and shaved body part for recognition during behavior tests. On the weaning day, immediately after separation from the mother, each infant was video taped for 5 minutes while alone in a 43 × 60 × 60 cm cage. Fifteen minutes after separation the four subjects were moved into one side of a 90 × 60 × 60 cm cage, bisected by a partition board. The other cage contained the nurse female. The nurse and weanlings were kept apart for a half hour, after which the partition was removed.

A blood sample was taken 24 hours after separation from the mother. The nurse was separated from infants by inserting the partition board and infants were captured at random. The experienced nurse did not show agitation during this procedure. Blood was drawn for the first infant within 1.5 minutes of capture, with all samples collected within 6 minutes. The subjects and nurse were video taped using a remote controlled Hitachi camera (HV-17AJ) under standard lighting of 500 lux. Taping occurred for 5 minutes before the blood sampling capture, and for 5 minutes after in the home cage. These blood sampling and video taping procedures were also done on days 7, 14, and 21 after maternal separation. On day 28 the nurse was permanently removed, followed immediately by 5 minutes of video taping. Blood sampling and before-after video taping were done 24 hours later (day 29), and on every seventh day through post-separation day 56. This yielded a total of 10 blood samples per subject and 11 video recording days.

**Cortisol Assay**

All 2.5 ml blood samples were taken between 10:50 and 11:00 a.m. from the femoral vein of un-anesthetized animals. The blood was centrifuged at 3,000 rpm for 15 minutes at 4°C, with the serum stored at −20 °C until assay. Serum cortisol was assayed by solid phase radioimmunoassay, using SPAC Cortisol kit Daiichi (Institute of Daiichi Radioisotope). Intra- and inter-sample coefficients of variation were under 5%.

**Behavior Measurement**
The following seven infant behaviors were coded from the video tapes (inter-observer agreement concordance*, in parentheses).

1. Affinitive Interaction with Nurse (.82): Held or groomed by the nurse, clinging or other physical contact by the infant to the nurse.

2. Depressive Behavior (.98): Withdrawal to a cage corner and sitting with drooping head, eyes pointed down or closed, trunk sagging forward, and no locomotion.

3. Fear (.71): Simple grimace or open-mouthed grimace as defined by Redican (1975, pp. 128–130), withdrawal from another infant’s approach.

4. Locomotion (.71): Vertical or horizontal movement greater than one body length.

5. Social Play (.65): Active and rhythmical movements such as chasing and being chased or wrestling-like actions, mounting others with shambiting, pushing, and pulling. Participants exhibit neither fearful nor aggressive expressions.

6. Aggression (.81): Brows-up and protruding lip or open mouth stare with forward inclining posture (Redican, pp. 109–116), or rushing toward another infant and pulling, pushing and biting him.

7. Social Contact (.88): All other physical contact between infants not included in the other categories.

Each category was scored by pressing a key connected to a NEC PC-9801 computer. The behavior of each animal as a focal subject was scored for 5 minutes of each video session. The average kappa for inter-observer agreement between all pairs of three observers over all behavior categories was .702 (Lehner 1979, pp 133–134). The results for each category were analyzed by percent of the 5-minute total observation time per session. Results for social play, aggression, and fear employed their mean percent duration for session before and after blood sampling. Results for the other categories used only data from sessions before sampling blood.

Statistical Analysis

The relationship between cortisol and depressive behavior was calculated for each subject by Pearson correlations of these two measures on days 1, 7, 14, 21, 29, 35, 42, 49, and 56. The behaviors were studied by ANOVAs with subjects and days of observations. Social contact, social play, aggression, and fear were tested again by ANOVA with subjects and before-after separation from the nurse in order to analyze the presence of nurse. In analyzing cortisol responses, 95% confidence intervals for the samples were calculated by the method of Bliss (1952).

RESULTS

Body Weight

*see NOTE on page 10
Body weight significantly increased during the observation period (\(F(10, 30)=4.2, p=.002\)), and there was individual difference among the infants with respect to change of the weight (\(F(3, 30)=21.3, p=.002\)) (Fig. 1). S2 weighed more than the other three infants before weaning. Body weight for S2, S3, and S4 increased from weaning throughout the two month test period. S1 lost weight toward the end of the nurse period and thereafter, being 14% below his weaning weight at the end of the observations.

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**Figure 1.** Change in body weight during study period: M is the maternal separation, N in brackets indicates the nurse separation period, asterisks denote the presence of diarrhea when weighing each animal. S1 was male and 20 weeks old at weaning, S2 was male, 20 weeks old, S3 was female, 19 weeks old, and S4 was female, 17 weeks old.

**Figure 2.** The change in serum cortisol concentrations after separation: B is the basal value taken one week before maternal separation. Black arrows indicate the samples on the day after the maternal separation, white arrows samples on the day after nurse separation. The dotted band and vertical bars give 95% confidence limits of the cortisol assay.
Serum cortisol concentrations for each subject are shown in Fig. 2. S1 presented the most extreme response pattern, with a large rise at separation from the mother (black arrow) and from the nurse (white arrow). Cortisol levels significantly changed in sampling dates \((F(9, 27)=4.2, p=.002)\). Individual differences were also observed, but not statistically significant \((F(3, 27)=2.5, p=.082)\). All S1 points, except that at day 14, were above the basal confidence band. Also, most S1 responses after nurse separation were greater than after maternal separation. S3 and S4 also showed a large increase at separation from the mother, with little response immediately after nurse separation. However, all S3 points after nurse separation were above the basal level, as were the final two values for S4. In contrast to the other subjects, S2 showed no immediate response to either separation, and little change from basal values over the entire test period.

Depressive behavior (Fig. 3) was conspicuous only for S1, who showed high levels immediately following, and the day after, both maternal and nurse separation, significantly different from the other infants \((F(3, 30)=3.5, p=.029)\). After nurse separation depression for S1 remained elevated for two weeks. The correlation between cortisol and depressive behavior for S1 was \(r=.78\) \((p=.012)\). S4 was the only other infant exhibiting any substantial level of depression, which occurred two and four weeks after nurse separation. Neither S2 \((r = -.31)\), S3 \((r = .60)\), nor S4 \((r = .04)\) showed a statistically significant correlation between cortisol and depressive behavior.

Locomotion (Fig. 4) varied between 20-40% of test time, except for observations immedi-
ately after maternal separation when S2, S3, and S4 exhibited the expected ‘agitation’ pattern. Significant individual differences (F(3,30)=7.74, p=.002) occurred due to the activity of S1 being substantially lower than that for the other infants on most test days.

Figure 4. The change in locomotion after separation: (See figure 3 legend for abscissa explanation).

Figure 5. Social contact measures for (a) infants with the nurse, and (b) among infants before and after nurse separation. Numbers 1 – 4 identify individual infants, vertical bars give standard errors.
Affinitive interaction with nurse consisted of contact to the nurse in the present observation, grooming being rare. So Fig. 5a shows that S4 tended to have more nurse contact than the other infants (F(3,9)=3.41, p=.066). Social contact among infants revealed significant individual differences (Fig. 5b, F(3,27)=3.52, p=.027) and change over test days (F(9,27)=2.55, p=.029). Social contact significantly increased after separation from the nurse (Fig. 5b, F(1,32)=5.84, p=.021). S1 exhibited the most infant contact while the nurse was present, with high levels shown by all infants except S3 after the nurse was removed.

Infant social interactive behavior is shown in Fig. 6. Play was highest for S2 (F(3,27)=4.40, p=.012), especially after the nurse was removed, while play was almost nonexistent for S4 (Fig. 6a). S4 showed no aggression, while aggression tended to increase for the other three infants after nurse removal (Fig. 6b, F(1,60)=3.50, p=.066). When the nurse was removed, S4 fear behavior increased markedly (Fig. 5c). Fear also increased for S3, but not for S1. S2 did not express fear after nurse separation.

![Figure 6. Infant-infant interactions involving (a) play, (b) aggression, and (c) fear before and after nurse separation. Numbers 1 - 4 identify individual infants, vertical bars give standard errors.](image-url)
Clinical Symptoms

S1 developed serious diarrhea two weeks after the maternal separation (Fig. 1). However, *Shigella, Salmonella*, and *Campylobacter* bacteria were not isolated from S1. In spite of the administration of antibiotics, S1 did not recovered from diarrhea. Electrolytes with vitamin complex were given orally to S1 during the period of diarrhea. S2 and S3 also showed some diarrhea, while S4 developed no diarrhea during the study period.

DISCUSSION

A large increase in serum cortisol following environment changes is often interpreted as a physiological indicator of psychological stress (Kaplan 1986). This interpretation is consistent with the results of many primate studies involving maternal separation (e.g., Smotherman et al. 1979, Coe et al. 1983, Vogt & Levine 1980). By this interpretation, only S1 showed a sustained psychological stress reaction. Only S1 had markedly elevated cortisol throughout the two month test period and only S1 lost body weight, which he did after both maternal and nurse separation. Of course, from these data, it is unclear whether psychological stress factors produced both cortisol and weight loss effects, or if the weight loss served to maintain high cortisol. However, it may be concluded that the nurse did not produce a good effect on weaning process of S1, since stress indexed by cortisol was very pronounced again after removal of the nurse.

Considering all subjects, cortisol-behavior response patterns were highly variable. In fact, four different patterns occurred. S1 had a pronounced cortisol response, correlated with high depressive behavior and low motor activity. S2 had no cortisol response, no depression, and high motor activity and play. This suggests that either maternal separation had no effect on S2, or the nurse offset all measurable effects. S3 exhibited cortisol reactivity, but little depression and no adverse behavioral reactions. S4 also showed a cortisol response, with delayed depression two weeks after nurse removal. Behaviorally, S4 had the most interaction with the nurse and a large increase in fear when she was removed. This suggests that the nurse was effective in reducing S4 maternal separation effects, but these effects were either reinstated or produced independently upon her removal. Anyway, we may need further observations in order to discuss the relation between the behavioral response in separation and the cortisol level. Coe et al. (1985) presented in their observation of squirrel monkeys that the intensity of protest is not always correlated with the level of adrenal activation. An increase in infant-infant contact occurred after the removal of the nurse, especially for S2 and S4. This suggests that peer contact may serve to attenuate the effects of separation from adult females (Caine & Reite 1981).

Given these results, it is clear that evaluating behavioral interventions such as the nurse female must involve both physiological and behavioral measures. If we had measured cortisol alone, we would have concluded that the nurse had little effect on reducing initial distress after maternal separation, as three of the infants (S1, S3, and S4) responded adversely after the separation. If we had used depressed behavior alone, we would have concluded that
the nurse eliminated maternal separation effects in three of the infants (S2, S3, and S4). On the basis of both physiological and behavioral measures we do conclude that (1) the nurse may have been unnecessary for infant S2, who showed no immediate or delayed adverse responses to maternal separation, (2) the nurse had little benefit for S1, and (3) the nurse may have offset maternal separation effects for S4, but her removal then produced adverse separation effects, such as increase of cortisol concentration, depression, fear, and social contact. Those results suggests that we need to discuss the effectiveness of a pregnant female as a caregiver which was reported by Rosenblum (1971) and Coe et al. (1978) (both cited in Coe et al. 1983) more precisely again in terms of individual difference.

Clearly, the effects of a nurse on relieving maternal separation distress are complex. Perhaps the most important factors concern differences among infants. As shown by Suomi (1987), some macaque infants appear to have a genetic disposition for adverse responses to environmental changes. S1 of this study may be an example of that because it showed conspicuously adverse responses to both environmental changes, maternal separation and removal of the nurse. S2 may represent the opposite extreme of being buffered against strong reactions to social-emotional disruption. However, another dimension concerns the quality of mother-infant relationship prior to separation, which may be important even under single-cage dyadic conditions.

To adequately address the husbandry, psychological well-being, and scientific issues raised in this study, nurse effects must be compared with effects when infants are separated without a nurse. Measures of mother-infant relationship quality (Suomi 1987) are also needed to assist in predicting which infants will respond adversely to nurse intervention and to explain when and how the intervention will be effective. Further, given the nurse's effectiveness in reducing diarrhea, immunological, viral, and bacterial measures need to be related to cortisol and behavior changes for an understanding of the physiological and psychological consequences of husbandry practices such as nurse intervention. Such measures are planned for TPC husbandry and behavior studies following up on the work reported here.

NOTE

The concordance was calculated by the formula, \( C = A / [A + (D1 + D2)/2] \), where \( C \) = concordance, \( A \) = number of agreement, \( D1 \) = number of disagreement for observer 1, \( D2 \) = number of disagreement for observer 2.

ACKNOWLEDGMENTS

We would like to express our gratitude to all the TPC staffs, especially to Messrs. K. Hanari, K. Shimizu, and M. Kono, all excellent animal technicians, also to Ms. M. Tokairin for her earnest patience in the inter-observer test, and to Dr. T. Yoshida for general advice to this project, especially in endocrinological field. We also express thanks to Drs. T. Minami, Osaka University, and K. Negayama, Mukogawa Women's University for critical reading of the m.s. Lastly we would like to acknowledge the deep understanding to the present study by Drs. S. Honjo, the former director of TPC, and F. Cho, the chief division
of breeding & rearing in TPC. The present study was supported in part by the Grant-in-Aid for Scientific Research (02801021) and the Grant (3-1-4-A-9) of the Human Science Foundation.

REFERENCES

要約
本研究は、保母ザル同居が離乳（母子分離）後の子ザルの心理的ストレスに及ぼす影響を検討するために行われた。4頭の幼体カニクイザルを生後17～20週で実母から分離し、未知の成体雌ザル（保母）と1カ月間同居させ、分離した。実母との分離1週間前から1週間毎に採血を行い、その直前の子ザルたちの行動がスコーバー化された。3頭の子ザルで、実母、保母、いずれの分離際にもコーチゾル濃度の上昇が見られた。内1頭は高頻度の抑鬱行動を現し、それは血中コーチゾル濃度が高い正の相関を示した。さらにその体重は14％減少した。遊びの多く見られた子ザルはコーチゾルの増加は見られなかった。今後、保母の同居しない子ザルとの比較が必要である。