Effects of climate-controlled housing on reproductive potential of rhesus monkeys

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Summary. The reproductive adaptability of newly acquired female feral rhesus monkeys was evaluated by comparison of their menstrual records and progesterone levels with those of females in captivity for 3½ years. Housing of the animals under conditions that simulated conditions of the breeding season in the wild resulted in a rapid adjustment with minimal periods of amenorrhoea or anovulation. Both groups of monkeys were similar with respect to menstruation and menstrual cycle length and progesterone levels.

Introduction

The 'seasonal breeding' of the rhesus monkey (*Macaca mulatta*) in natural and laboratory habitats has been well documented (Hartman, 1931; Van Wagenen, 1945; Kaufmann, 1965; Koford, 1965; Valerio, 1969; Hendrickx & Kraemer, 1970; Wilson, Fradkin & Hardman, 1972). Variation in the breeding season between troops has been related to the geographical conditions (Washburn, Phyllis & Lancaster, 1965; Hendrickx & Kraemer, 1970). It has been reported that feral animals new to captivity experience extended periods of infertility (Kriewaldt & Hendrickx, 1968; Dukelow, Harrison, Rawson & Johnson, 1972) which are characterized by absence or irregularity of menstruation.

Previously, conditions for housing primates have not taken into consideration the source of the captured animals. Uniform recommendations have been made for maintenance of temperatures of 21–26°C with no more than 1°C variance, a relative humidity of 40–50% with minimum fluctuation and regular air changes with no recirculation of inside air (Whitney, Johnson & Cole, 1973). The temperature conditions, but not the humidity recommended, have been adhered to in several laboratories; a fall breeding season was still observed (Johansson, Neill & Knobil, 1968; MacDonald, 1971; Valerio, Pallotta & Courtney, 1968; Wilson *et al.*, 1972).

The fact that artificial lighting conditions of 12 h light/24 h were common to all the above-cited studies led to the premise that the amount or timing of lighting may be important factors to minimize the adaptation time of rhesus monkeys new to such conditions. Valerio *et al.* (1968) found that animals new to the laboratory showed seasonal reproduction in the first year, but this was eventually lost. We therefore studied the effect of modulation of lighting, temperature and humidity, in a manner similar to that in the animals' habitat, to see if there was a reduction of adaptation time for new arrivals to the colony.

Materials and Methods

Animals

The female rhesus monkeys studied weighed 3.8–8.1 kg. The control group consisted of 30 feral animals which had been in captivity in the University of Wisconsin Biotron for 3½ years. The experimental group contained 18 newly imported adult females (Primelabs, Farmingdale, New Jersey, U.S.A.), obtained in July. All animals were housed individually in a controlled environment which simulated the light, temperature and humidity of a 20-day period of the breeding season of their native India. This information was gathered from weather stations in 3 cities in northern India forming a triangle around the area where these animals were captured. At programmed time intervals, temperature, humidity, fluorescence and incandescence were integrated to correspond to those of an average day in the area described. The computer programmes gave daily temperature and humidity variations of 12–23°C and 45–50%, respectively. The lighting duration ranged from 13 h 15 min to 15 h 45 min throughout the 20-day cycle. This 20-day cycle was continued throughout the year. Air was passed through a coarse, then a fine filter system. The entire room air volume was recirculated 3 times/min. Fresh air was circulated at the rate of one room volume/4 min. The programme for one such day is shown in Table 1. The animals were fed a diet of Purina Monkey Chow (Ralston-Purina Co., St. Louis, Missouri, U.S.A.) supplemented with fruit. Water was available *ad libitum*.

Table 1. Details of the climatic conditions used over one 24-h period for newly imported rhesus monkeys to simulate conditions in the wild

Time (h)	Temperature (°C)	Humidity (%)	Fluorescence (%)*	Incandescence (%)*
01:00	15	60	0	0
01:45	14	60	0	0
02:25	13	60	0	0
05:00	13	60	0	10
06:10	13	60	0	20
06:30	14	60	0	30
06:50	15	60	0	40
07:50	16	60	0	75
08:30	17	60	0	100
09:10	18	58	0	100
10:00	18	58	30	100
10:50	19	54	100	100
11:45	20	54	100	100
12:45	21	52	30	100
13:45	21	49	0	100
15:00	21	46	0	75
15:15	21	46	0	40
17:30	21	48	0	30
17:40	21	48	0	20
17:45	20	48	0	20
18:00	20	48	0	10
18:25	20	50	0	0
19:15	19	50	0	0
20:15	18	52	0	0
21:15	17	55	0	0
21:55	16	57	0	0
22:35	15	60	0	0
23:15	14	60	0	0
23:50	13	60	0	0

* % of available lighting.

After arrival at the colony, medical and menstrual (July–March) information were recorded for each animal. Menstruation was detected by examination of the animal and its cage for blood. The intensity and length of bleeding as well as the length of the cycle were recorded. The length of the cycle was calculated using the first day of bleeding as Day 1 and the day preceding the onset of the next bleeding period as the final day. Hormonal data for an entire menstrual cycle were obtained for animals exhibiting at least 2 regular cycles. Daily blood samples (5 ml) were obtained by femoral venepuncture at approximately the same time each morning starting at 08:00 h. Iron B complex was administered intramuscularly as dictated by the haemoglobin levels of the animals. The serum was separated by centrifugation at 2000 *g* for 10 min and stored at -10°C until all the samples for one cycle could be assayed for progesterone.

Progesterone assay

Progesterone concentrations were measured by modification of the radioimmunoassay of Bielert *et al.* (1976). Triplicate serum aliquots of 0.05 ml were added to 15 ml conical test tubes and brought to 0.5 ml with phosphate-buffered saline (PBS, pH 7.0). To this mixture 5.0 ml petroleum ether (Fisher Pesticide Grade) were added and the samples were placed in a mechanical shaker for 10 min before centrifugation for 10 min. The extracts were dried under filtered air in a 45°C water bath.

The antibody for progesterone (Holly Hill Farms, Hillsboro, Oregon, U.S.A.) was diluted in PBS–0.1% gelatin to a concentration permitting approximately 50% binding. There was no cross-reaction with oestriol, oestradiol- 17β , oestrone, cortisone, androsten-3,17-dione, cholesterol and testosterone, but reactions were found (progesterone 100%) with 5α -pregnan-3,20-dione (40%), 5β -pregnan-3,20-dione (10%), desoxycorticosterone (9.9%), cortisol (2.5%) and 20-dihydroprogesterone (1%). Dextran-coated charcoal in PBS gel was used to separate the bound from the unbound steroid. In addition to the unknown samples, each assay included duplicate 0.05 and 0.1 ml pooled serum from female rhesus monkeys for inter-assay and dilution factor checks, and also blank tubes containing only PBS.

The curve for extracted standards was consistent with that for unextracted standards. The mean recovery for the tritiated progesterone added to the serum and carried through the procedure was $94.0 \pm 4.7\%$ ($n = 25$). Measurements with the 0.05 and 0.1 ml samples of pooled

Text-fig. 1. A composite graph showing mean $\pm$ s.e.m. concentrations of serum progesterone in 40 menstrual cycles of control and experimental rhesus monkeys. The data are arranged about Day 0 which is the day before the rise of the progesterone concentrations above 1 ng/ml serum.

serum gave proportional values of 38.5 ± 3.2 and 76.4 ± 7.6 pg, respectively ($n = 12$) and the inter-assay variation was less than 10%. Intra-assay precision demonstrated a standard deviation for 35 samples in triplicate of ± 3.1 pg. The sensitivity of the assay was <5 pg and estimations of blank values with 0.05 ml charcoal-stripped serum were <5 pg ($n = 30$). Since this value was less than the sensitivity of the assay, the blank values were not subtracted from the progesterone values of the unknown samples. Differences between means were checked statistically by Student's t test.

Text-fig. 2. Variations in progesterone patterns seen in laboratory rhesus monkeys. (a) In this 30-day menstrual cycle there was premature degeneration of the corpus luteum. The animal had had 2 abortions and was excluded from the composite graph in Text-fig. 1. (b) A 47-day ovulatory menstrual cycle; this cycle length was consistent in this animal, and similar multiple peaks of progesterone were seen in other monkeys. (c) A 28-day anovulatory menstrual cycle of an animal that experienced menstrual bleeding.

Results

There were no differences between the two groups in length of the cycle or duration of menses. The menstrual cycle (mean $\pm$ s.e.m.) was 28.5 ± 2.2 (range 22–28) days in the control group and 27.9 ± 2.4 (range 21–43) days in the experimental animals. The length of bleeding (mean $\pm$ s.e.m.) was 2.7 ± 1.0 (range 1–5) days in the controls and 3.8 ± 1.3 (range 1–7) in the experimental monkeys. One animal in each group had cycles of >40 days but experienced ovulation and thus potentially fertile cycles (Text-fig. 2b). Examination of the progesterone values showed that 41 of the 48 animals exhibited ovulatory cycles (Text-fig. 1). Values were similar in both groups except for slightly higher values in the control animals on Days -4 and -3 caused by 2 females which had >0.5 ng progesterone/ml serum. Values in all the other females were <0.3 ng/ml during the follicular phase (before Day -1). The day before progesterone values rose to >1 ng/ml was designated Day 0 and this was 14.2 ± 4.0 days from the onset on menstruation in the cycles considered (range 5–30 days). Values increased to high levels by Day 4, remained high for several days and then declined slowly from about Day 9. Three of the experimental animals exhibited progesterone values above 10 ng/ml and in 26 of the 40 animals more than one progesterone peak (i.e. higher than median values and for >1 day) was recorded, but the highest was usually that which occurred first in the cycle. Of the 7 animals deemed anovulatory because progesterone concentrations did not rise above 1 ng/ml serum (Text-fig. 2c), 3 were experimental and 4 were control monkeys.

Discussion

In contrast to previous reports of newly captive animals experiencing amenorrhoea for extended periods, the present data indicate that in controlled environmental conditions that simulated the natural breeding season rhesus monkeys adapted well and were similar to animals kept in captivity for $3\frac{1}{2}$ years with respect to menstrual cyclicity, duration of menstruation and progesterone levels. These data compare well with those previously reported for the rhesus monkey (Hartman, 1932; Neill, Johansson & Knobil, 1967; Valerio, 1969; MacDonald, 1971; Bosu, Johansson & Gemzell, 1973; Niswender & Spies, 1973). Blood samples for progesterone assay were taken from all animals between September and March. The breeding season in Northern India has been reported to be November to January (Southwick, Beg & Siddiqi, 1961) and 21% of the control and 6% of the experimental animals were bled in this period. During September to October, considered part of the breeding season of captive animals (Van Wagenen, 1945; Valerio *et al.*, 1968), the proportions were 32 and 56% respectively. In February to March, 46% of the control and 39% of the experimental monkeys were sampled throughout one menstrual cycle. The fertility of rhesus monkeys in these months has been reported to be very low (Valerio *et al.*, 1968). Of the observed anovulatory animals, 2 controls were sampled in October and 2 in February, and 1 experimental was sampled in October and 2 in March. These anovulatory cycles were not, therefore, considered to be seasonal. All 7 animals subsequently menstruated and had ovulatory cycles. Cases of menstrual bleeding with anovulatory cycles have been noted previously (Hartman, 1932; Bosu *et al.*, 1973). No seasonal amenorrhoea was detected in any of the animals during the year of study.

The dramatic rise in progesterone occurs with the development of the corpus luteum; thus, the day before this rise, Day 0 (14.2 ± 4.0 days), is similar to that reported for ovulation on Day 13 (range 10–16) by Johansson *et al.* (1968), and to that occurring 1–2 days after the mid-cyclic oestrogen peak (Hotchkiss, Athenson & Knobil, 1971; Bosu *et al.*, 1973). The luteal phase appears to be more consistent in length than the follicular phase, even for the animals exhibiting cycle lengths of >40 days (Text-fig. 2b).

With the data presented here, it is clear that the humidity, and timing and type of lighting are

important factors in reducing the acclimatization period of the rhesus monkey in captivity and eliminating seasonal effects on the colony. However, it is impossible to ascertain which of the factors is the most important. The exposure time and intensity of the lighting might be important because these conditions differed most from those previously reported for housing rhesus monkeys (Johansson *et al.*, 1968; Valerio *et al.*, 1968; MacDonald, 1971; Wilson *et al.*, 1972), but more work is required to confirm this.

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