REPRODUCTION AND BEHAVIOUR OF THE LESSER MOUSE LEMUR (MICROCEBUS MURINUS, MILLER 1777) IN CAPTIVITY

by

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ABSTRACT

A detailed knowledge of the behaviour and reproduction of any exotic species of mammal plays an important role in the successful maintenance and breeding of that species in captivity. Data from the field are particularly important in evaluating results in captivity. Where complete field data are not available, problems arising in captivity can only be assessed by taking successfully breeding individuals as the norm and using them as a guideline when studying reproductive problems.

This study has been designed to establish the reproductive parameters of the lesser mouse lemur, Microcebus murinus, using data from the field and successfully breeding individuals to this end. Aberrations arising in captivity were examined and an attempt was made to identify their causes.

During the course of this study data have been collected on the annual variations of behavioural and physiological parameters. Information on male and female reproductive physiology has been collected and hormone assays have been conducted on urine samples from oestrous, pregnant and non-pregnant females. Data on infant birth-weight and development have also been recorded. Special attention has been paid to reproductive behaviour, particularly birth and maternal care. In addition, a study has been made of social behaviour and communication. The relationship between social behaviour, status and reproductive success has been examined.

Having established the "normal" condition it was possible to examine deviations from this norm. Under certain circumstances environmental stimulation was observed to either stimulate or suppress reproduction. Suppression of reproduction is discussed in the light of physical and social environmental influences.

Data on pathology have also been collected and adrenal weight and histology recorded. An attempt has been made to integrate post-mortem findings with observations on reproduction and behaviour.

The normative data collected in this study are discussed in terms of their adaptive significance and the deviations from the normal condition are viewed in terms of stress. An attempt is then made to indicate the positive influence which this and similar studies could have on captive animal management.

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CHAPTER I

INTRODUCTION

The research undertaken on the lesser mouse lemur, Microcebus murinus, during the course of this study is intended to be of both general and scientific interest, in that it is hoped that the study will have some bearing on the husbandry and breeding of exotic species.

The data presented in this thesis are of obvious use to others involved in similar captive breeding projects in which colonies of exotic species are maintained for scientific research. However, the implications that such work could have on the zoo world should not be forgotten. The zoo both as a locale for research or as an institution benefitting from research work, tends to be very much neglected by the scientific community. As part of the research for this thesis was undertaken under the auspices of the Zoological Society of London and of the Jersey Wildlife Preservation Trust, it is felt that the beneficial relationship between research and the zoo world should be emphasised. First, however, a few words need to be said on the topic of zoos themselves.

The exhibition of wild animals in zoological gardens is now a worldwide phenomenon. Fairfield Osborne (Crandall,1964), one-time President of the New York Zoological Society, has pointed out that "the whole subject of wild animals including methods of keeping them has been of engrossing interest throughout the world for many centuries even, one might say, since the earliest days when man captures wild animals to domesticate them". However, if the only function of zoos was to be the entertainment of their human visitors, there would be very little argument to support their continued existence. In order to justify its existence in the modern world the zoo should also be a vehicle of conservation in which viable populations of various animal species should be maintained in order to ensure against their extinction. Today some of the better zoos are beginning to aim towards this ideal.

The progressive zoos of today are far removed from the menageries of yesterday. No longer do they seek to operate as a kind of living museum where the most important aim is to display one example of as many species as possible, but rather they tend to try to establish breeding groups of various species. As a result methods of husbandry and breeding of their charges have improved beyond recognition. Nevertheless, the breeding records of most zoos leave much to be desired.

Probably one of the greatest obstacles to successful breeding in zoos is stress. In general terms so little is known about the habits of most exotic species under natural conditions that the diet, social groupings and caging conditions used for a particular species are derived through a process of trial and error. This lack of knowledge together with the constraints imposed on husbandry by finance and the necessity to exhibit the animals to the public results in the animals being housed in less than optimal conditions and this results in stressing of the inmates. It is in these fields of providing information about exotic species and of investigating the relationship between housing conditions (including group structure and diet) and reproductive success that scientific research can be of use to the zoo.

The subject of this thesis, the lesser mouse lemur, Microcebus murinus, illustrates well the problems encountered in captive breeding projects. The reproductive output of the study colony as a whole was well below maximum and various problems arose in the health of the animals. As a species the mouse lemur is not endangered, although it is accorded protection by the International Convention on the Trade in Endangered Species. Nevertheless, it is rarely seen in captivity and is virtually unobtainable from Madagascar. Furthermore the problems encountered by conservation projects in Madagascar (Griveaud & Albignac, 1972) indicate that there is no guarantee that its existing status will be maintained.

Under natural conditions the mouse lemur leads a semi-solitary existence (Martin, 1972a, 1973). The problems of social stress encountered in groups of these animals are, therefore, greater than those associated with more social species. One of the early reports on the behaviour of mouse lemurs (Shaw, 1879) described them as shy, wild and untameable and referred to their quarrelsome nature and their tendency to fight fiercely in captivity. In addition, data collected in the present study indicate that daily and annual temperature variation and annual variation in the timing of "dawn" and "dusk" are important to the well-being and successdul reproduction of this species. These factors are rarely considered in animal husbandry and may be of importance in other species.

During the course of the study, data have been collected on the normal reproduction and behaviour of the mouse lemur as well as on the problems which arise in captivity. The data on reproduction and behaviour are important as they not only provide invaluable information for others who are breeding mouse lemurs but they also are interesting in relation to evolutionary theory. The prosimians as a group retain many ancestral characteristics (Martin, 1973) and the mouse lemur, in particular, is considered by some authors to be closest to the ancestral condition (Charles-Dominique & Martin, 1970). Thus, the data obtained may also throw some light on the reproduction and behaviour of the ancestral primate.

Although some studies have already been conducted on the reproductive physiology of the mouse lemur; notably those by Petter-Rousseaux (1962, 1964, 1974, 1975, Perret, 1974, 1977), Martin (1972b, 1973) and Andriantsiferana et al. (1975), very little work has been conducted on its behaviour. Some details on the behaviour of the mouse lemur in the field were provided by Martin (1972a, 1973) but otherwise little has been published. Therefore, laboratory studies are necessary to fill the gaps in our knowledge of the behaviour of this animal. Even if plentiful field data were available, laboratory studies would still be necessary as detailed behavioural data would be extremely difficult to obtain from a small, nocturnal, arboreal species under field conditions.

This thesis is divided into four sections. The first section deals with normal reproduction. Data have been collected over three breeding seasons during the course of this study and over four seasons by Dr. R.D. Martin. During these seven seasons information was collected on various reproductive parameters: testis size; oestrus, including inter-oestrus periods; gestation length; litter size and infant development. Such extensive data on oestrus, gestation and infant development have not previously been published. These data for the successfully breeding individuals in the study colony are considered together with those obtained in other laboratory studies (Petter-Rousseaux, 1962, 1964, 1974 and Andriantsiferana et al., 1974) and in the field (Martin,1972a, 1973). Where the data from all these sources agree the basic reproductive parameters of the species are considered as established.

Under natural conditions mouse lemurs are strictly seasonally breeding animals (Petter-Rousseaux, 1968) and there is clear evidence from previous studies that the timing of the breeding season is primarily controlled by daylength (Petter-Rousseaux, 1970, 1974). The relationship between reproduction and photoperiod is also examined in the first section, and data collected in this study confirm the relationship. Annual variations in other physiological and behavioural parameters are also investigated and particular emphasis is laid on those activity variations which can be correlated with adrenal activity. By this correlation a behavioural measure of adrenal activity is established.

The first section also includes information on reproductive behaviour, mating, birth and maternal care. The data on the behaviour at parturition and early maternal care are the first published for this species. This is primarily due to the fact that birth and early maternal care all take place within the nest-box. These observations, therefore, had to be undertaken with the aid of viewing equipment sensitive to infra-red light. The reproductive behaviour of the mouse lemur is compared with that of other species of primates, notably Galago senegalensis, with a view to deducing from behaviours common to the two species those behaviours which might have been exhibited by an ancestral primate form. In addition, the influence of hand-rearing on behavioural development, particularly on the development of normal reproductive patterns, is discussed. This information could be of particular interest to those studying the mouse lemur in the future as it will be necessary to know to what degree the behaviour of hand-reared animals can be considered as normal and whether it would be valuable to incorporate such animals in a study.

The second section of this thesis is devoted to the behaviour of captive mouse lemurs. The first chapter deals with activity patterns and social behaviour. The variations of behavioural activity over the day, over the year and between individuals are examined as well as the occurrence of specific behaviours. Detailed observations of social interactions have also been made, both in the group situation and during introduction experiments. The results of these observations are compared with those obtained for other prosimians and many similarities are noted and discussed. In addition, the relationship between social status and reproductive success is examined and differences in social behaviour within groups of mouse lemurs are discussed and related to the reproductive potential of the group.

The second chapter in this section is devoted to the detailed examination of communication in the study species. Visual, olfactory and vocal communication are all investigated. Body posture, tail movement and ear position are examined in terms of visual communication. The types of marking behaviour used by the mouse lemur are examined as are the frequency of marking and the places marked. In addition, a histological examination was made of those areas of skin which are rubbed against the substrate during marking behaviour in order to determine whether specialised scent glands were involved in marking. Vocal communication was found to be well-developed in the mouse lemur and a total of four infant and thirteen adult calls were identified during the course of the study. Three of these calls seem to be involved in the control of reproduction and, therefore, are of relevance to the third section of this thesis in which reproductive problems are examined.

Having established the basic reproductive parameters of the mouse lemur in the first section of this thesis, the third section deals with reproductive abnormalities or deviation from the norm arising in captivity. A chapter on pathology is also included in this section in order to determine whether reproductive problems can be associated with an overall disease syndrome arising in captivity. However, before continuing it must be noted that all deviations from the normal pattern of reproduction arising in captivity need not necessarily be disadvantageous. For example, transfer of animals to new cages during the non-breeding season can, in some instances, induce the animals to come into breeding condition out of season: this can lead to successful mating in some circumstances. As this phenomenon seemed difficult to duplicate under controlled conditions, resort had to be made to the literature for supporting evidence. An extensive literature search ensued which indicated that similar phenomena have been observed in other species although apparently no follow-up work seems to have been undertaken. This is unfortunate given the tremendous advantages which could accure to captive breeding projects if this phenomenon were fully researched and exploited.

However, most of the reproductive abnormalities arising in the mouse lemur in captivity have a negative effect. They include suppression of testis development in the males and abnormal oestrus patterns, refusal to mate, failure of conception, abnormal maternal care, abortion and pseudomale behaviour in the females. These findings are very similar to those noted in other species maintained in situations of social stress: for example, on mice maintained in overcrowded conditions (Christian, 1955, 1956, 1971, Southwick, 1955) and for the tree shrew, Tupaia belangeri, subjected to fighting stress or housed with a dominant individual (von Holst, 1972, 1974). The relationship between these findings in the mouse lemur colony and stress is examined in some detail and recourse is made to post-mortem findings for further evidence. Adrenal weight and histology are considered and the various pathological conditions diagnosed are compared with those reported in other species under conditions of stress.

The final section of this thesis is the discussion which endeavours to draw all the results together into a comprehensive whole. A relationship is sought between various behavioural parameters and the stimulation or inhibition of reproduction. The various pathological findings are also examined in order to determine whether these may be linked to reproductive suppression and to establish the extent to which they may be considered indicative of stress. Direct links between methods of husbandry and reproductive suppression or pathological conditions are also sought. An attempt is made to determine whether there is one underlying mechanism involving adrenal stimulation which, at one end of the spectrum, can stimulate reproduction and at the other can inhibit it and lead to disease. To this end the theory of stress is invoked.

The groundwork of the relationship between stress, adrenal activity, inhibition of reproduction and susceptibility to disease was laid down by Selye (1950). According to his classical theory of the process of adaptation to stress, an individual which is subjected to long term stress undergoes a process of physiological adaptation in order to maintain homeostasis. During adaptation, functions which are of less immediate importance to the organism, for example reproduction, are temporarily sacrificed to achieve this end. This theory could explain the inhibition of reproduction observed in the mouse lemurs. However, a problem could then arise when it is noted that cage transfer, which is also a stressor, can stimulate reproduction. This dichotomy is resolved when it is remembered that Selye's original hypothesis was formulated in terms of "chronic stress". It is considered in this thesis that chronic stress due to environmental conditions leads to the observed reproductive suppression in accordance with the classic theory. However, the reproductive stimulation observed in other circumstances is considered to be the result of "acute stress". It is the difference in the action time of the stressor that accounts for the difference in response. Thus, one underlying mechanism, adrenal stimulation, could lead to both phenomena.

However, if the discussion were rendered solely in terms of stress much data collected during the course of the study would be greatly undervalued. It is in the nature of this type of study that a limited amount of data are collected on a very wide range of topics. In the case of the mouse lemur study one is dealing with a small number of animals and data collection is influenced by their seasonal nature. Thus, for example, collection of data on reproduction is limited to a few months of the year and to those few individuals which are apparently reproductively normal. Data on the inhibition of reproduction are also limited by the small numbers of individuals and the limitations this imposes on experimental work. As a result, data were collected on a wider range of topics than might be the case in the conventional thesis, both in the hope that the findings might facilitate the interpretation of the reproductive results and because, as is stated earlier, so little is known of the behaviour or pathology of this species that all information may be of use to future studies on the mouse lemur.

The main criticism of this type of work must arise out of the definitions of "normal" and "abnormal". In the absence of complete field data it is impossible to establish when an animal is behaving and reproducing normally. However, this lack of knowledge is common in captive breeding projects involving exotic species. This thesis illustrates the only method of evaluating captive results in the absence of field data. This involves a decision as to what the "normal" condition of the species is to be during a project. This decision is influenced by the long-term aims of the project. Once the "norm", which may be formulated in terms of longevity, resistance to disease, reproductive success etc., has been decided upon, then deviations from this desirable condition can be examined and the causes thereof established and eliminated. Whilst this approach might not be acceptable to the purist it is the only way of undertaking a study on reproductive and behavioural abnormalities arising in captivity and, therefore, its use is valid in this thesis and in other captive breeding projects.

Finally, it is felt that the approach adopted in this thesis can perhaps serve as a model for others who are working with limited numbers of animals. The paucity of specimens should not be allowed to discourage such study. Should such discouragement become accepted little or no research would be undertaken on the rarer exotic species. These are the very species for which, by virtue of their rarity, research is essential to ensure their survival for posterity. If one adopts the broad approach to research illustrated in this thesis not only is much interesting information collected on a variety of topics but also this data can be formulated into a general picture of the biology of the species concerned, which in turn can improve husbandry and provide basic information for future laboratory or field studies.

CHAPTER II

MATERIALS AND METHODS

1. The Study Species

The lesser mouse lemur is a small-bodied, omnivorous, nocturnal prosimian native to the island of Madagascar. The mouse lemurs, genus *Microcebus*, comprise at least two species, the rare Coquerel's mouse lemur, *Microcebus coquereli*, and the more abundant lesser mouse lemur, *Microcebus murinus*.

Two forms of lesser mouse lemur are well-known:- a rufous form with small ears which inhabits the rain forest areas along the east coast of Madagascar and a grey form with larger ears which inhabits the remaining forested areas of the island.

A third form has also been reported (Petter,1962,cited by Martin,1972a). This form is similar to the rufous form described above but has large ears. This animal is rare and little is known about it, other than that it is known to occur in West Madagascar sympatrically with the grey form.

In the past, all three forms of the lesser mouse lemur were accorded species status. The grey mouse lemur, otherwise known as the Rat of Madagascar (Buffon, 1798),was given the latin name, *Microcebus murinus*, the rufous form known as Smith's mouse lemur was named, *Microcebus smithii*, and the third form was apparently known as the dormouse lemur and named *Microcebus myoxinus* (Forbes, 1894).

Today the two well-known forms of mouse lemurs are considered as subspecies (Hill, 1953, Napier & Napier, 1967) and referred to as *M.m.murinus* and *M.m.rufus* respectively. The rufous form is also referred to as *M.m.smithii* by some authors; for example Napier & Napier (1967). The third form or dormouse lemur of Forbes remains at present unclassified.

However, some authors, notably Martin (1972a, 1973), consider the rufous and grey mouse lemurs to be separate species and evidence collected during the course of this study would tend to support this view.

1.1. The Study Colony

Both of the above common forms of lesser mouse lemurs were maintained in the study colony.

The colony was established in 1969 with 10 individuals (3 males and 7 females) of each type. Since then the colony has expanded to 30 individuals. The grey mouse lemurs have prospered and in 1977 numbered 22 individuals including 12 males. The rufous mouse lemurs have been maintained with rather less success and now number only 8 individuals. Of this total of 30 animals, 67 per cent are captive-born including 6 full second generation offspring. All animals were moved in the autumn of 1974 from University College to the Wellcome Institute, Regent's Park.

2. Housing Conditions

Three types of housing were used in the colony. These were the basic cube cages, a "harem" cage and an observation room. All cages were fitted with a number of fine branches for climbing. Table 1 indicates the kind of caging used to house each individual over the study period.

2.1. Basic Cages

The basic cage (fig.1) was 75 cm cube and an individual animal was housed in each module. The cubes were arranged in blocks of two or three. The wire walling between adjacent modules allowed visual, vocal, olfactory and some tactile contact between individuals. Each module had one sliding nest-box attached to the outside to facilitate the trapping of animals (fig.2) and one

TABLE 1

Caging Conditions

	University College	Wellcome Institute			
		Room 1		Room 2	Observation Room
Year	Cube cage	Harem cage	Cube cage (double unit)	Cube cage (treble unit)	Observation Room
1973-1974	All RF2, RM7, MF7 & MM3 housed in separate room, not included in study.				
1974-1975		MM4 MF1 MF2 MF5 MF7	RF1 & RM3 RF10 & RM4 MM1	RF3,RF2[d],RM7 Bonnie[b], Clyde[b], MF9 Sam[b], Plato[b], -	MF6 MF8 MM3
1975-1976		MM4 MF1[d] MF2 MF7[d] MF12[b]	Sam & Bran[b] MM10[b] & MM11[b] RF1[d] & RM3 RFJ[a] & RM4	RF3,RF10,RM7 Bonnie,Clyde,MF9 Henry[b],MM1,MF5 Plato, MM9[b], Branwen[b]	MF6 MF8 MM3

a indicates that the animal arrived from another collection during the previous year.

b indicates that the animal was born during the preceding breeding season.

d indicates that the animal died during the course of the year.

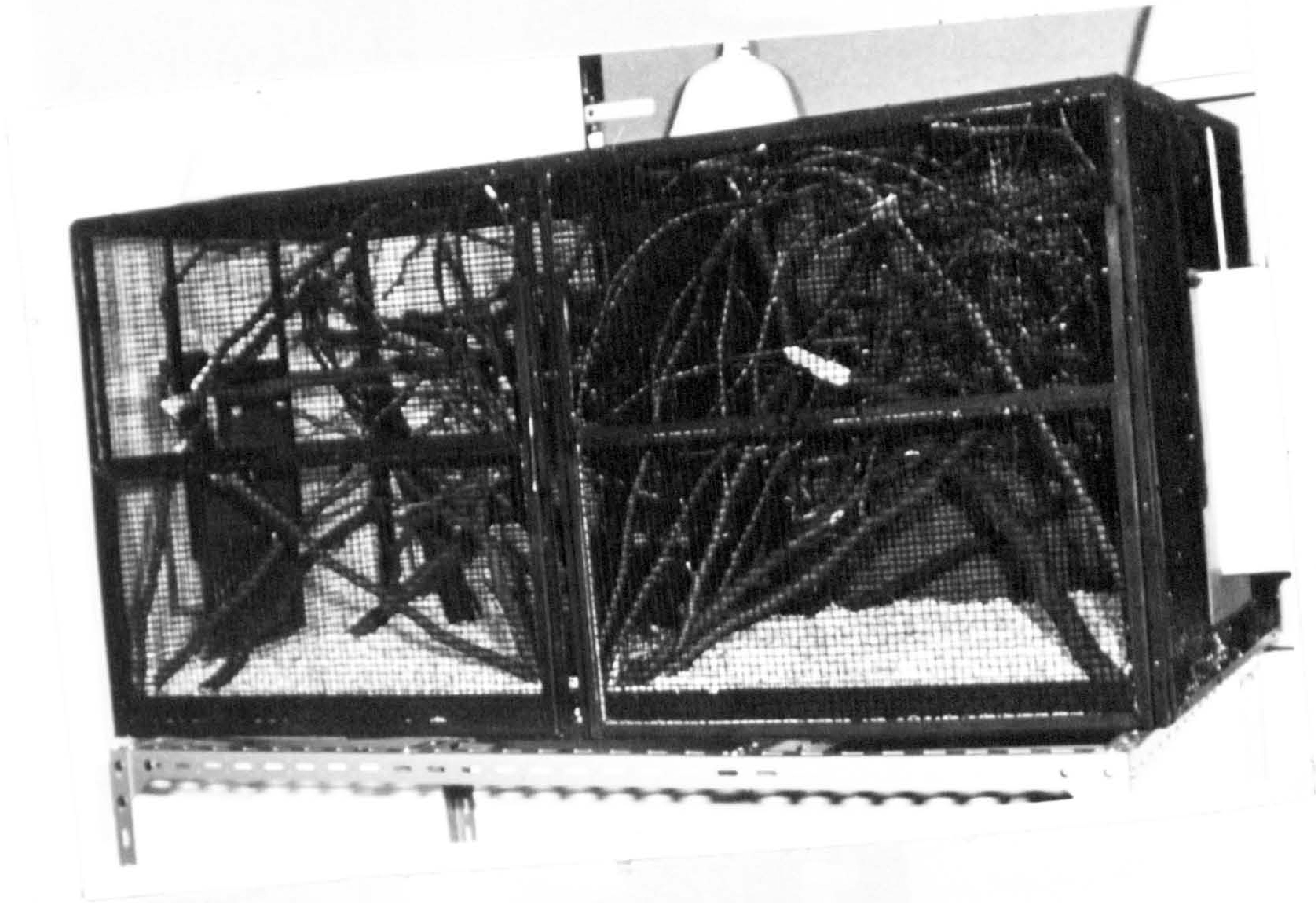

Figure 1

Two basic cage modules

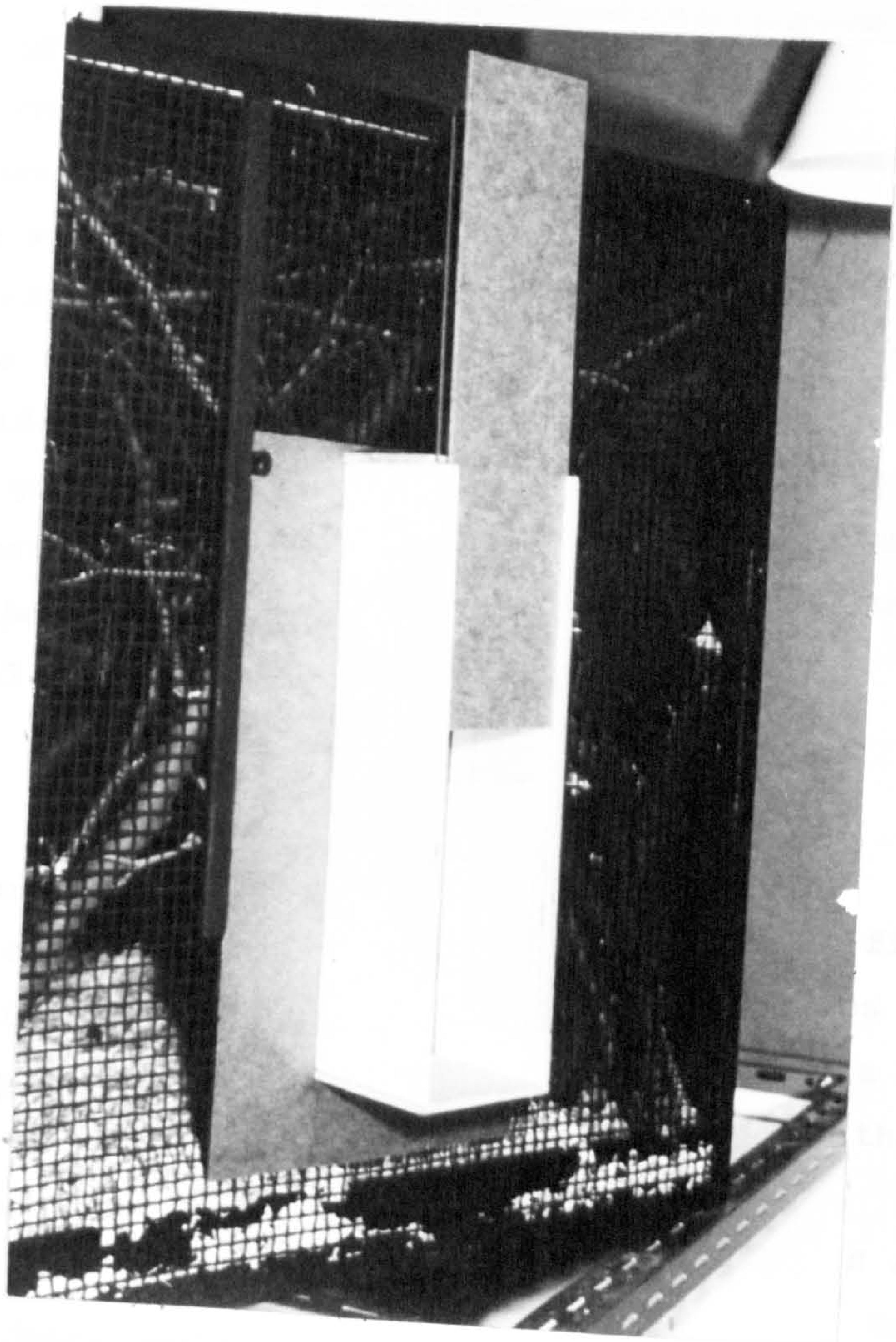

Figure 2

Nest-box attached to basic cage

or more ground nest-boxes or tubes were provided for the animals to hide in. Most of the colony animals were maintained in this type of housing when housed at the Wellcome Institute (1974 onwards). When the colony was based at University College (prior to 1974) the basic cage was the only type of housing available. However, there was some modification to this basic cage design during the University College period. During this time there were some multi-module units in use in which there were no partitions between the individual modules. These double or triple units were used to house groups of animals as well as individual specimens. During the period when the current study was in progress the only animals to be housed together were the two female pairs, MF1 and MF2 and RF1 and RF3.

2.2. The "Harem" Cage

The "harem" cage (fig.3) consisted of a unit of five individual cages, each of 150 x 75 x 75 cm. Above the cages was an inter-connecting passage to which access was given by a sliding door in each cage; in addition, each cage had access to the adjacent cage or cages via a sliding door in the wall.

The walls of the individual cages were solid and therefore there was no contact with neighbouring animals. However, all the sliding doors could be replaced as required by "sliders" made of cage wire which enabled visual, vocal, olfactory and some tactile communication between neighbours and with animals in the inter-communicating passage.

In these cages, nesting facilities were provided by the use of plastic drainpipes which were supported in a vertical position in the cage. Wood wool was pressed into the bottom opening (fig. 4). The diameter of the drainpipes corresponded closely to the average diameter of tree-hollows used by grey mouse lemurs in the wild (Martin, 1972a, 1973).

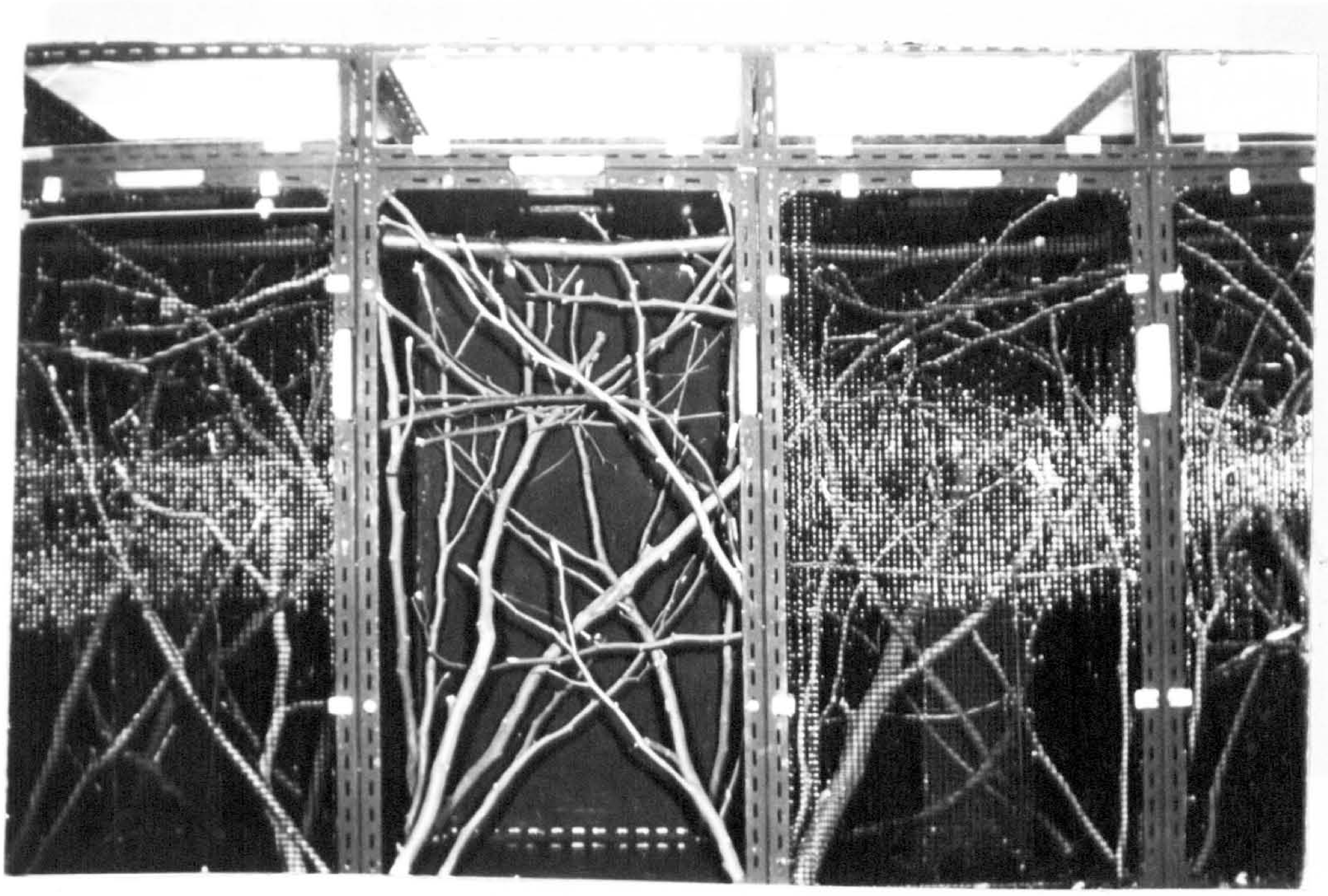

Figure 3

The harem cage

Figure 4

Drainpipe nesting-tube

2.3. The Observation Room (fig.5)

Three individuals, two females and one male, were kept in an observation room. The room measured 3.2 x 2.4 x 1.65 m and was partially filled with branches. A window from the room gave into a small chamber in which an observer was able to sit.

Nesting facilities were provided by plastic drainpipes which were rather longer than those used in the "harem" cage. Twigs were placed inside each section of the drainpipes to facilitate the animals' movement up and down the tubes.

2.4. Temperature and Humidity

As far as possible the ambient temperature of all rooms was maintained between 22° and 28° C. With the exception of the Observation Room, where temperature was thermostatically controlled at a constant 25° C, all rooms had an auxillary heater which came on with the white lights (see lighting below). This led to a higher temperature being maintained during the artificial day (23°-28° C) than during the night period (22°-25° C).

Each room, with the exception of the Observation Room, was supplied with a humidifier. Relative humidity was maintained above 40 per cent. However, even with a humidifier the humidity never rose above 50 per cent.

2.5. Lighting

As the study species is nocturnal the animals were maintained under a reversed lighting regime so as to facilitate behavioural observation during the "night". Night lighting was provided by means of 40, 60 and 100 watt red light bulbs. This provided a level of illumination in the darkened room of between 0.5 and 1.5 foot candles per square foot (i.e. between 5 and 15 lux). This may have been somewhat bright, as Kavanau and Peters (1976a,b) in their work on other nocturnal primates (namely the lesser

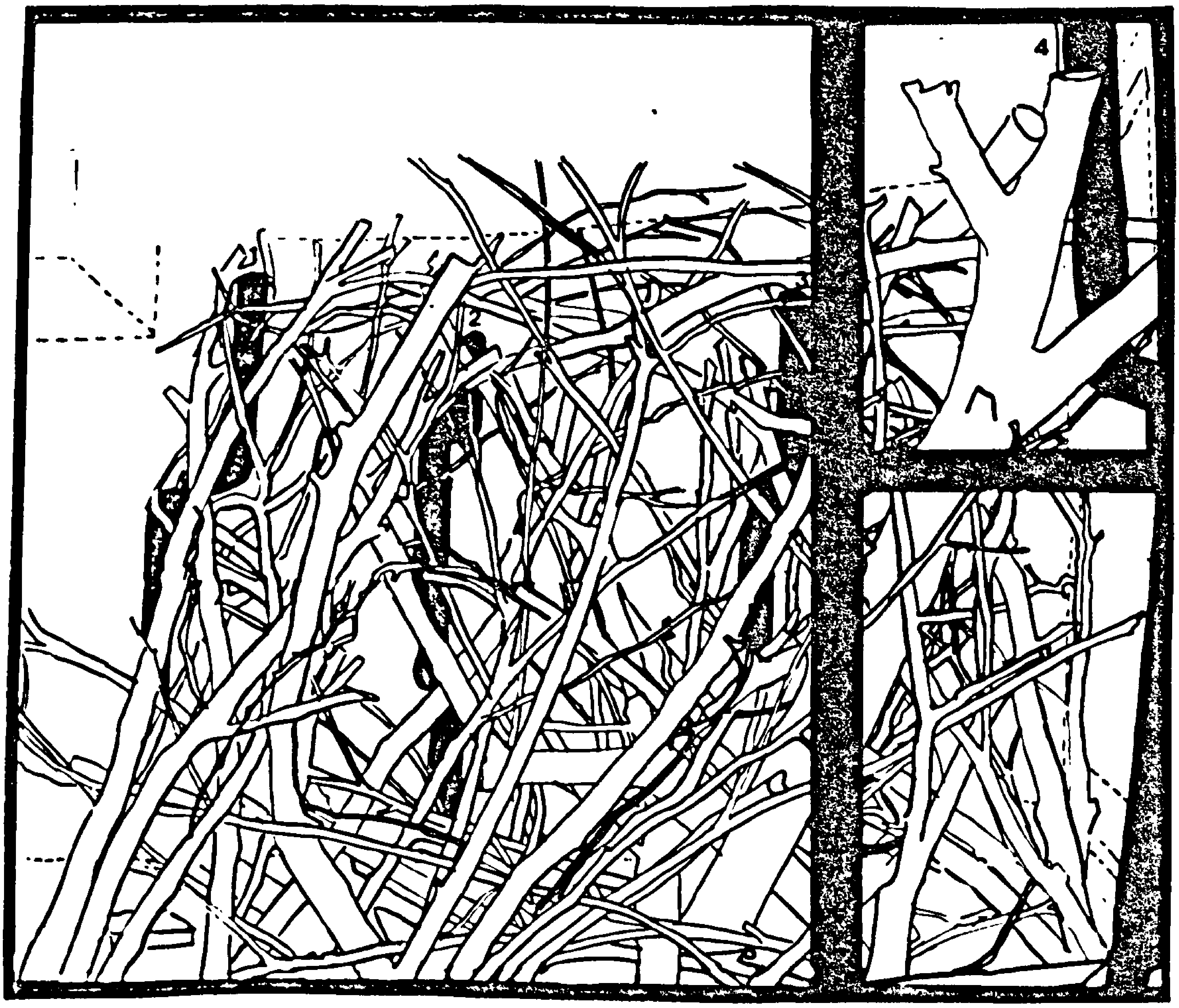

Figure 5

Plan of the Observation Room

Numbers have been allocated to differentiate between the four drainpipe resting tubes.

bushbaby, Galago senegalensis, the slow loris, Nycticebus coucang and the owl monkey, Aotus trivarigatus) have reported that these species are active at much lower light intensities (between 0.042 and 2.5 lux). The relatively high light intensity used during the artificial night did not seem to adversely affect the behaviour of the animals, which were generally only active during the red light period.

Daylight was provided by means of fluorescent tubes and the changeover between day and night was accomplished by means of an automatic time-switch. The onset of the night phase was timed to occur between 11.00 a.m. and 2.00 p.m. This enabled the animal staff to feed the animals and clean the cages in the morning while the animals were still asleep.

2.5.1. Light Cycle

The length of time during which the animals were subjected to white light each day was varied over the course of the year so as to simulate the seasonal changes in daylength occurring in Madagascar (fig.6), although no "twilight" period was included. Normally a twelve-month light cycle was used but in some instances a nine-month cycle was used in which the same variation of daylength was compressed into a nine-month period (fig.6).

Maximum daylength was 13 hours and minimum daylength was 11 hours.

2.6. Diet

The animals were fed on a diet of mixed fresh fruit, carrots, tomatoes, lettuce, raisins and nuts. Insect food in the form of mealworms and locusts was given four times a week. Fresh milk and pink mice were provided once each week and Vionate vitamin additive was sprinkled sparingly over the food twice a week.

During the winter daylength period, care has to be taken to reduce the quantity of food given to prevent obesity.

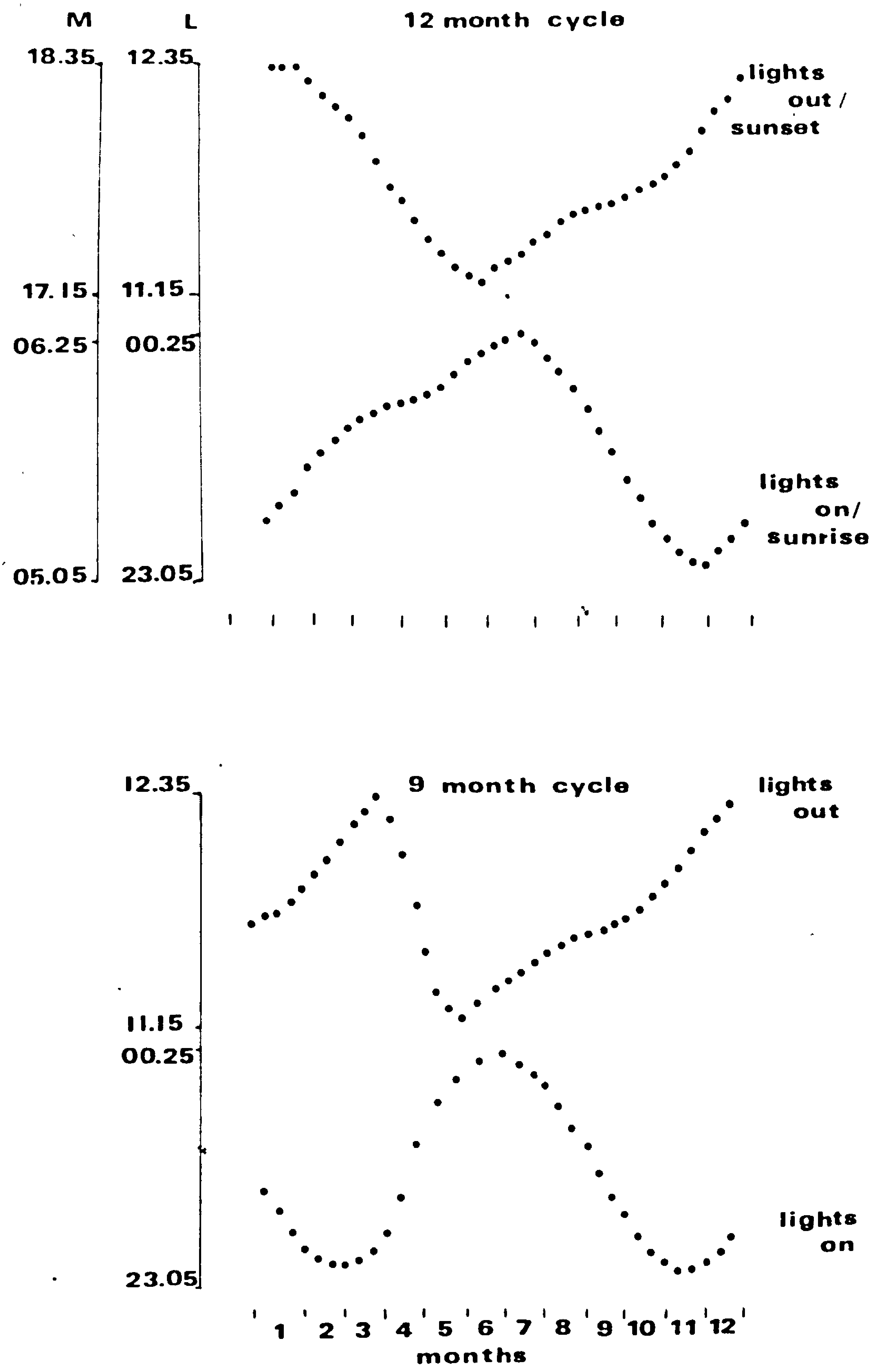

Figure 6

Light cycles

The times on the M axis of the top figure indicate the actual time of sunrise and sunset in Madagascar. The times on the L axis indicate the times of lights on and lights off in London.

2.7. Jersey Zoo

Three grey mouse lemurs, two females and one male, which were housed together in the nocturnal house in Jersey Zoo were also included in the study. These three animals were housed together in a cage measuring 2.3 x 3.0 x 2.8 m. Temperature, humidity and diet were essentially similar to those in the main study colony. The light regime differed in that there was a period of "twilight" during which the white lights of the cage were switched off, but those outside the cage were left on. In addition, although daylength was varied over the course of the year, rate and pattern of change were not based on those naturally occurring in Madagascar. An artificial pattern was employed (fig. 7).

3. Methods

3.1. Condition Checks

All animals in the handling cages, that is the basic cube cages, were caught once a week and weighed using a 0-300g Pesola spring balance. At the same time their tail size was estimated by visual comparison with a size chart (fig. 8). The testis size of the males was estimated by visual comparison of the scrotum with a size chart (fig. 9). The vulval condition of the females was also recorded: the state of swelling and colouration and the presence of a vaginal opening were noted.

Both the tail and testis size charts were derived from actual measurements. The accuracy of this method for estimating testis size was evaluated by comparison with actual measurements of the scrotum (table 2) and by comparison with the estimates of other observers. On both these counts, this method appeared to be reliable and accurate.

During these routine condition checking procedures the females were examined to determine whether they were pregnant. This was usually done by means of abdomenal palpation to determine whether

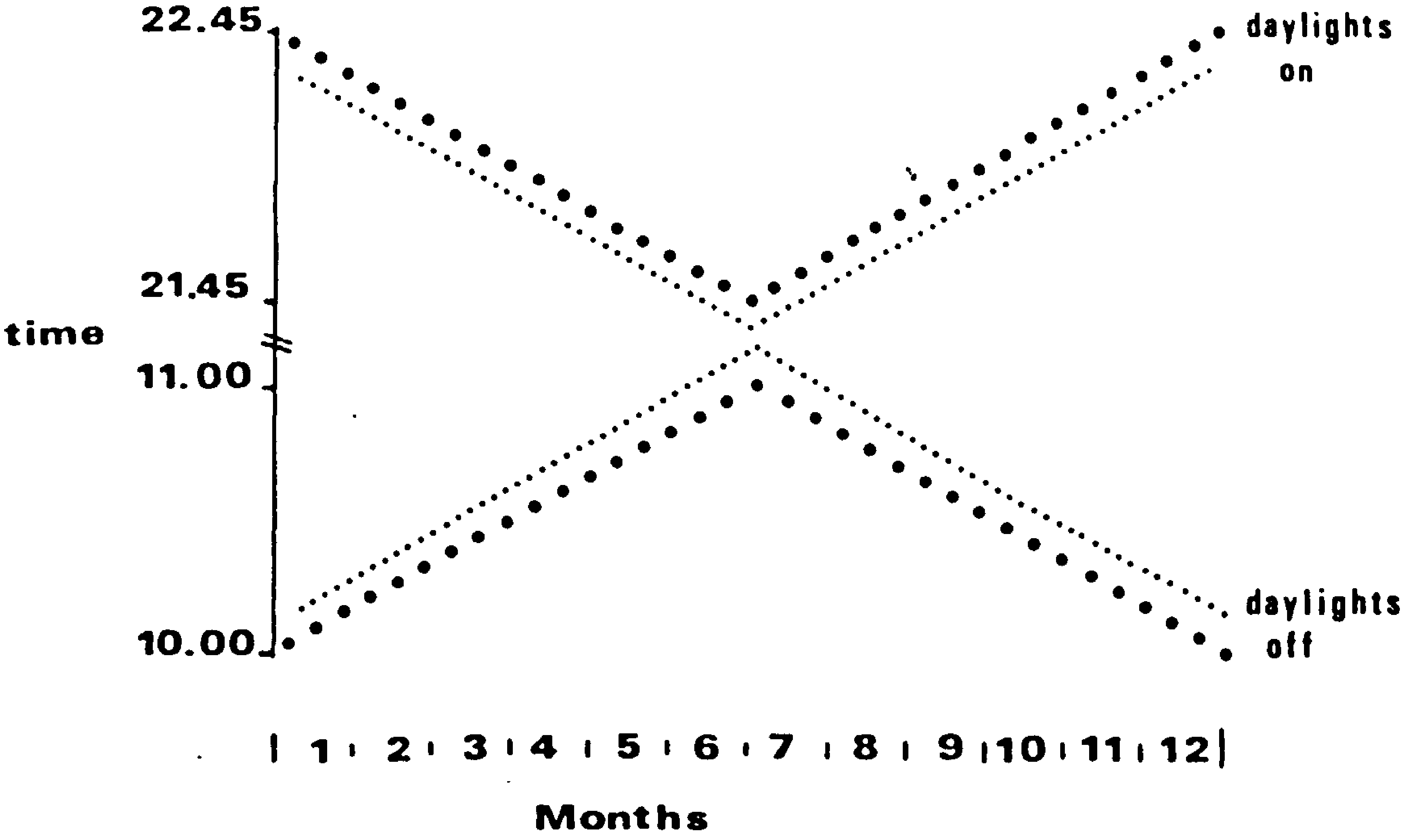

Figure 7

The Jersey light cycle

The "twilight" period is indicated by the space between the large and small dots

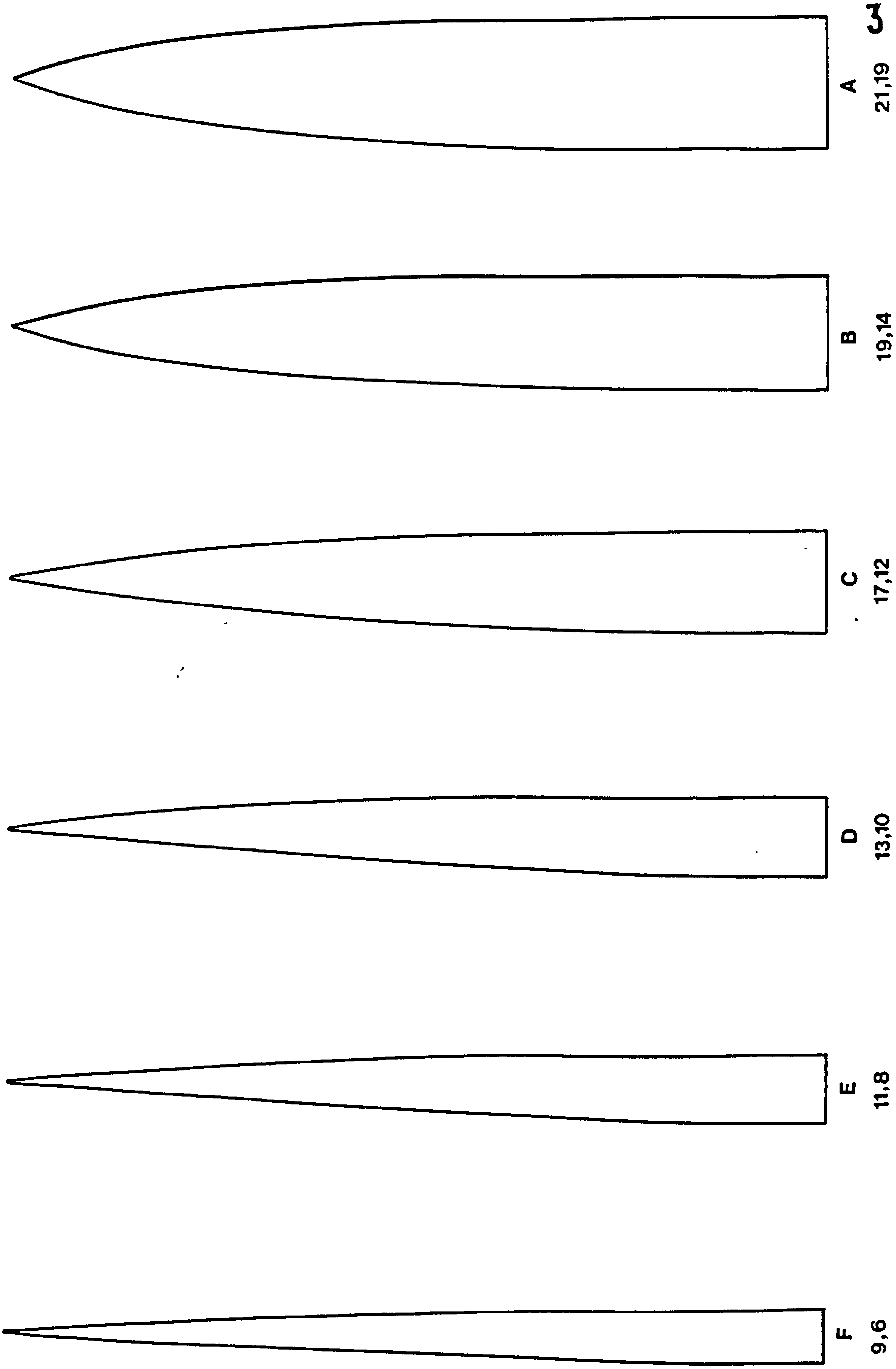

Figure 8

Tail size chart

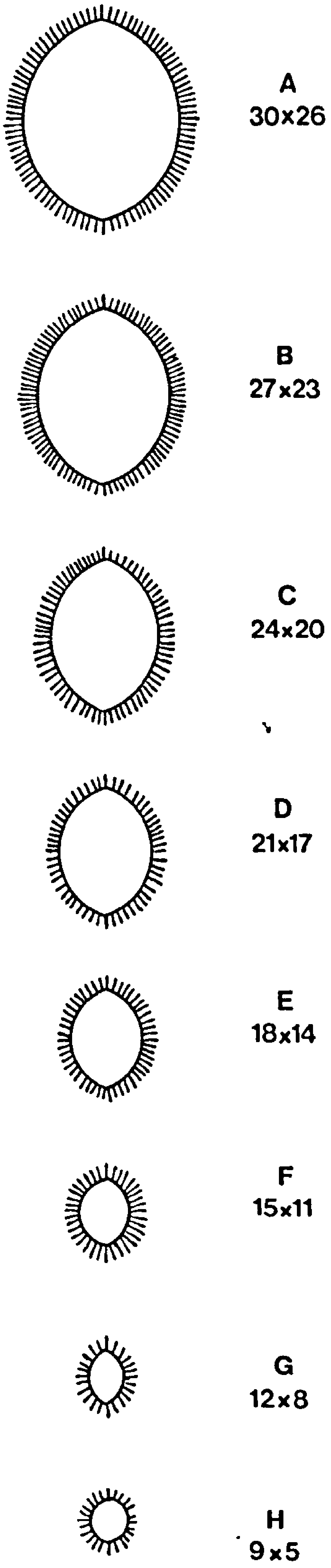

Figure 9

Testis size chart

Numbers represent length x breadth (mms).

TABLE 2

Testis Size: - comparision of visual estimates with measured size

Size	Actual Conversion	Estimated Scrotal Size	Actual Scrotal Measurement
A	30 x 26 mm	A - B	27 x 24 mm
B	27 x 23 mm		
C	24 x 20 mm	C - D	24 x 20 mm 20 x 18 mm
D	21 x 17 mm		
E	18 x 14 mm	E - F	19 x 16 mm
F	15 x 11 mm		

the uterine horns were enlarged. However, in the non-handling situation (see below) only visual examination of the belly for signs of swelling was possible. Both these methods proved reliable during the latter half of pregnancy.

Non-handling cages were established to facilitate condition checking without subjecting the animals to handling stress. Animals housed in the non-handling cages, that is the Observation Room and the "harem" cages, were therefore not weighed on a regular basis. These animals were trained to jump onto the bars of their cages for a food reward (fig.10). When the animals were in this position it was possible to check tail size, scrotum size and vulval condition in the same way as for the handled animals. In addition, the food bowls of the animals in the non-handling cages were placed on the scale pans of Salter balances. This enabled some estimate of the body weight of these animals to be obtained whilst they were feeding. However, in most instances it was found that the animals would go to great lengths to avoid standing on the scales and would usually reach down to the food while maintaining a grip on some convenient branch with their feet.

3.2. Histological Observations

3.2.1. Vaginal Smears

Vaginal smears were obtained by a lavage technique. Approximately 0.5 cc of sterile saline was flushed in and out of the vagina of a female mouse lemur using a plastic Pasteur pipette. This flushing procedure was repeated several times.

A few drops of this saline solution were then dropped onto a microscope slide. The slide was allowed to dry in the air and was then treated with "Dryfix", a polyethylene glycol solution (Disbrey & Rack,1967).

The resulting smears were then either stained with haematoxylin and eosin or with Papanicolaou stain (1942). The numbers of each

Figure 10

Condition checking of trained animals in non-handling cages cell type present in the smears were then estimated visually and scored as to whether that type was very rare, uncommon, common or very numerous in the particular smear.

3.2.2. Organs

The organs removed at post-mortem were fixed (unless otherwise stated in the text) with Bouin's solution (Disbrey and Rack,1967), mounted in paraffin wax and stained (unless otherwise stated) with haematoxylin and eosin.

3.3. Behavioural Observations

In addition to the behavioural observations undertaken on the study colony individuals, a series of observations were undertaken at Jersey Zoo. To accomplish this a series of five-day long visits were made at monthly intervals over a six-month period and one additional visit was made three months after the completion of the six-month period.

Only the names of the behaviours recorded will be mentioned here and in the result section. A full description of all behaviours observed is provided in Appendix I.

3.3.1. Activity Cycles

Data on activity cycles were collected from the animals in the group-housed condition: i.e. the three specimens in the Observation Room and the animals housed in Jersey Zoo.

In order to examine daily and annual activity cycles, the occurrence of eight easily observed behaviours was recorded. These were: still, move, groom self, groom other, feed, eliminate, nest-box and marking. In addition it was noted when any of the animals were in physical contact or proximity (i.e. within one mouse lemur length apart). All of these behaviour categories, with the exception of the proximity scores, were mutually exclusive. For example, if an animal was feeding in the nest-box entrance, only feeding would be scored. The occurrence of these activities was scored at one-minute intervals during a twenty-five minute observation period.

These observation periods were organised into "blocks". Each block consisted of twenty-five observation periods - five at each of the five observation "times". These times were:-

1. within one hour of the onset of red lighting,
2. 2.00 pm.,
3. 6.00 pm.,
4. 8.00 pm.,
5. within one hour prior to onset of white lighting.

The sequence of observation times within each block was randomised using a Latin Square technique.

Four blocks of observations were carried out during each year. Blocks I and II were implemented when the daylength was 11 hours. Block I was completed before the period of shortest daylength and Block II was undertaken after this period. Blocks III and IV were carried out when the daylength was 13 hours. Block III was undertaken before the longest daylength period and Block IV was carried out after that period. The whole series of observations was repeated over a second year in the London group but not in Jersey where the group of mouse lemurs was only established in suitable accommodation during the final year of the study. Thus, a second series of observations was not possible.

The method of data collection chosen, in which only those behaviours occurring at a specific instant in time were recorded, would not be particularly accurate if one was dealing with behaviours of brief duration. However, the behaviours which were being recorded, with the possible exceptions of elimination and marking behaviour, tended to persist for prolonged periods of time and, as such, the method of data collection employed gave a relatively good estimate of the percentage of time the subject spent on each behaviour. In addition, although this method does not give an accurate estimate of the percentage of time spent occupied in brief behaviour patterns, the data collected are adequate to allow for comparison of the frequencies of various behaviours during different observation periods.

3.3.2. Social and Maternal Behaviour and Birth

These behavioural observations were made directly or from video tape recordings and the data were collected on to check sheets, written down directly or spoken into a cassette recorder. The video equipment used in most situations was a Sony Portapac portable videotape recorder. The recorded tapes were either played back at normal speed using the portapac or, in the cases where detailed analysis was required, a Shibaden videotape recording deck with a slow motion facility was employed.

To secure observations of birth or maternal behaviour in the nest-box, special equipment had to be used: all behaviour in the nest-box was observed with the aid of infra-red light and special infra-red sensitive equipment.

To facilitate this, the back wall of the nest-box was replaced by a sliding wall permeable to infra-red light. The wall was constructed with three Kodak Wratten infra-red filters sandwiched between two sheets of perspex.

The lighting was provided by a mains-operated 70W infra-red light source manufactured by Plasma Electronics.

Activity within the nest-box could be observed using a hand-held, battery operated infra-red viewer, also produced by Plasma Electronics, or by the use of a closed-circuit arrangement.

The closed-circuit arrangement was comprised of an infra-red video camera connected to a television monitor outside the animal room. The television monitor in turn could also be connected to a video recorder to enable the recording of behaviour on videotape. Birth was only recorded using the closed-circuit arrangement as the presence of an observer within the animal room could disturb the mother.

A Hansen frequency technique was used for most social and maternal observations. An electronic timer was used which gave an audible signal at thirty second intervals and all the behaviours occurring within each thirty second interval were scored.

As Altmann (1974) has indicated, this method does not provide a true measure of frequency but is rather a measure of the frequency of intervals that include some amount of time spent in that behaviour. However, the use of this method for comparison either between groups of animals or when comparing the same group over time is valid.

3.3.2.1. Introductions

Before the onset of an introduction experiment the sliding doors to the inter-communicating passage were removed from the cages of the animals involved. Each introduction lasted 45 minutes; timed from the moment when the individuals first saw each other. This method of timing was used because the subjects would often remain in their cages for 10 or 15 minutes after the sliding door had been opened.

Data from these introductions were recorded onto video tape for later analysis of visual communication signals or were recorded directly onto check sheets using the Hansen frequency technique. To allow for better analysis the introduction sequences were randomised using a Latin Square technique.

3.3.2.2. Group Behaviour

Social behaviour in groups of mouse lemurs was followed over 45-minute observation periods which were distributed through the first half of the night. Data were either collected onto check sheets using the Hansen frequency technique or, more frequently, observations were made on a continuous basis using a cassette recorder to record a spoken commentary of all behaviours observed. The timer mentioned in section 3.3.2. above was used to provide a time base for the observations.

The observations collected in a continuous fashion were analysed by computer using the Primate Program devised by Humphreys (1974). This analysis provided information on the relative frequency of behaviours and on the probability of two behaviours occurring in sequence.

3.3.2.3. Early Maternal Behaviour: the nest-box stage

Information collected on early maternal behaviour included data on the amount of time the mother spent with her infants in the nest-box as well as mother-infant behaviour in the nest-box. Data on the amount of time spent in the nest-box were collected by direct observation over 30-minute periods using a cumulative stop-watch. The watch was started when the mother left the nest-box and stopped again when she returned.

Observations of behaviour in the nest-box were facilitated by the use of the infra-red equipment described above (section 3.3.2.). The Hansen frequency technique was used over 30-minute observation periods. The main behaviours recorded were suckling and cleaning. However, notes were made on all behaviour observed. The amount of time spent in the nest-box was generally only recorded during the first hour after the red lights came on. Data on behaviour in the nest-box were collected at this time and during two later periods, two and four hours later respectively.

3.3.2.4. Later Maternal Behaviour: post nest-box stage

Data on later maternal behaviour were recorded during a series of daily observations implemented between 30 and 90 days after birth. During 30-minute observation periods, data were collected on a continuous basis. All interactions occurring during the observation periods were noted and the individual which initiated and terminated each bout of mother-infant contact was also recorded. In addition, a description was made of all activities occurring within each interaction unit (i.e. the interval between the approach of one individual to another and the departure of one of the individuals).

3.3.3. Communication

3.3.3.1. Marking Behaviour

Marking behaviour was studied in twelve individuals, nine females and three males. Six of these individuals, one male and five females were housed in the harem cage unit. The other six individuals were housed in two groups of three, one in the Observation Room and one at Jersey Zoo. The frequency of marking was recorded and, with the exception of the Jersey Zoo group, the areas marked were also noted.

The areas within the Observation Room which were used for marking were delineated by tracing the place where the marking occurred onto a prepared map of the branch layout. This same map was employed to collect information on cage use, viz. routes and resting sites. In order to augment this information on cage use a record was kept as to which individual used which nest-boxes and feeding sites. In the harem cages no data were collected on cage use. Data on marking sites were obtained by comparing the marked branch with a corresponding numbered branch on a photograph of the cage. The branch number and the type of marking behaviour observed were recorded onto a check-sheet.

In the"harem"cage recording of areas marked was combined with a record of marking frequency. All bouts of marking within each 30-minute observation period were recorded.

In both the Observation Room and Jersey Zoo, data on marking frequency were collected during the social behaviour observation periods.

3.3.3.2. Visual Communication

A Sony Portapac Videotape Recorder was used to tape the interactions of mouse lemurs during a series of introductions.

Analysis of these tapes was made using the slow motion apparatus described above (section 3.3.2.). Using this technique information was collected on ear, body and tail positions associated with these interactions.

3.3.3.3. Vocal Communication

Tape recordings were made of as many different mouse lemur calls as possible. All recordings were made using a Nagra tape recorder and BASF low noise tape. As mouse lemur calls are generally of high frequency, the fastest tape speed of 15 inches per second was used.

The calls were analysed using a Kay-sonagraph. Half or quarter speed playback was used in all instances.

Some attempts were made to play back various calls to the mouse lemurs. Generally, however, the quality of the played-back recording was poor due to the absence of suitable speakers and therefore the animals failed to respond in most instances.

3.3.4. Statistical Techniques

The statistical procedures used during the course of this work have, in as far as possible, been restricted to the most basic tests; t-tests, F-tests and chi-squared tests. Therefore, little explanation of method is required. However, such tests did not suffice in all instances and recourse had to be made to Analysis of Variance, Cluster Analysis, linear regression and various non-parametric tests.

3.3.4.1. Analysis of Variance

In order to assess the influence of subject, time, and month on activity levels a three-way analysis of variance technique was used.

Due to the quantity of data analysis had to be made by computer. The SPSS subprogram ANOVA (Kim & Kahout, 1970) was used to this end. This method of analysis was found to be particularly suitable as ANOVA can cope with uneven cell size and even empty cells in the data matrix. The reasons why this facility was necessary will be discussed later (section 4).

In the event that the results from the analysis proved significant, post-hoc comparisons by means of t-tests and concordance tests (see section 3.3.5.4.) were used to evaluate any interesting differences within the data-matrix.

3.3.4.2. Single Link Cluster-Analysis

This method of analysis has been employed to investigate which social behaviours occur together in sequence and to compare these sequences between groups. The advantages of this method of analysis are that:-

a) it is simple to apply and understand and it produces a visual representation of the data;

b) it is a continuous method i.e., small changes in the data do not give rise to discontinuous changes in the dendrogram.

Method (Morgan *et al.*, 1976): the first step in this method of analysis is to construct a table indicating the number of occasions that one behavioural event succeeded another, for example:

Preceding Behaviour	Succeeding Behaviour					
	Sniff	Assoc.	Nest	Follow	Chase	Total
Sniff	28	41	0	29	3	101
Assoc.	10	30	0	2	0	42
Nest	0	0	0	10	0	10
Follow	58	39	5	7	0	109
Chase	4	33	0	2	4	43
Total	100	143	5	50	7	305

From such data, a triangular table of similarities can be constructed; "similarity" in this study is the likelihood of two behaviours occurring in succession. Taking the data from the example above this is next transformed into a table of proportions by dividing each element in the row by the row total, for example:-

	Sniff	Assoc.	Nest	Follow	Chase
Sniff	0.28	0.41	0.00	0.28	0.03
Assoc.	0.25	0.74	0.00	0.005	0.00
Nest	0.00	0.00	0.00	1.00	0.00
Follow	0.49	0.38	0.05	0.07	0.00
Chase	0.10	0.75	0.00	0.05	0.10

The table is then symmetrized using the method of Altmann (1968, cited by Morgan <u>et al.</u>, 1976), which is to sum corresponding off-diagonal terms; for example the similarity between sniff and associate is (0.41 + 0.25). The similarity table is now:-

	Sniff	Assoc.	Nest	Follow	Chase
Sniff		0.66	0.00	0.77	0.13
Assoc.			0.00	0.385	0.78
Nest				1.05	0.00
Follow					0.05
Chase					

From the similarity table a dendrogram is constructed. First the most similar pair is determined: in this case it is nest and follow with a similarity of 1.05. These two are linked up on the dendrogram. The second most similar pair is determined (associate/chase) and these are also linked up on the dendrogram. Sniff links to follow at 0.77. This forms a cluster of sniff, nest and follow at a similarity of 0.77. Sniff links with associate at 0.66, and this completes the dendrogram.

Comparison of Dendrograms (Jenkinson,1973): in order to compare dendrograms the similarities in the above example are replaced by ranks. Thus:-

1.05, 0.78, 0.77, 0.66, 0.385, 0.13, 0.05, 0., 0., 0.,

are replaced by:-

1, 2, 3, 4, 5, 6, 7, 9, 9, 9,

respectively.

A value d' is a measure of comparison of two such ranked matrices.

$$d' = \sum_{i<j}\sum \left| a_{ij} - b_{ij} \right|$$

where i is the number of rows and j is the number of columns. To ensure that 0<d'<1, the value $\sum\sum \left| a_{ij} - b_{ij} \right|$, is multiplied by a constant. The constant used by Jenkinson was:-

$$\frac{1}{\max\left(\sum_{i<j}\sum a_{ij}\right)}$$

For a dendrogram containing 13 objects this constant is $\frac{1}{819}$, and for a dendrogram of 14 objects it is $\frac{1}{1040}$.

Jenkinson also deduced a table of significance for d', this is:-

Significance level	Number of Objects 12	13	14
1%	0.178	0.184	0.173
5%	0.234	0.219	0.213
10%	0.246	0.236	0.229

3.3.4.3. Linear Regression

Linear regression was calculated either with a calculating machine or by using the SPSS multiple regression subprogram (Kim & Kahout, 1970). In the event that it proved necessary to determine whether several regression lines approximated to the same line an F-test was used. This test determined whether each individual line differed significantly from the variance of a mean regression line, which took into account the data from all the separate regression lines which were of interest.

3.3.4.4. Non-Parametric Methods

Four non-parametric techniques were employed in this study. They were:the Spearman rank correlation coefficient, the Kendall rank coefficient, the Mann-Whitney U test and the Kendall coefficient of concordance (all are discussed in Siegel,1956).

Non-parametric statistics are particularly useful in behavioural studies as they do not make the numerous assumptions about parameters required by parametric statistics. Thus, they are useful when the data for analysis either are not truly numeric or when they do not meet the assumptions required by a parametric test: for example, the observations must be drawn from a normally distributed population. The observations must be independent and, in cases where a comparison is to be made of two or more sets of data, these data must be drawn from populations with the same variance.

The non-parametric statistics chosen for this study are the most powerful available for the data. A powerful test is one in which there is a small probability of rejecting the Null Hypothesis when it is correct but a large probability of rejection when it is incorrect. When discussing the power of a test the figure quoted represents the power-efficiency of that test compared to its parametric counterpart. Thus, if a test is 90% as powerful as its parametric counterpart, then to equate the power of both tests it is necessary to draw 10 cases for the non-parametric test for every 9 cases for the parametric test.

The Spearman and the Kendall ranking tests are measurements of correlation, that is to say they are used to ascertain whether two sets of data are related and the extent to which they are related. Both of these tests have a power efficiency of 91%. In the case of the Kendall ranking test, the cumulative significance of the level of correlation of several sets of rankings is of interest. To this end z is calculated for each set of rankings (Siegel, 1956) and then summated to give z total. This figure is divided by the square root of the number of sets of ranking to give z' which is then looked up in a table of z values.

The coefficient of concordance differs from these two methods of measuring correlation in that it measures the relation among several sets of data instead of between two. The significance of the coefficient, W, obtained in this test was obtained from Pearson & Hartley (1958), table 46.

The Mann-Whitney U test on the other hand, is used to test whether two independent sets of results differ significantly from each other, i.e.: it acts as a non-parametric alternative to the t-test. It is one of the most powerful non-parametric tests with a power-efficiency of approximately 95% for moderate sized samples.

3.3.4.5. Significance

Throughout this thesis a probability level of 5% or lower is taken as significant.

3.3.4.6. Means

Throughout this thesis all means given are qualified by standard errors or, where insufficient data are available for the calculation of the standard error, by ranges.

4. Problems

A number of problems were encountered during the course of this study and, as these problems were rooted in the nature of the study and the choice of study species, it is felt their inclusion is necessary in order to explain some of the discrepancies in the data presented. The difficulties arose because of the paucity of specimens available for study. This resulted in limiting the amount of data which could be collected - a problem which was exacerbated by the seasonal nature of the species in question. As the animals were only really active over part of the year, the collection of data in the fields of reproduction and behaviour was restricted. In addition, one was dealing with a species which does not breed readily in captivity and, even if successful reproduction is achieved,only two young are produced by each female each year. This meant that the number of animals available for study remained small, although the former point resulted in the collection of data on reproductive suppression.

This situation resulted in difficulties in duplicating observations and made experimental work on stress impractical. However, the most important problem arose when several events of interest occurred at the same time, as it was necessary to select which was the more important to the study as a whole. For example when a visit to Jersey coincided with a month allocated to the study of activity patterns it was not possible to complete all the activity pattern observations. Seasonality resulted in several females giving birth within a few weeks of each other which meant that it was not possible to study the maternal behaviour of all the females daily at the appropriate times.

It is felt that the difficulties encountered do not detract from the work as a whole, given that valuable data were collected on a little-known species.

Further, similar types of problems are encountered by most researchers who study exotic species in captivity. For example, in most of these cases the number of specimens available for study is small. It is felt that the value of this kind of study should be emphasised because,although the data collected are not extensive enough for exhaustive analysis to be undertaken or for firm conclusions to be drawn, they do provide necessary basic information which is of use in future studies and captive breeding projects.

SECTION A

NORMAL REPRODUCTION

This section seeks to establish the basic reproductive parameters of the lesser mouse lemur. Data collected from successfully breeding individuals in the study colony over seven breeding seasons plus information from other captive colonies and field studies are used to this end.

CHAPTER III

PHOTOPERIODISM

Jolly (1972a) has stated that "... many or most primates have a breeding season". However, as she also pointed out, a great deal of data is necessary to distinguish between a season of births, in which reproduction is confined to one part of the year, and a peak of births, where sporadic reproduction occurs throughout the year with the majority of births confined to a few months. This problem of differentation does not occur in the mouse lemur, where seasonality is very clearly defined.

Seasonal reproduction is a common phenomenon amongst the lemuroid and lorisoid groups, particularly in those prosimians which inhabit areas of marked seasonal variation in climate (Doyle, 1974). This currently held view of prosimian reproduction runs contrary to that held by Hill (1953) who indicated that most prosimians were polyoestrous throughout the year.

In Madagascar mouse lemurs are reported to breed between the months of September and March (Petter-Rousseaux,1962, 1968, Martin, 1972a, 1973) regardless of the climatic zone in which different individuals live. This is equally as true for the rain forest dwelling rufous sub-species as for the grey sub-species which inhabits the more arid regions, although it must be noted that few data are available for rain forest mouse lemurs. This breeding activity corresponds roughly with the rainy season. Thus, while the females may be pregnant during the latter part of the dry season, lactation and the maturation of the young take place during a period of maximum food availability.

Petter-Rousseaux (1970, 1974, 1975) has demonstrated that reproductive activity in the mouse lemur is influenced by daylength. In the laboratory, seasonal variation of daylength is manipulated artificially (see Chapter II, section 2.5.). The pattern of lighting used in the twelve-month cycle is almost identical to that occurring naturally in Madagascar (fig.6) except that the twilight period is eliminated. Under natural conditions most nocturnal primate species so far investigated (e.g., the sportive lemur, Lepilemur mustelinus leucopus, the fork crowned lemur, Phaner furcifer, (Pariente,1974), the loris, Nycticenus coucang, the lesser galago, Galago senegalensis, and the owl monkey, Aotus trivirgatus, (Kavanau & Peters,1976a,b) commence their activity at specific light intensities. With varying degrees of cloud and foliage cover, the time of emergence from the nest can vary from place to place and day to day. This variation in time of onset of activity is eliminated under these captive conditions without obvious detrimental effect. It has proved possible by further manipulation of the light schedule to reduce the annual cycle to one of nine months (Martin,1972b)(fig.6).

The data presented in this chapter were essentially collected during this study. However in some instances the inclusion of data from other sources has proved relevant and thus they have been included.

1. Reproductive Cycles

During the period of short daylength the mouse lemurs are in non-breeding condition. The females do not come into oestrus, the testes of the males are small and spermatogenesis does not occur (see Chapter IV, section 1.1.).

Testicular growth occurs as daylength increases and is complete two months after the shortest day (figure 11). When the males' testes are maximally developed the females start coming into oestrus. In captivity the first oestrus cycles are also observed about two months after the shortest day (figure 11).

The females are seasonally polyoestrous; thus, if they do not conceive during their first period of oestrus, they can come into oestrus again. Several periods of oestrus are possible during one breeding season. However, three is the number most commonly recorded

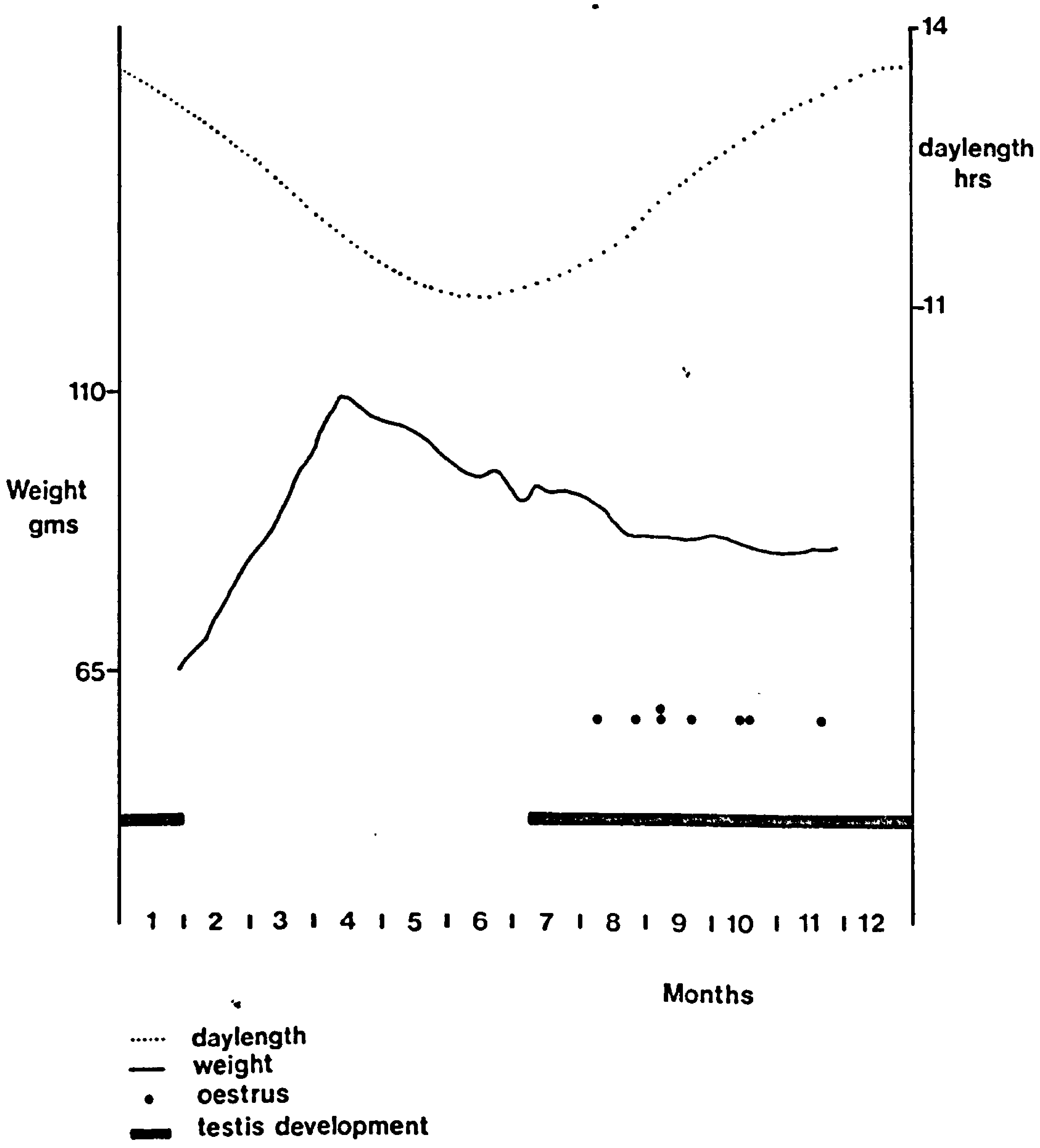

Figure 11

The relationship between daylength, body weight, testis development and oestrus: 12-month cycle

Body weight from a typical specimen

in those females which do not conceive.

The breeding season persists until the longest day. After this, as the days grow shorter, the animals return to the non-breeding condition. The last period of oestrus observed in captivity occurred about the time of maximum daylength. The males' testes begin to regress within a month of the longest day and rapidly resume their resting state (fig.11).

The males remain in breeding condition for between 4 and 6½ months. The duration of testicular development appears to be related to the sub-specific affiliation of the male concerned (see section 5. of this Chapter).

2. Weight Cycle

In addition to seasonal variation in reproductive condition, the mouse lemur also exhibits considerable variation in body weight over the course of the year (figs. 12 and 13). It is during the non-breeding season that body weight is at its greatest. During this time the body weight of the adult grey males ranges between 96 - 150 grams (mean = 124.9 ± 5.9 grams, n=9) and that of the adult grey females between 90 - 138 grams (mean = 111.3 ± 4.5 grams, n=11). These data correspond to maximal body weight obtained during the short daylength period. The corresponding figures for the rufous mouse lemurs are: males, mean = 82.4 ± 5.1 grams, n=7, range 63 - 100; females,91.5 ± 4.2 grams, n=8, range 70 - 103. (This difference is discussed further in section 4.3. of this Chapter).

In the study colony, weight loss commenced just prior to shortest daylength, when using a twelve-month lighting regime (figure 11). Body weight was at its lowest during the breeding season. The body weight of the grey males ranged between 57 - 117 grams (mean = 72.4 ± 4.5 grams, n=14) and that of the non-pregnant grey females between 69 - 90 grams (mean = 75.1 ± 2.75 grams, n=14) at this time. Corresponding figures for the

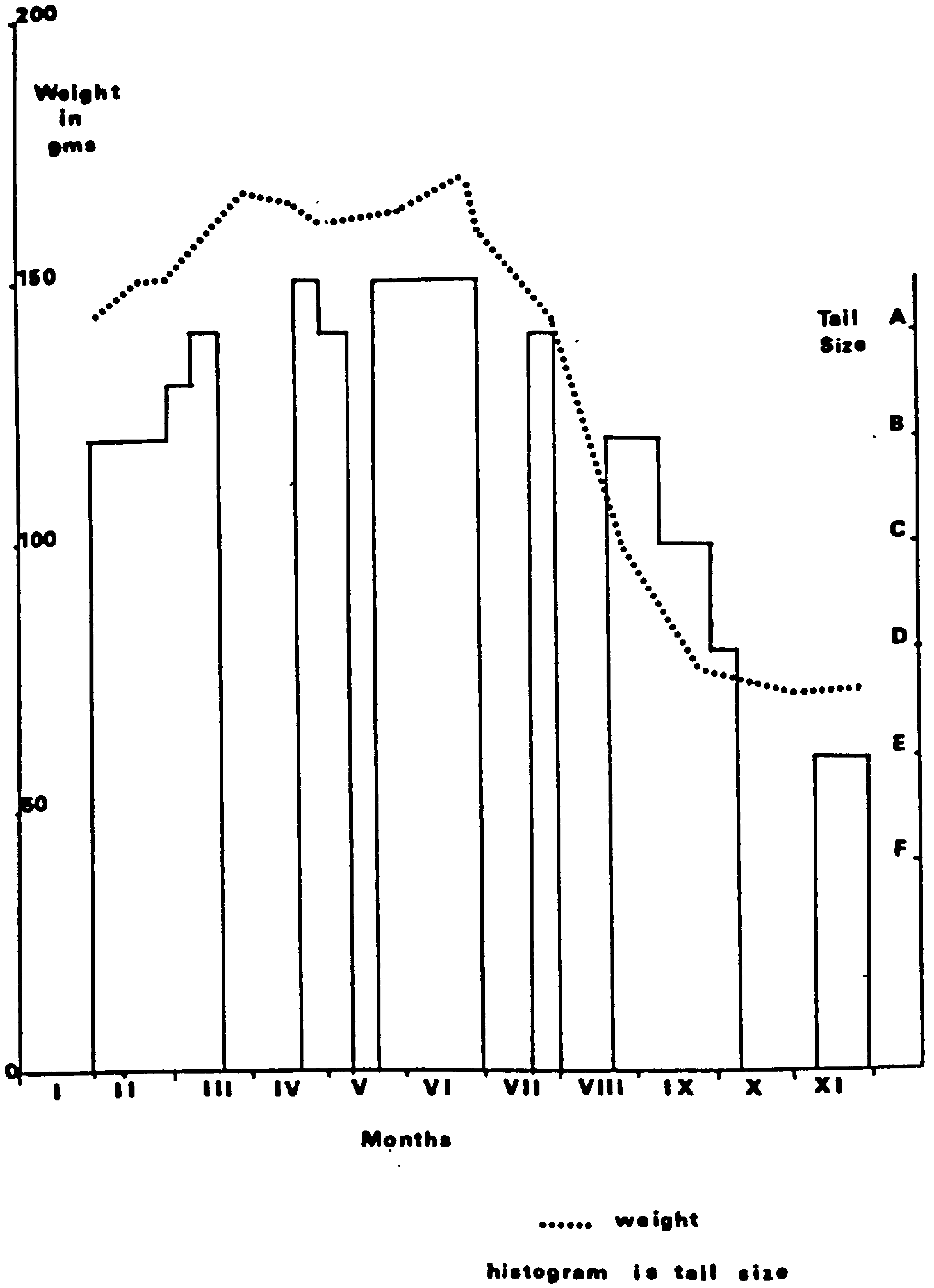

Figure 12

The relationship between body weight and ~~tail-size~~

in a typical individual

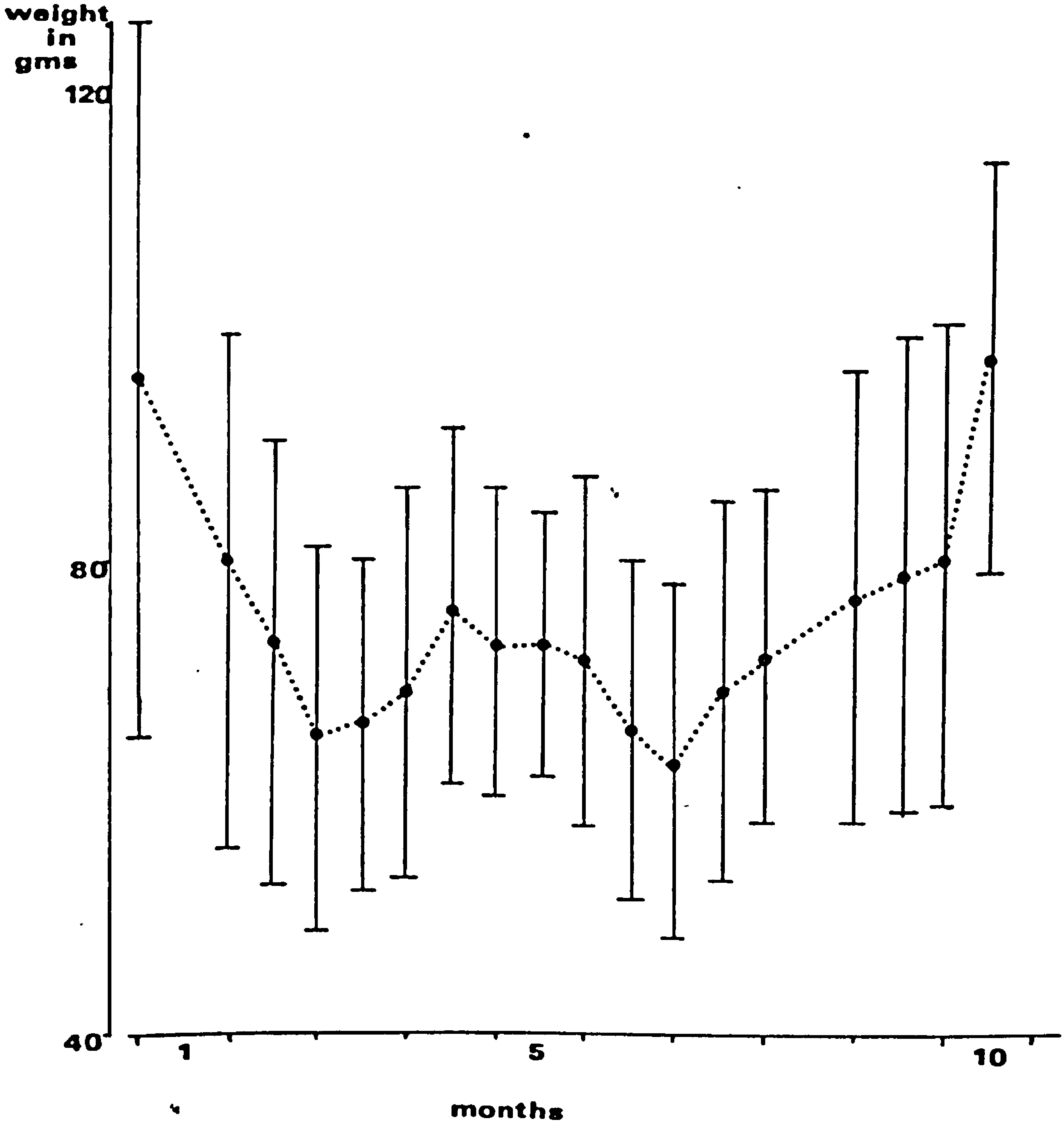

Figure 13

The annual variation in body weight (mean weight & standard deviation) of the colony as a whole during the first year of the study

Data from the first year only were used as transfer to the Wellcome Institute (see Chapter VII) and the use of a nine-month light cycle confused later results.

rufous mouse lemurs are: males, mean = 53.6 ± 1.4 grams, n=8, range 50 - 61; females, mean = 56.5 ± 2.0 grams, n=8, range 50-62.

Body weight increases again with decreasing daylength. There is a burst of feeding activity following the longest day (see section 3.) which probably initiates this weight gain.

During the non-breeding season, much of the accumulated body fat is stored in the tail. Storage of tail fat is most noticeable in the older individuals, particularly the females. In these individuals tail size may reach an A+ as estimated using the tail size chart (fig.8 and Chapter II, section 3.1.). Thus tail size can be seen to follow a similar seasonal pattern to that of body weight (fig.12). However, it must be noted that males, with the exception of those chronically obese animals (see Chapter IX, section 2.5.1.), never exceed a tail size of C/D. It must also be noted that these chronically obese animals belong invariably to the grey sub-species and account for the magnitude of the weight ranges given above. It is these males which weigh 150 grams in the non-breeding season and 117 during the breeding season.

2.1. Weight of Juvenile Animals

During the non-breeding, short daylength period, succeeding their birth, juvenile mouse lemurs do not increase their body weight to the degree attained by the adults. The body weight of these animals in the short daylength period was as follows: grey males had a mean weight of 74.5 ± 4.3 grams (n=7), range 58 - 93 grams. The two grey females weighed 86 and 110 grams and a rufous male weighed 64 grams.

Despite the difference in body weight exhibited by the young animals, particularly the males, during their first short daylength season, by the following breeding season their weights were indistinguishable from those of the rest of the colony; by this time they were sexually mature and capable of reproducing (see Chapter IV, section 4.4.).

3. Annual Cycles of Behavioural Activity, Adrenal Activity and Activity of Other Endocrine Organs

Perret (1972, 1974, 1975) and Perret et al.(1971) have demonstrated that the adrenal, thyroid and pituitary glands of the mouse lemur all exhibit annual variation in their activity. These glands are more active during the period of increasing and long daylength and less so during decreasing and short daylength.

In addition to the above mentioned cycles of body weight, and of reproductive and endocrine activity, the mouse lemur also exhibits an annual cycle of behavioural activity. Onservations on the level of behavioural activity have indicated that, in spite of considerable individual variation in activity levels (see Chapter V, section 1.2.3.),a clear annual pattern of activity emerges. Activity is lowest during the short daylength period. At this time some of the older, fatter individuals were rarely observed to indulge in any activity at all although actual dormancy was not observed.

The percentage of the "night" spent in activity increases with increasing daylength to reach a maximum one month after the longest day. This annual cycle of activity is particularly marked for feeding behaviour and results in a clear peak in feeding behaviour frequency in batch observation IV, i.e. the month succeeding that of the longest day (fig.14). The level of feeding behaviour at this time (batch observation IV, see Chapter II, section 3.3.1.) is significantly higher than that recorded just after shortest daylength (batch observation II, see Chapter II, section 3.3.1) as can be seen from table 3.

The pattern which the level of feeding behaviour shows over the year is very similar to the pattern of adrenal activity, as measured by adrenocortical width and adrenocortical nuclear diameter (Perret, 1972, 1974, see fig.14). The results of feeding levels obtained in the study colony were compared with these

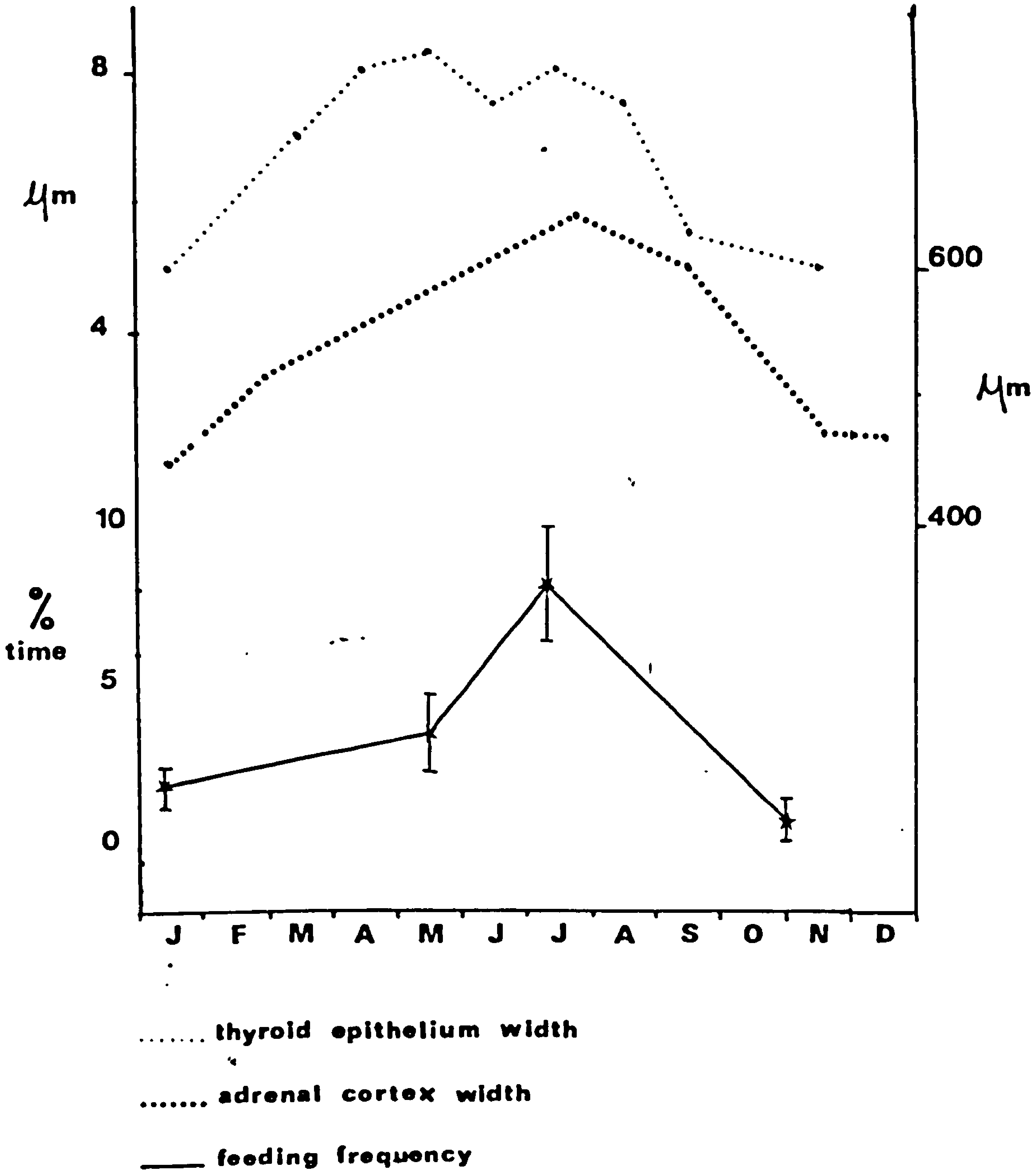

Figure 14

Annual variation in thyroid activity (thyroid epithelium width top left scale), adrenal activity (adrenal cortical width right scale) and percentage of time spent in feeding (bottom left scale)

Thyroid and adrenal data were obtained from Perret (1972).

Table 3

Comparison of feeding levels in Batch observations II and IV

This table shows the mean level of feeding for each of the individuals in the observation room over two years.
For full description of batch observations see Chapter II, section 3.3.1.

Animal	Year	Batch II (just after shortest day)	n	Batch IV (just after longest day)	n
MF6	1975	0.64±0.38 (0,8)	25	2.83±0.74 (0,12)	24
	1976	0.5±0.485 (0,8)	16	0.21±0.20 (0,4)	19
MF8	1975	3.20±1.06 (0,24)	25	7.67±1.71 (0,28)	24
	1976	3.75±1.56 (0,20)	16	6.53±1.89 (0,28)	19
MM3	1975	2.24±1.24 (0,24)	25	13.33±2.49 (0,40)	24
	1976	1.25±0.58 (0,8)	16	8.42±2.28 (0,36)	19

Figures in brackets indicate the range.

t-test comparing the means of Batch II and Batch IV for 1975 and 1976 combined

Animal	Mean of II	n	Mean of IV	n
MF6	0.585±0.30	41	1.67±0.465	43
	t = 1.97 (significant p = 0.1)			
MF8	3.415±0.90	41	7.16±1.27	43
	t = 2.38 (significant p = 0.05)			
MM3	1.85±0.80	41	11.16±1.78	43
	t = 4.77 (significant p = 0.05)			

The difference in feeding frequency between Batch II and IV for MF8 and MM3 is significant at the probability level accepted in this thesis. However, it is only significant for MF6 at a somewhat higher probability level.

n = no. of observations in all cases.

measures of adrenal activity using Kendall's τ rank test (Siegal, 1956, Kendall, 1962) and were found to be significantly similar ($p \leq 0.05$, table 4).

4. Observations with Practical Implications for Husbandry Techniques

Although all the data presented here impinge on husbandry techniques, it is felt that the following two points deserve the special consideration of people dealing with mouse lemurs or, indeed, any other species maintained under an artificial lighting regime.

4.1. Artificial Manipulation of the Natural Twelve-Month Cycle

As mentioned previously, some reduction of the annual cycle of the mouse lemur can be obtained by manipulation of the light clock (fig.6).

When using the nine-month cycle shown in figure 6, both the onset of weight loss and of reproductive condition are somewhat later with respect to daylength than is the case with the twelve-month cycle (fig.15). Weight loss commences more than one month after the shortest day and testis development more than two months after. Using the twelve-month light regime,weight loss commences before the shortest day and testis development is complete two months after (see sections 1 and 2 above).

From the results it appears that the use of the nine-month cycle acts principally to reduce the length of the breeding season. In both the twelve-month and nine-month cycles the inactive phase lasted 5½ months. Using the nine-month cycle the breeding season would have been substantially reduced had not the period of maximum daylength been extended to accommodate pregnant females. Even when extending the period of maximum daylength, the duration of testis development was one month shorter than with a twelve-month cycle.

Table 4

Kendall's Rank Test. Comparing Feeding Frequency With Adrenal Activity

Months	Oct.	Jan.	May	July	s	$z = \frac{s}{var.s}$	N
Adrenal activity	min.	increasing		max.			
Feeding							
MF6							
yr 1	-	0.6	0.3	3.0	+1	0.522	3
yr 2	0.3	0.5	1.1	0.2	0	0	4
MF8							
yr 1	-	3.2	3.4	7.7	+3	1.567	3
yr 2	1.0	3.75	6.2	6.5	+6	2.04	4
MM1							
yr 1	-	2.2	6.1	13.	+3	1.567	3
yr 2	2.0	1.2	6.0	8.	+4	1.359	4
Jersey female			4.5	11.6	+1	1	2
Jersey male			4.0	7.6	+1	1	2

$$\frac{\sum z}{m} = \frac{9.055}{\sqrt{8}} = 3.20 = z'$$

This result demonstrates a significant correlation $p \leq 0.05$

No. of cases = m = 8

$$var.s = \frac{N(2N+5)(N-1)}{18}$$

where N = no. obs.

$\sum z = 9.055$

s is a measure of the similarity between the rank order of the feeding results and the rank order of adrenal activity.

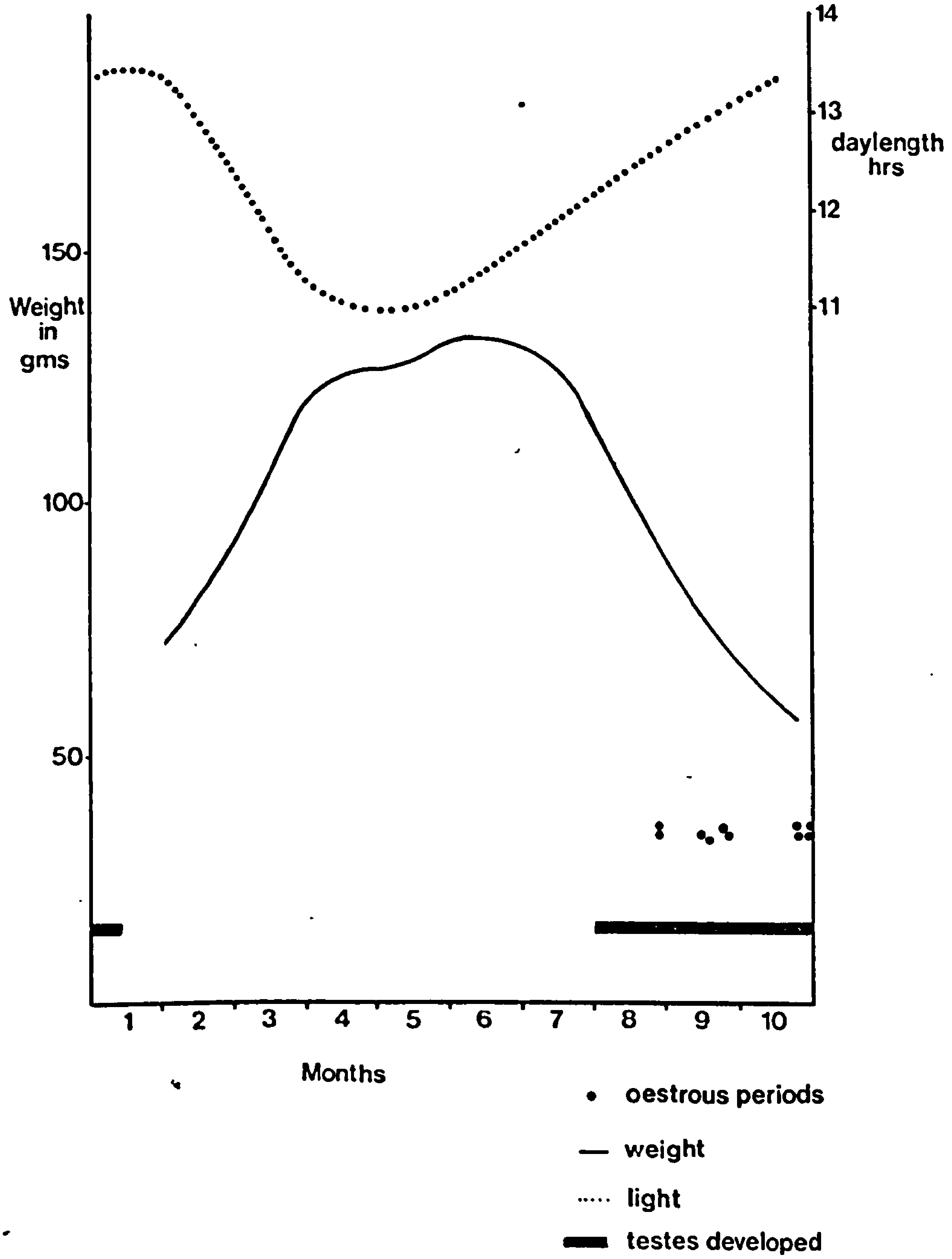

Figure 15

The relationship between daylength, body weight, testis development and oestrus: 9-month cycle

A typical body weight curve is illustrated

4.2. Poor Photoperiodic Alteration

When photoperiodic variation is important in sychronising various circannian rythms it must be remembered that it is not known if it is the time of "dawn" of "dusk" which is important. In the Clore Pavilion at the London Zoo four mouse lemurs were maintained under an artificial light regime which was varied only by altering the timing of "dawn". When three of these four animals were examined for reproductive condition they were found to be out of synchrony with each other and with the light regime (table 5).

5. Differences between Red and Grey Mouse Lemurs

During the course of the above observations it was noted that not only were the rufous mouse lemurs a much lighter weight animal than the greys (fig.16) but also that the duration of their reproductive season, as measured by duration of testis development, was shorter than that of the grey mouse lemurs (table 6).

6. Discussion

Photoperiod has long been known to control endocrine function in many species of animal including man. For example, the ancient Egyptians were well aware of the somatic changes which accompany blindness and portrayed them in their works of art, although they did not attribute these changes to disturbances of metabolism due to the lack of influence of light on endocrine secretory functions (Fuchs, 1964). Fifty years ago, Rowan (1925) reported that the breeding and activity of the slate coloured junco were set in motion by small consecutive changes of daylength in Spring.

It would seem, as Jōchle (1964) stated, that the eye acts like a photocell, correlating changes in the "internal clock" with those of the "external clock" of light-darkness change in the environment. This would appear to be achieved by direct action of the optical system on the hypothalmus. The observation of

Table 5

Condition of Mouse Lemurs in Clore Pavilion of the London Zoo

Animal	Date of Check	Daylength	Expected Reproductive Condition	Weight	Oestrus/ Testis Size	Actual Reproductive Condition
Grey Female	28 Nov.	Incr.	Breeding	70g	no oestrus	Poss. breeding
Grey Male	26 Nov.	Incr.	Breeding	85g (fat)	small testes	non-breeding
Rufous Male	8 July	Just Incr.	Coming into Condition	50g	large testes	full breeding condition

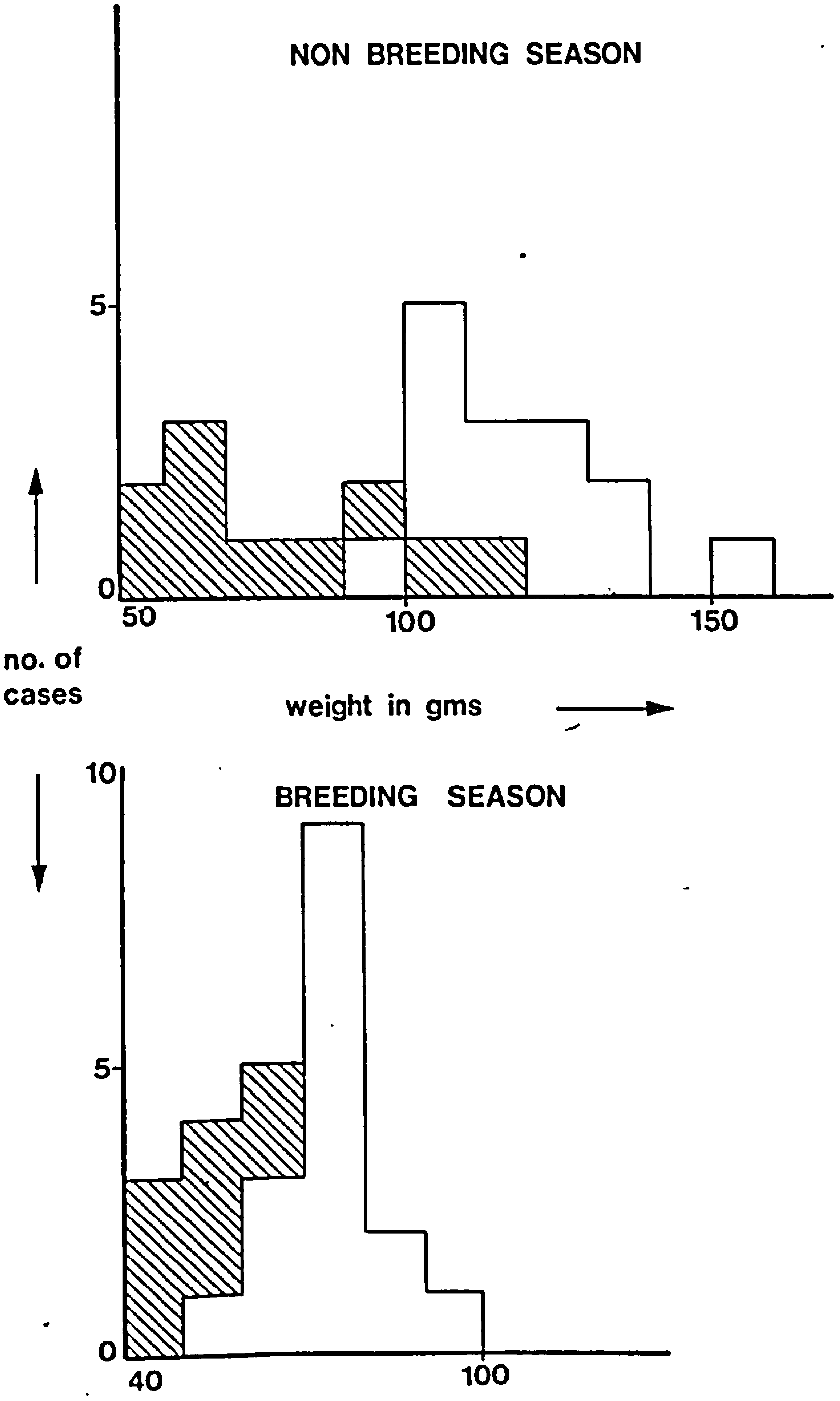

Figure 16

Weight distribution of red and grey mouse lemurs

Shaded area represents the red mouse lemurs.

Table 6

Comparison of the Duration of Testis Development in Fertile Males*

		Onset Date (week beginning)	End Date (week beginning)	Duration (months)
Grey Males	MM4	before 19 Oct. 73	18 April 74	6
	Sam	15 Jan. 75	17 July 75	6
	MM4	6 Jan. 76	21 July 76	6½
Rufous Males	RM3	19 Oct. 73	28 Feb. 74	4
	RM3	5 Jan. 76	17 May 76	4½

During the year of the transfer to the Wellcome Institute (1974-75) the old breeding grey male (MM4) exhibited two periods of testis development from before 9 October until 30 October and from 17 February to 16 June, a total of 5 months. In the same year the rufous breeding male exhibited only one period of testis development from before 9 October until 20 November, 2 months.

The small sample size arises as a direct result from restricting the data to fertile males. The inclusion of infertile males would confuse the issue as they exhibit shorter periods of testicular development (See Chapter VIII, section 1.).

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*For further discussion of fertile males see Chapter VIII, section 1.

retinohypothalamic connections in various mammal species (Scharrer, 1964) would seem to confirm this.

From the results presented in this chapter it can be seen that there is a close association between daylength, reproductive activity, body weight, endocrine activity and behavioural activity in the mouse lemur. These results confirm the relationship between photoperiod and reproduction postulated by Petter-Rousseaux (1970, 1975). The data on this relationship have been expanded in this study partly as a result of quantification, particularly of testis size, and by the inclusion of behavioural data.

By using the twelve month lighting schedule, the onset of reproductive activity observed in the study colony mimics that observed in the field. Petter-Rousseaux (1968) reported that the onset of testicular development in the males was in August and that, from the condition of pregnancy observed in some females in the field, mating could be deduced as having taken place as early as the end of August (two months after the shortest day).

Both the cycle of loss and gain of body weight and concomitant changes in tail size and the duration of testicular development confirm the findings of other authors working under laboratory conditions: Petter-Rousseaux (1974), Russell (1975) and Perret (1974) respectively. In addition, the duration of testicular development corresponds to that reported in the field by Petter-Rousseaux (1968). However, it must be noted that the body weights of mouse lemurs in captivity exceed those found in the wild by Petter-Rousseaux (1974) (see Chapter IX, section 2.5.1.).

The absence of a definite dormant period in this species confirms the field observations of Martin (1972a) who found that the mouse lemur was unlike the dwarf lemurs, Cheirogaleus sp., in this respect, and the observations of Petter (1962) who found that mouse lemurs were only markedly inactive when the temperature fell to 18 C. Temperatures never fell this low in the study colony.

Annual fluctuation in the level of activity of the endocrine glands is a common finding amongst seasonally inactive species. Observations on the thyroid glands of various seasonally inactive species, particularly hibernating ones, indicate the occurrence of annual cycles of activity similar to those found in the mouse lemur; this is true of the dormouse , *Eliomys quercinus*, the ground squirrel, *Citellus tridecemlineatus*, and the hedgehog, *Erinaceus europaeus*, (Lachiver, 1964, Hoffman and Zarrow, 1958 and Pinatel *et al.*, 1970). Similarly annual cycles of adrenal activity have been observed in the same species (Zalesky, 1934, and Parkes and Deansley, 1966). In these species maximal adrenal activity also coincides with maximum daylength.

In addition to the cycles of reproductive, endocrine and behavioural activity discussed above, Russell (1975) and Andriantsiferana and Rahandraha (1973b) have reported an annual variation in the thermoregulatory ability of the mouse lemur. During the breeding season the body temperature is maintained at 37 - 39°C during the "night", whereas it is maintained at about 6°C above ambient temperature during the non-breeding season. In this case the ambient temperature was 21°C (Russell, 1975).

These various cycles obviously have a role to play in fitting the mouse lemur for survival in its natural biotope and all are apparently directed towards one goal which is to reduce the demands of the mouse lemur on its environment to a minimum during the dry season, short daylength period, when food availability is limited. This is achieved by hormonal variation which in turn influences behaviour. Thus, the burst of feeding behaviour which occurs at the end of the rainy season (see section 3. above) allows for energy to be stored as fat; the fact that thyroid activity is lower than maximum at this time (Perret,1976) enhances this effect. As daylength decreases, decreasing adrenal activity leads to diminished feeding and decreased thyroid activity leads to a lower metabolic rate. This combines with lowered body temperature and lethargy to ensure that the energy requirement of the mouse lemurs is substantially reduced. However, juvenile mouse lemurs do not appear to exhibit these cycles. They appear to be much more active than the adults during the non-breeding season and, according to observations made by Andriantsiferana and Rahandraha (1973a), their food intake is not reduced. Further observations by Andriantsiferana and Rahandraha (1973b) indicate that juvenile mouse lemurs regulate their body temperature better than the adults. This observation together with that made in the present study, that juvenile mouse lemurs do not exhibit a similar weight gain to that of the adults during the non-breeding season, would tend to indicate that juvenile mouse lemurs do not substantially reduce their energy requirement during the dry season. However, the behaviour of the adults means that the limited food which would be available at this time is left at the disposal of the juveniles.

The possible relationship between feeding frequency and adrenal activity, as denoted by adrenocortical width and nuclear diameter, needs further expansion as obviously the monitoring of feeding behaviour or food intake could be a useful tool for measuring adrenal activity if the correlation between these two factors is a good one. This relationship finds further support from other observations made during the course of the study. Firstly, ACTH administration was observed to cause elevated levels of feeding in an experimental animal (Chapter VII, section 3) and secondly, subordinate animals appear to consume more food than dominant ones. This observation came to light during a period when the total food intake of several specimens was measured. Each day during this period, excess quantities of food were given. The food was weighed each day prior to being given to the animals and the following morning that left unconsumed was also weighed. From the results obtained it appeared that the dominant female, MF2, consumed a mean of 38.3 ± 2.8 grams of food (n=25), while two subordinate females, MF5 and MF12, consumed a mean of 48.4 ± 3.8 (n=24) and 42.5 ± 2.7 (n=25) grams of food respectively. This difference is significant in the case of MF5 ($p \leq 0.05$) although not so in the case of MF12 (see table 7). This is supported further by the measured food intake of two other dominant females, MF7 and MF9. Food intake was measured on four occasions in these females and the consumption was as follows: MF7, mean intake 35.4 grams, range 27 - 44 grams, and MF9, mean intake 27.3 grams, range 6.5 - 40.8 grams. In all cases the body weight of individuals, as estimated from tail size, appeared to be approximately the same.

As adrenal activity is higher in subordinate individuals a relationship is apparent between adrenal activity and appetite. However, the increased food intake in subordinate animals may not result from direct stimulation of appetite. It was also noted during the course of the study that socially low-ranking females were also hyperactive. Thus, this excessive activity could also have stimulated greater food intake.

The correlation between food intake and adrenal activity is not unknown in the literature. Young (1973) has reported that corticosteroid administration is usually effective in increasing appetite in most species, and Takahashi and Takahashi (1976) have reported a parallel shift in the circadian rhythms of adrenocortical activity and food intake in blinded rats and rats exposed to continuous illumination. However, other reports indicate that adrenocortical activity as a result of stress decreases appetite (van Bers, 1973). This dichotomy will be examined further in the final discussion.

The observation that the nine-month lighting schedule only led to a reduction of the breeding season and that the duration of the non-breeding season remained constant in both the nine and twelve-month regimes may be an indication that the breeding season in the

Table 7

Comparison of food intake in dominant and subordinate individuals

Subject	MF5		MF2		MF12
Status	subordinate		dominant		subordinate
Tail size	D		D		DE
Food intake (grams)	48.4±3.8		38.3±2.8		42.5±2.7
No. of obs.	24		25		25
t		2.13		1.08	
significance		$p \leqq 0.05$		not significant	

mouse lemur is succeeded by a refractory period during which reproductive activity cannot be stimulated by changing photoperiodic length. The existence of such a refractory period was postulated by Petter-Rousseaux (1970, 1975), and the observations made on the mouse lemur in this study parallel those made by Ortavant et al. (1964) on the ewe. They reported that ewes exposed to a six-month cycle of illumination exhibit two periods of oestrus a year but these occur during the period of increasing daylength as opposed to during the period of decreasing daylength, which is the normal situation as observed under natural conditions. This result they take as an indication of a refractory period. This refractory period depends on the species or breed of the animal concerned and also on the preceding photoperiodic treatment. This latter observation may also be true in the mouse lemur.

From the results presented in this chapter it can be seen that photoperiod is important in synchronising many annual cycles in the mouse lemur; notably those of reproduction, body weight, behavioural activity and various endocrine functions. This control can be achieved in one of two ways. Either cycles in environmental lighting may act directly on the animal to produce cycles of behaviour and reproductive activity, or they may act as a synchronising agent, or Zeitgeber, of an endogenous rythm. In the latter case the absence of variation in photoperiod will not extinguish the natural rythm. It is photoperiodic change acting as a Zeitgeber which occurs in the annual cycles of the mouse lemur. This can be deduced from the fact that mouse lemurs kept under constant lighting still exhibit a sexually active phase (Russell, 1975, Petter-Rousseaux, 1975). However, this may not be synchronised with the sexual activity phase of other individuals. This kind of lack of synchrony was also demonstrated by the individuals kept in the Clore Pavilion (see section 4.2 above), which indicates that the timing of "dusk" is important for maintaining synchrony in the mouse lemur.

If photoperiod acts only as a synchronising agent, this would explain the comparative failure of the nine-month cycle and also an observed lack of response to a sudden change in the light clock at Jersey Zoo. If the endogenous endocrine cycles are annual ones, then manipulation of the rate of photoperiodic change will only be able to alter those cycles within certain limits. Thus, results obtained by Petter-Rousseaux (1970, 1974) when transferring mouse lemurs between Madagascar and Paris and when manipulating the light regime in various ways, may be due to some other overriding effect, such as adrenal stimulation due to the stressful effect of such changes (see Chapter VII).

SUMMARY

1) Mouse lemurs are strictly seasonal in their reproduction. The annual variation in their reproductive activity is controlled by photoperiodic change. During the winter, short daylength period, the animals are reproductively inactive. As daylength increases reproductive activity commences; the testes of the males enlarge and the females begin to come into oestrus. Reproductive activity continues until the longest day, after which the males' testes regress and the females cease coming into oestrus.

2) The annual reproductive cycle is paralleled by a cycle of body weight loss and gain, a cycle of endocrine activity and a cycle of behavioural activity. During the winter, short daylength period body weight is greatest and endocrine and behavioural activity are lowest. Body weight decreases with increasing daylength and both endocrine and behavioural activity increase and are maximal at about the longest day. After this time behavioural and endocrine activity decrease and body weight is gained.

3) It is possible to reduce the twelve-month cycle to a nine-month one by manipulation of the light cycle. However, such manipulation acts principally to reduce the breeding season, while the non-breeding season remains about the same length. The occurrence of a refractory period is postulated.

4) The alteration of the timing of both "dawn" and "dusk" are essential to ensure normal reproductive activation.

5) A relationship between adrenal activity and feeding behaviour is postulated. This relationship is deduced from the observation that the annual variation in feeding behaviour reflects the annual changes in adrenal activity and from the observation that socially

subordinate animals consume more food than dominant individuals of approximately the same body weight. This supposition is supported by data from the literature and by the results of ACTH treatment (see Chapter VII). Thus it is suggested that the monitoring of feeding behaviour can provide a good indication of adrenal activity and so of stress.

6) It is postulated that photoperiod acts as a Zeitgeber, or synchronising agent for reproductive activity. This explains many anomalies including the comparative failure of the nine-month cycle.

7) It was noted that the rufous mouse lemurs had a significantly lower body weight than the greys and that the duration of reproductive activity was shorter in the red mouse lemurs than in the greys.

CHAPTER IV

REPRODUCTION

Research into the basic reproductive parameter of any species provides the fundamental building blocks for both captive breeding and conservation projects involving that species. Data provided by such studies are required to provide a scientific basis for decision making in both of these situations. With the decline in the availability of exotic species due to the decreasing numbers in the wild and to the international convention on the import and export of endangered species, captive breeding projects take on a new importance. In order to remain viable propositions, zoos and research establishments must be able to replenish their stocks by breeding. Data on photoperiodism and seasonality, such as that discussed in the preceding chapter, have an obvious impact on breeding projects. Not only do they allow one to anticipate breeding activity and to synchronise such activity, but also they enable manipulation of reproductive activity (for example the nine-month light cycle) to allow for maximum reproductive output. The impact of data, such as presented in this chapter, may be more discreet but it is nevertheless invaluable. Data on the parameters of male fertility may, for example, be used to enable the selection of the best male for breeding purposes. In the female, data on oestrous cycle and gestation length are of importance to the general management of a captive colony in that they furnish information on the optimum time to introduce animals for mating, or indicate when a birth is to be expected so that, if necessary, routine husbandry procedures can be adjusted. Data on litter size and the attainment of sexual maturity are also invaluable, particularly when considered in combination with the duration of the breeding season and with the lengths of inter-oestrus periods and gestation. From these data necessary information can be obtained not only for the long term planning of a captive breeding colony; i.e., how many individuals are required to maintain a viable group and how many individuals will be spare, but also the status of that species in the wild can be determined from similar considerations.

Generally this kind of information on reproduction is only readily available in captivity and hence the need for this study.

Some of the data presented in this chapter are not new but they do differ from those currently available in the literature by virtue of the larger sample sizes employed and are important in that they confirm and expand upon existing information. Other data, such as the quantification of testicular size and all the behavioural information, are new and as such increase our knowledge of reproduction in the mouse lemur.

1. Male

The testes of the male mouse lemur undergo seasonal variation in size and activity (see Chapter III,section 1.). This phenomenon is not unique amongst lemurs: Hill (1953) has reported it as common to all lemurs and Petter-Rousseaux (1962) observed that it was more marked in the smaller bodied forms. In the following section testicular changes as observed in the mouse lemur colony are reported.

1.1. Testicular Development

Testis size was assessed on a regular basis by visual comparison of the scrotum with a size chart (see Chapter II, section 3.1., fig.9).

During the resting season all males exhibit a scrotal size of F/G or G, (15 x 11 - 12 x 8 mm). Size H (9 x 5 mm) scrota were only observed in immature males prior to their first breeding season.

All males in the colony exhibited an increase in testis size during the period of increasing daylength. However, this increase was rather more marked in some of the males (see Chapter VIII, section 1.). During the reproductive season, breeding males had scrota of A/B or B (30 x 26 - 27 x 23 mm) in size: this represents a seven-fold increase in the area of the scrotum in these animals. The non-breeding males in the colony had scrota of size C or D (24 x 20 - 21 x 17 mm, representing a three to four-fold increase in scrotal area) during the breeding season in most cases, although some individuals exhibited smaller scrotum sizes (see table 8). It is interesting to note that testes of the size exhibited by most of the non-breeding males are not necessarily infertile. A fertile mating was observed in the study colony between a male with a C/D sized scrotum and a female exhibiting an unseasonal oestrus as the result of a cage transfer (see Chapter VII, section 1.2.1.). However, under normal circumstances these males have not been observed to copulate.

The duration of the phase of testicular hypertrophy and atrophy is very short in these animals, the process of testicular growth or regression being completed within 4-6 weeks (fig.17). This observation implied a rapid change in hormone levels. Unfortunately, it has not proved feasible to collect male urine on a regular basis so as to follow these hormone changes by assay techniques.

1.2. Testis Histology

It was not the policy in this study to sacrifice individuals in order to facilitate histological study of their organs. Thus all histological data must needs rely on those individuals which died of natural causes during the course of the study and on material collected by other workers. In the case of testis histology, data rely on the examination of the testes of four individuals (two exhibiting active spermatogenesis; these specimens originated in the London and Jersey Zoos, and two exhibiting regressed testes; these individuals originated in the study colony). These observations confirm that the testes of male mouse lemurs are

Table 8

Maximal testis size of male mouse lemurs during the breeding season

	Grey Mouse Lemurs		Rufous Mouse Lemurs	
	Male	Size	Male	Size
Season 1973-74	→MM4	A - B	→RM3	A - B
	MM1	C	RM4	B - C
Season 1974-75	→MM4	A - B	RM3*	B - C
(Transfer year)	MM1	C - D	RM4*	B - C
(see Chapter VII)				
	→'Sam	A - B	RM7	C - D
	'Clyde	C - D		
	'Plato	D		
	MM2	C - D		
Season 1975-76	→MM4	A - B	→RM3	B
	MM1	C - D	RM4	B - C
(Sam transferred see Chapter VII)	Sam	B	RM7	D
	Clyde	D	'Henry	D
	Plato	D		
	MM2	E		
	'Bran	B - C		
	'MM10	B		
	'MM11	B		
	'MM9	B - C		

Arrows indicate breeding males.

* These males did not come into breeding condition during breeding season. The sizes given are the maxima obtained as a result of the transfer to the Wellcome (See Chapter VII).

' Indicates males born previous season.

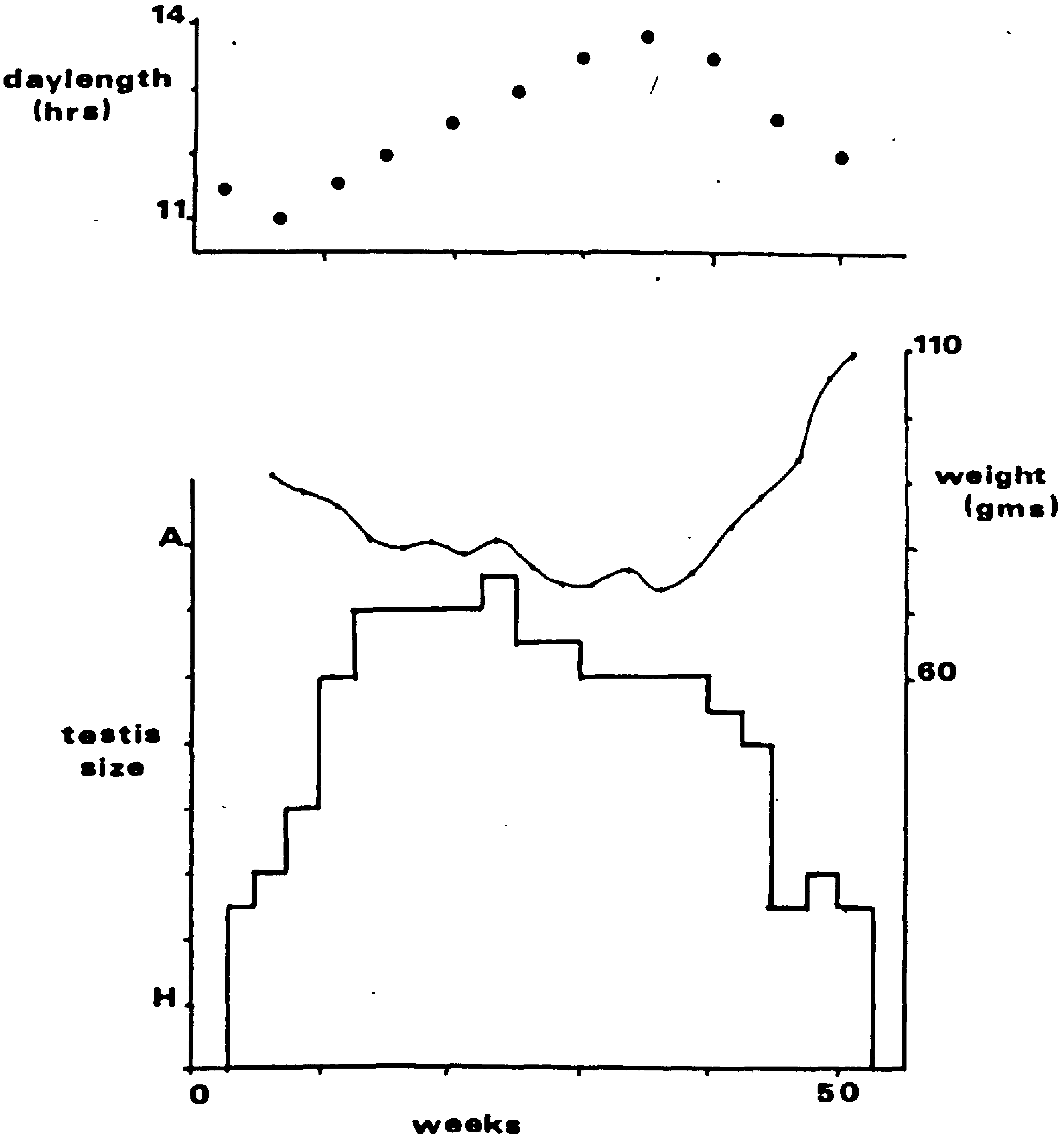


Figure 17

The cycle of testis development

This figure shows the annual cycle of testis development (histogram) in a typical male. The relation between this cycle, body weight (graph) and the light cycle is indicated.

regressed in the non-breeding season (fig.18) and that no spermatogenesis occurs at this time. The tubules are collapsed and their lumina are full of fibres. The Leydig cells are few and their nuclei are small and degenerate.

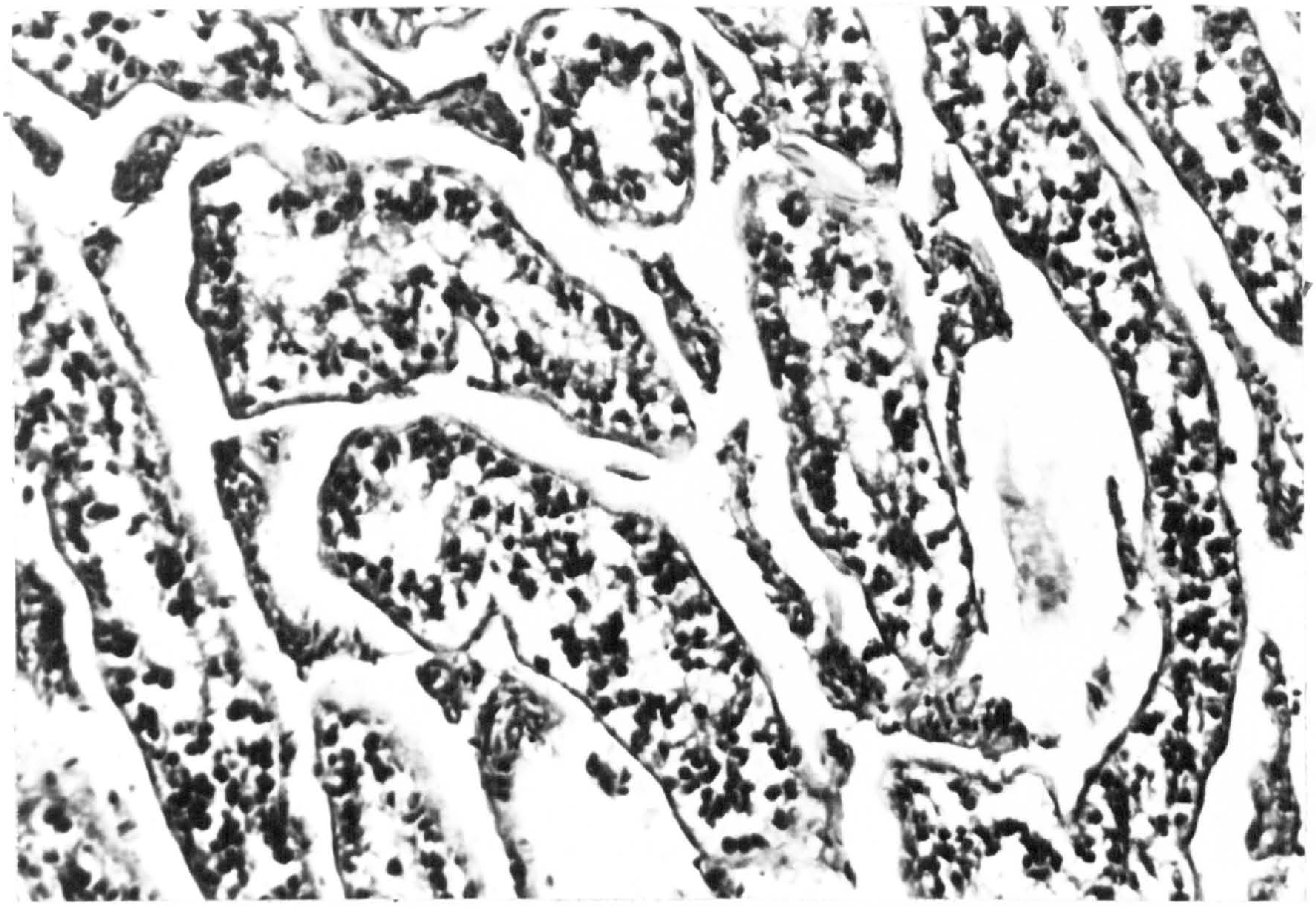
2. Female

The female mouse lemur is seasonally polyoestrous. If conception does not occur the female may come into oestrus three or four times during the course of the breeding season.

2.1. Oestrus

The vagina of the female mouse lemur is imporforate except during oestrus and at parturition. Prior to vaginal opening at oestrus the vulval area develops a red colouration and a swelling is formed. The duration of this swelling phase was found to range between 3-14 days (mean 5.4±0.8 days, n=15) in the study colony. When the vulval swelling is maximal the vaginal orifice appears. After the opening has developed the vulval swelling first loses its colouration and after this the swelling gradually subsides and the vagina is sealed. The flattening phase in the study colony was found to last between 1 and 6 days (mean 3.3±0.3 days, n=13).

Ovulation, as indicated by vaginal smear (see section 2.3, this chapter), and receptivity to the male usually occur on the second or third day after vaginal opening, when the vulval condition is white and swollen. However, females have been observed to mate and conceive on the first day of vaginal opening when the vulval condition is red and swollen (see table 9).



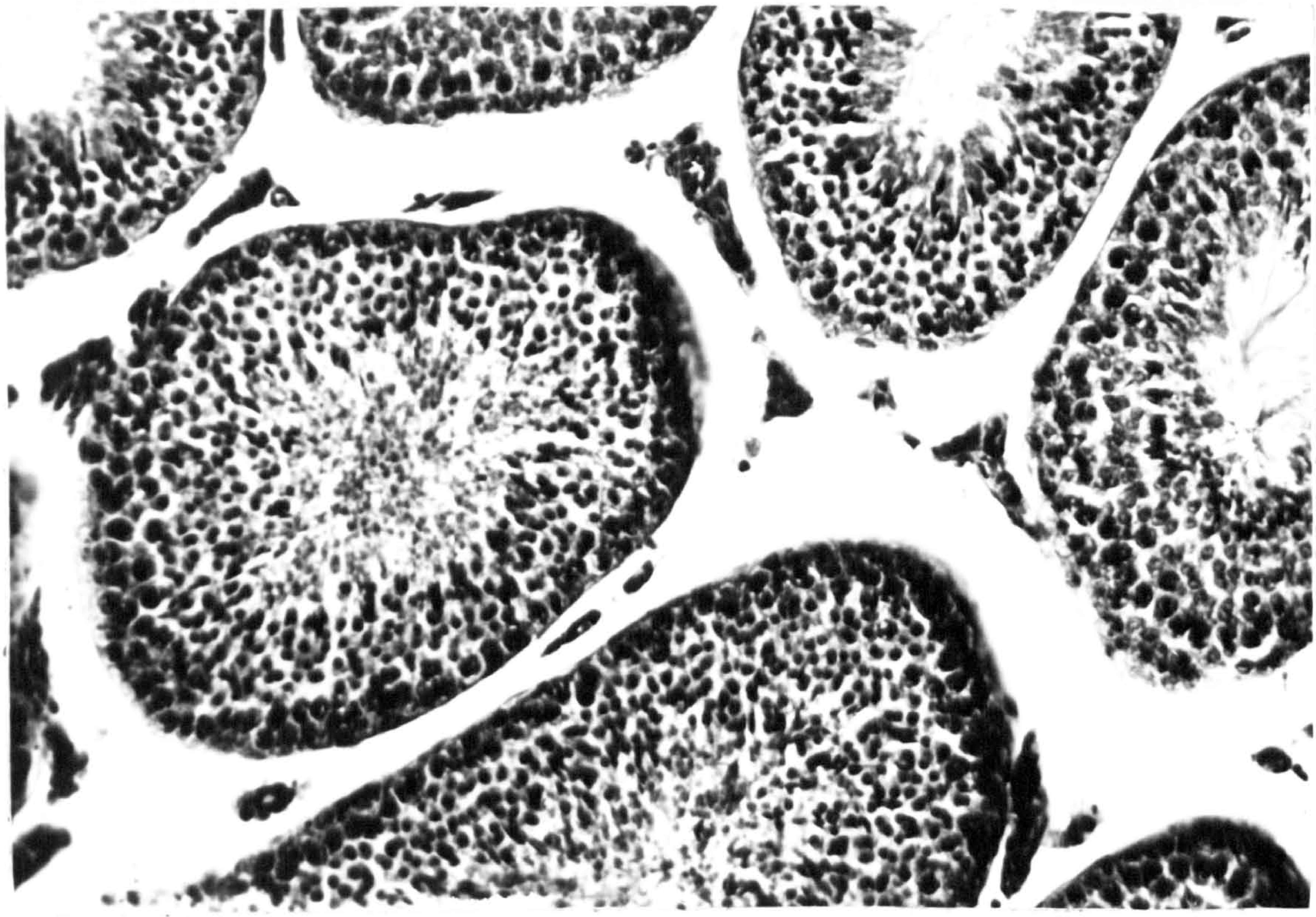


Figure 18

Testis histology
(x250)

a - is a section from an inactive testis; size F-G (Fig. 9)

b - is a section from an active testis; size B.

Table 9

The occurrence of mating in relation to vaginal opening

	The day after vaginal opening on which mating was observed				
	Day 1	Day 2	Day 3	Day 4	Day 5
Number of observations	1	4	3	0	0

2.2. Vaginal Plugs

In this species a vaginal plug is occasionally formed. However, during the three years in which this study was in progress only one vaginal plug was found. It occurred in association with the first oestrus of the season of the female concerned.

The vaginal plug discussed above was later found in the female's nest-box and was removed for histochemical investigation. The plug was embedded in paraffin wax, sectioned and then stained with PAS-Alcian Blue (Disbrey & Rack, 1970). This staining technique indicated a non-acidic polysaccharide structure. No sperm were observed in this plug and the female concerned did not become pregnant.

2.3. Vaginal Smears

Vaginal smears have been obtained from five females using a lavage technique. Four cell types were identified in these smears using Papanicolaou stain (1942):-

1) Basal epithelial cells - these are small, rounded cells with a well defined nucleus and blue/green staining cytoplasm;

2) Larger epithelial cells - the nucleus is still present in these cells but the cytoplasm stains a pink/red colour;

3) Keratinised cells - these are large, irregular shaped cells with a pink/red staining cytoplasm and no nucleus;

4) Leucocytes

The proportions of these cells vary through the period of vaginal opening. On the day of opening, the vaginal fluid contains large epithelial and keratinised cells. On the second or third day, that is the days of maximal female receptivity, the fluid contains mostly keratinised cells. Leucocytes appear in the fluid on the second or third days and become more numerous during the remaining period of opening. The keratinised cells become less numerous and disappear from the smear prior to vaginal closure. Small basal epithelial cells appear in the smears from day two onwards and gradually become more numerous prior to closing (table 11, fig.14).

2.4. Inter-Oestrus Periods

Data on inter-oestrus periods have been collected over three breeding seasons in the current study and prior to this study data were collected for three seasons by R.D. Martin. These data have been combined with those of Petter-Rousseaux (1962) and Andriantsiferana et al. (1974) to yield comprehensive data on the frequency of occurrence of oestrus in this species (table 10, fig.20).

Table 10

Inter-Oestrus Periods

Observer	No. of Seasons	Mean	No. of Observations	Mode
Martin	3	51.4±1.5	34	51
The Author	3	50.4±1.6	34	39,56
Petter-Rousseaux (1962)	?	50.45±1.5	20	52
Andriantseferana et al. (1974)	2	49.4±2.6	61	37
Combined		51.0±1.2	149	54,55

Table 11

Vaginal Smears

Female	Day	Epithelial Cells	Cornified Cells	Leucocytes	Small Epithelials
RF1	1	++++	+		
	2	++	++++	+	+
	3		+++	++	+
	4		++	+++	+++
RF2	1	++++	+		
	2	+++	+++	+	
	3		++++	+	++
	4		++	+++	+++
	5		+	+++	+++
RF10	1	++++			
	2	+++	+++	+	+
	3	no smear			
	4		+	+++	+++

+ present, ++ few, +++ many, ++++ v.many.

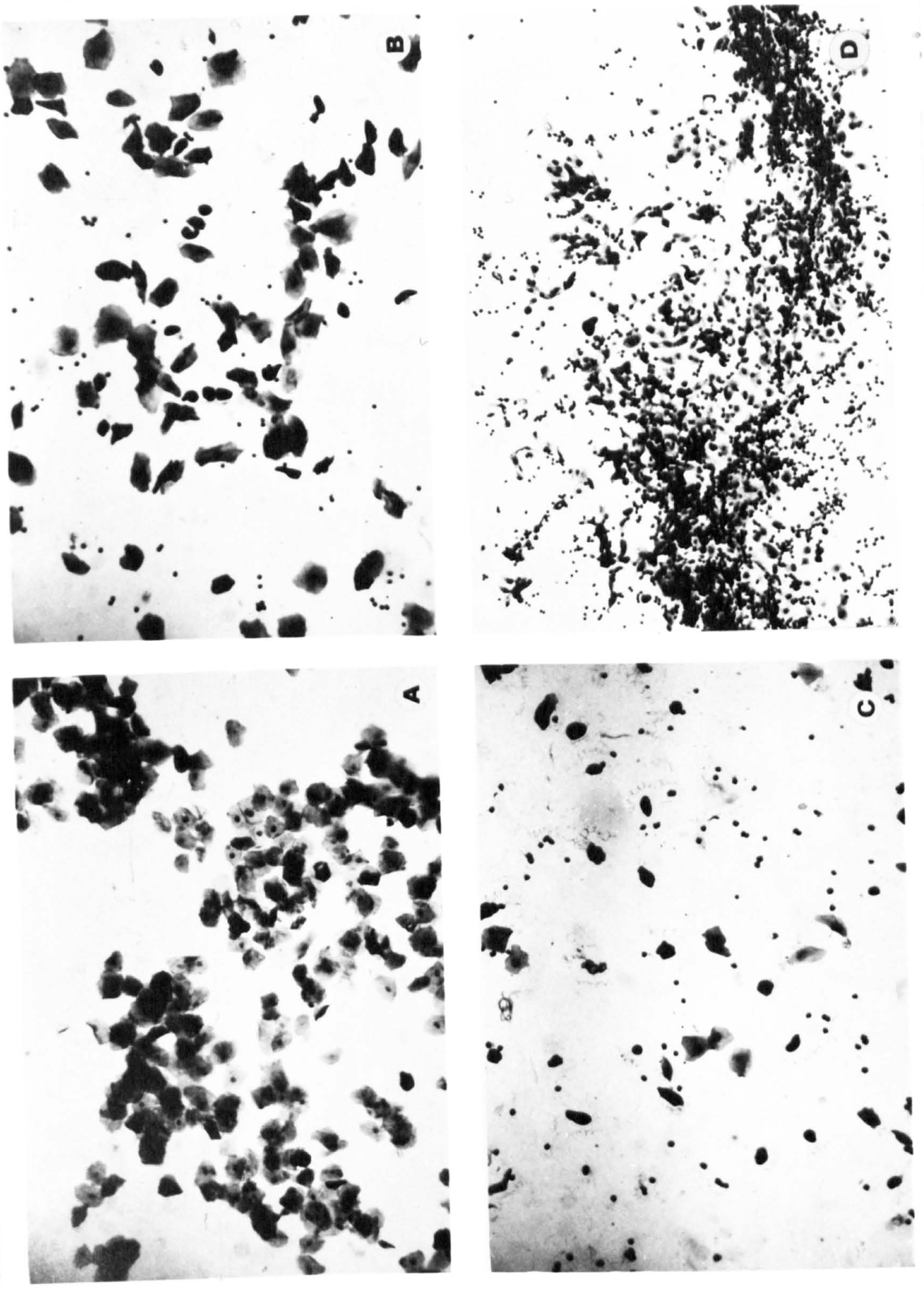


Figure 19

Vaginal smears

A - day 1; large epithelial and keratinised cells: B - day 2 (oestrus); keratinised cells with some leucocytes: C - day 3; only few cells but many leucocytes: D - day 4; numerous leucocytes and small epithelial cells.

Stain Papanicolaou.

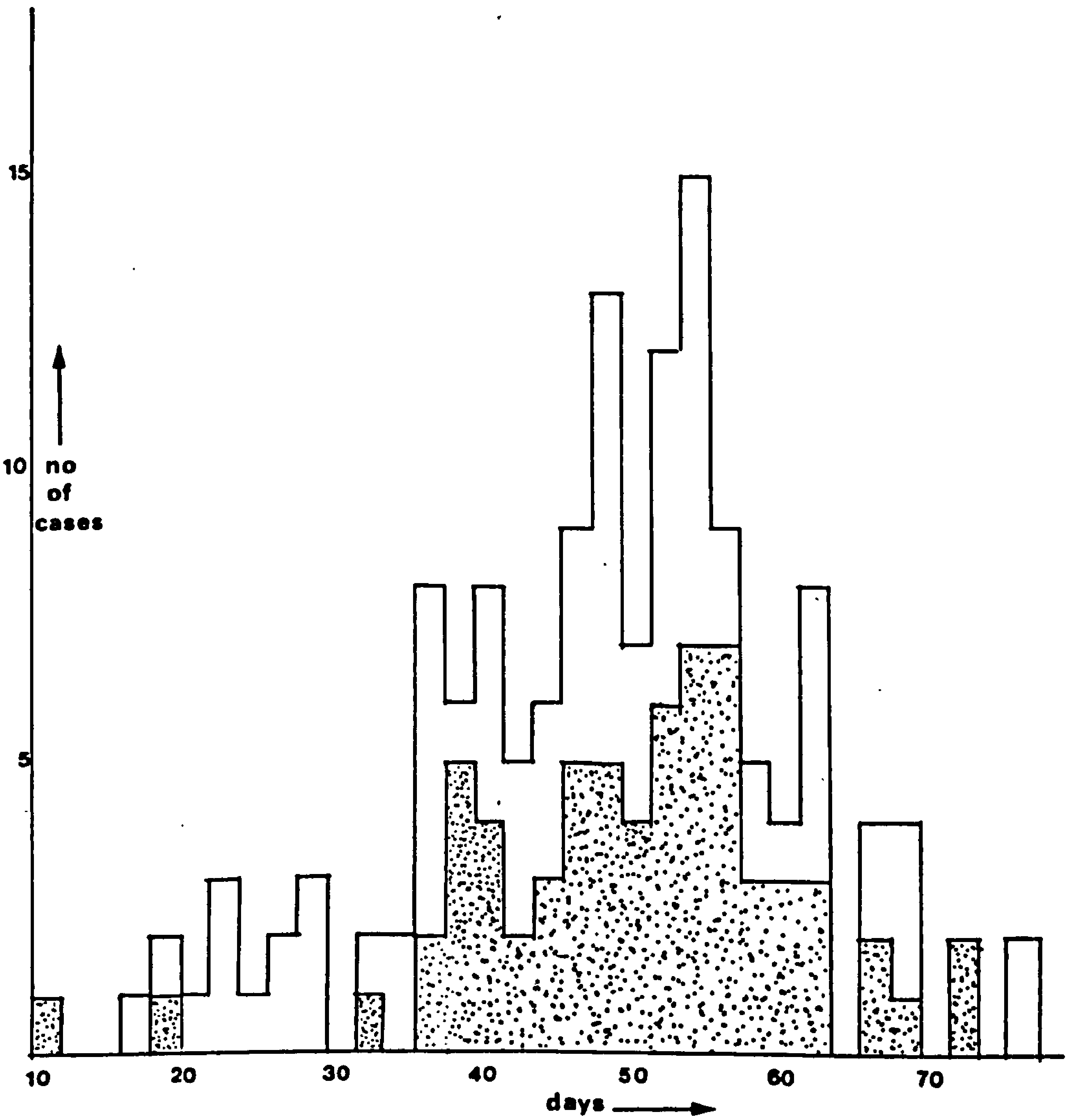


Figure 20

Inter-oestrus periods

Combined results from the study colony (stippled area) and data published by Petter-Rousseaux (1964) and Andriantsiferana *et al.* (1974).

Inter-oestrus periods of short duration, namely those of between 10 and 40 days, usually appear to be associated with abnormal oestrous periods. For example, one of the periods of oestrus in the London colony, which was separated from the succeeding oestrus by a period of only 10 days, involved a vaginal opening associated with no vulval swelling. The occurrence of abnormal oestrus will be discussed further in a later chapter (see Chapter VIII, section 2.2.3.).

Normal oestrus periods are generally separated by inter-oestrus periods of 44-60 days. The longer inter-oestrus periods represented on the right hand side of the histogram are of a duration indicative of post-abortion or post-partum oestrus and it is possible that pregnancy occurred but was not recorded in these females.

2.5. Post-Partum Oestrus

In the study colony post-partum oestrus has only been observed to occur in association with infant death or with the removal of the infants for hand-rearing. In these cases oestrus occurred either between 7-19 days post-partum, when the infants were lost on the day of birth (5 cases), or between 2-7 days after the loss of a suckling infant (2 cases).

2.6. Synchrony of Oestrus

From figure 21 it can be seen that the occurrence of oestrus was synchronised in the female mouse lemurs of the study colony. In many instances two or more females were observed to come into oestrus within a week of each other. In some cases this resulted in all the females caged in one room simultaneously exhibiting some sign of oestrus, ranging from a small red closed swelling to a collapsing swelling and a sealed vagina (see section 2.1. above). From these data it is apparent that synchrony of oestrus arises between females housed in separate cages as well as between those caged together.

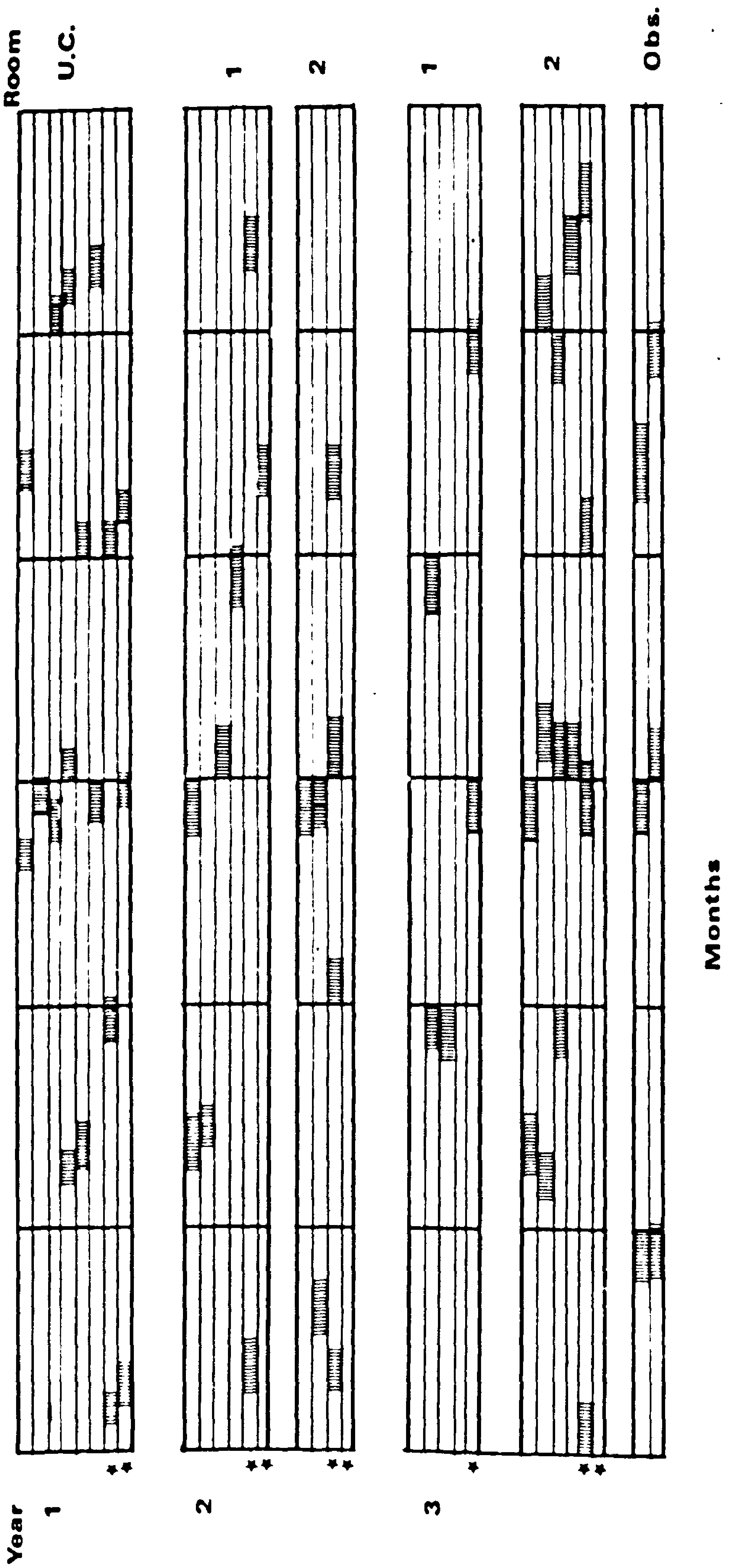


Figure 21

Synchrony of oestrus

Each female is represented separately, stars indicate _rufus_ females, shaded areas indicate the occurrence of oestrus.

2.7. Gestation Length

Gestation lengths have similarly been recorded over three breeding seasons in this study and prior to that for three seasons by R.D. Martin.

Gestation length was estimated from the second day after vaginal opening, or from the day on which mating was observed. The gestation lengths thus measured ranged between 57 and 66 days for both subspecies combined. The mean duration of gestation for the grey subspecies only between 1971 - 1973 was 62.3±0.65 days, n=6 (Martin, unpubl.data) and between 1973 - 1976 it was 61.5±0.4 days, n=14. Figure 22 represents the pooled results of both studies.

Additional data were collected by R.D. Martin for the breeding season of 1977. The mean gestation length for the three pregnancies which occurred was 62.7 (range 62-64) days.

2.7.1. The difference in gestation length of red and grey mouse lemurs

If the mean gestation lengths of the *rufus* and *murinus* mouse lemurs are compared, that of the *murinus* subspecies appears to be significantly longer (table 12).

2.8. Weight gain during Pregnancy

It has been the policy during this study not to handle pregnant females towards the end of their gestation period. Data on weight gain during pregnancy are therefore from two sources: firstly from information collected by Martin during his work on the study colony of mouse lemurs prior to the commencement of the current study and, secondly, from deductions which can be made from data on weight gain derived from weights recorded during early pregnancy which were collected during the current study. Martin's data indicate a weight gain of between 25 and 35 grams (mean 26.1±1.1 grams, n=8)

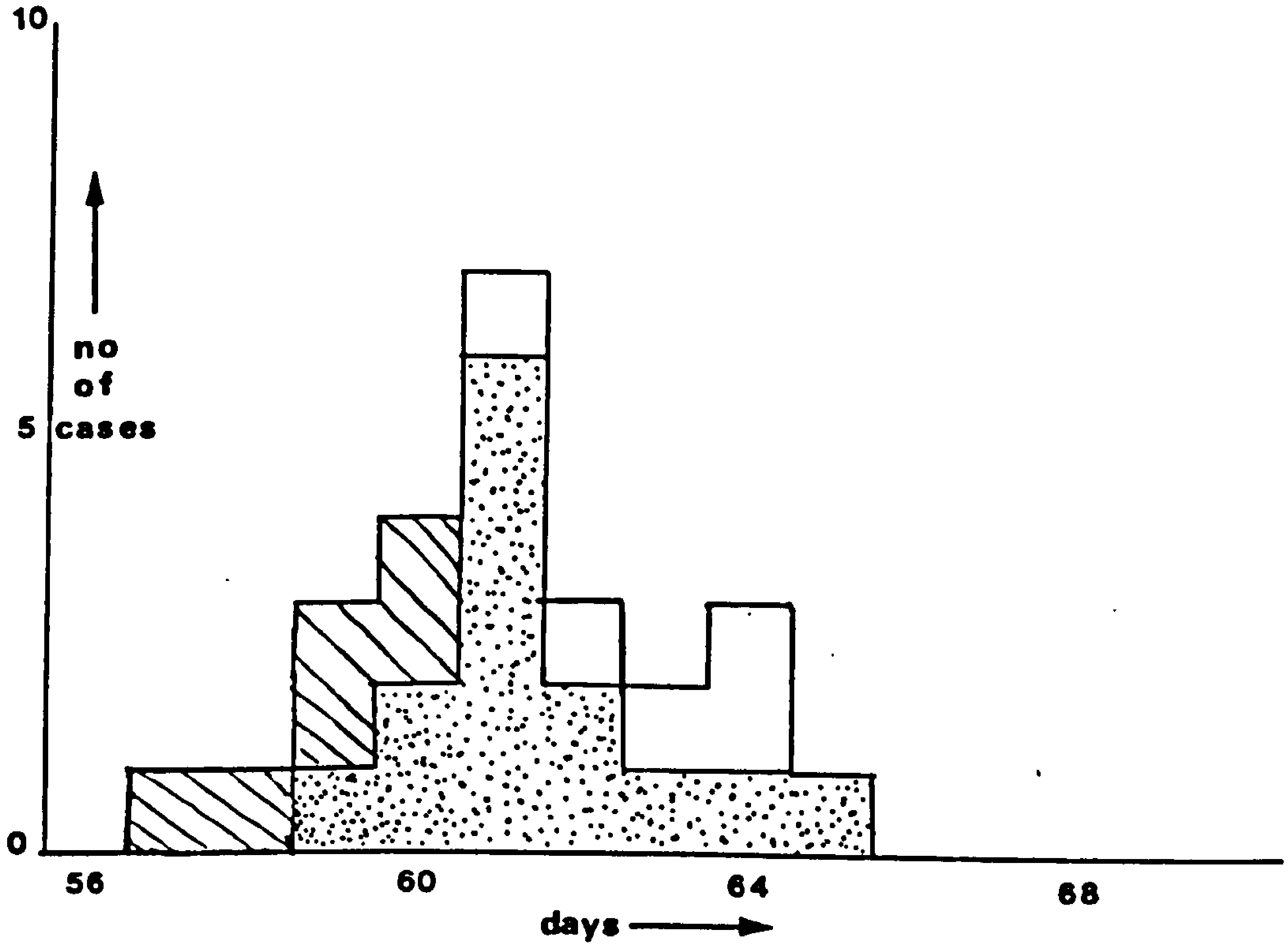


Figure 22

Gestation length in the study colony

Stippled area, data collected during this study for grey mouse lemurs. Other data obtained by R.D.Martin: cross-hatched area, data from rufous mouse lemurs; clear area, data from grey mouse lemurs.

Table 12

Comparison of the Gestation Lengths of Rufous and Grey Mouse Lemurs

	Rufous Gestation in Days	Grey Gestation in days
	57 (2 cases)	60 (1 case)
	59 (4 cases)	61 (1 case)
		62 (1 case)
		63 (1 case)
		64 (2 cases)
	1.0	1.6
Mean	58.3±0.4	62.3±0.65

t = 5.2. Thus there is a significant difference in gestation lengths ($p \leqq 0.05$).

The data for this table were collected by R.D. Martin (1970-1973) as only one birth of the rufous subspecies was recorded during the current study.

as measured by loss of weight at parturition (5 cases). He also observed a lapse of three weeks between conception and the onset of weight gain. Weight gain was recorded up to the 45th day of gestation in some cases and was taken as being the increase in weight over that measured at the last oestrus. These data together with Martin's are presented in table 13.

2.9 Placentation

Placentation in the mouse lemur was formerly thought to conform to the typical strepsirhine pattern (Luckett, 1974). In general the lemur and loris group differ in their placentation from all other primate groups in that they exhibit diffuse epitheliochorial placentation similar to that found in ungulates, whilst in the haplorhine primates placentation is of a discoidal or bidiscoidal, haemochorial type (Amoroso, 1952). In epitheliochorial placentation there is no erosion of the maternal tissue, no organic continuity between mother and foetus and, as no maternal tissue is lost at parturition, it is said to be non-deciduate. The attachment between mother and developing foetus is by means of villi. These are simple or branched structures, clothed by a single layer of cellular trophoblast, which come into apposition with the endometrium and lock into crypts or depressions therein (Hill, 1929).

The most recent paper on mouse lemur placentation, that by Reng (1977), has re-examined this traditional view. She has found that there is an area of trophoblastic invasion into the uterine mucosa at the placental centre in the mouse lemur. Outside of this area of penetration, as in the normal epitheliochorial placenta, the cytotrophoblast rests upon the uterine epithelium. The mouse lemur placenta is therefore of a mixed type, the central syndesmochorial labyrinthine area is surrounded by an epitheliochorial ring shaped placenta. This is very similar to the placental arrangement described for Galago demidovii (Gérard, 1932, cited Amoroso, 1952).

Table 13

Weight Gain During Pregnancy

Weight at Conception	Weight during Pregnancy	Weight Gain	Stage of Pregnancy	Number of Known Infants	Comments
79 grams	80 grams	1 gram	30 days	2	
70 grams	90 grams	20 grams	37 days	3	
70 grams	80 grams	10 grams	40 days	2	
93 grams	106 grams	13 grams	40 days	2	
87 grams	94 grams	7 grams	41 days	2	first pregnancy
64 grams	80 grams	16 grams	50 days	2	
72 grams	81 grams	9 grams	50 days	1	rufous mother
58 grams	106 grams	43 grams	50 days	3	1 low birth weight infant
75 grams	84 grams	9 grams	50 days	?	infants never found
87 grams	98 grams	11 grams	58 days	2	first pregnancy, same mother as above
66 grams	85 grams	17 grams	60 days	2	1 low birth weight infant

2.10. Hormone Assays

Urine samples were collected from most of the females on a regular basis during handling procedures. During handling most female mouse lemurs urinate. Samples were collected by the simple expedient of holding the female over a sample tube. It was hoped that these samples would lend themselves to hormone analysis and thus afford a method of detecting pregnancy in its early stages.

An attempt was made to assess levels of chorionic gonadotrophin and LH in the mouse lemur urine by using a human HCG immunoassay technique. When negative results were obtained with this technique an attempt was made to estimate total oestrogens in the urine using the radioimmunoassay technique described by Seaton et al. (1976) and by Martin et al. (1975). No peaks in oestrogen were found in association with either oestrus or pregnancy (see table 14).

3. Reproductive Behaviour

3.1. Mating

Mating has been observed on six occasions. The observations involved three different males MMJ, MM4 and MM3 and six different females (the two Jersey females, MF6, MF7, MF5, and Bonnie); of these, two of the males and five of the females were wild caught and the remaining male and female were hand-reared. In addition a videotape recording was made of one of the instances of mating to allow for more accurate observation. The following observations were made in all instances and it is interesting to note that hand-rearing makes no appreciable difference to sexual behaviour in this primate species.

Prior to female receptivity at oestrus the male will show considerable interest towards her. He continuously approaches her giving the trill call repeatedly (Chapter VI, section 3.2.) and tail-lashing vigorously (Chapter VI, section 1.1.). At this

Table 14

The Concentration of Oestrogens in the Urine of Female Mouse Lemurs

Reproductive Condition of the Female	Oestrogen Concentration (ng/mg creatinine)
Within one week of oestrus	16.3; 6.1; 19.7; 9.5; 29.7; 28.4; 22.3
Oestrus: vulval condition:-	
swollen, closed	19.3; 14.2
red, open	19.2; 28.7; 44.2
closing	30.4; 8.7; 12.5; 18.2
Pregnant: duration of pregnancy:-	
0-10 days	13.4
10-20 days	30.5; 7.0
20-30 days	18.3; 13.8; 8.5; 12.3; 28.4; 28.7
40-50 days	13.7; 5.9
Non-breeding season	19.6; 10.1; 12.6

stage the female is very aggressive towards the male and will attack and chase him.

Peak female receptivity is characterised by very frequent anogenital rubbing and mouthwiping on her part (Chapter VI, section 2.2.). At this time no aggression is directed towards the male. He approaches the female giving the trill call. Tail-lashing is observed in some instances and the male sniffs the female's flanks and anogenital area. If the female does not threaten or attack him he will proceed to groom her vigorously, gradually moving closer to her until he can mount. Once mounted, he continues to groom the back of her neck and utters soft trill calls. The female utters no audible vocalisation in association with the mating except threat calls which often terminate mating.

During mating the male grasps the female firmly around her waist with his forearms; his feet either grip the substrate or the female's hocks. The latter position is adopted when the female is climbing or moving about the cage.

Although the female may utter threat calls during mating, the male was never observed to do this even when provoked. On one occasion a male in a cage adjacent to that containing the mating pair was observed to reach through the bars, seize the mounted male's tail, pull it through the bars into his own cage and bite it persistently. The recipient of this act made no response while mounted but commenced threat behaviour once mating terminated.

3.2. Birth

Actual birth has been observed on two occasions during the course of the study. This was accomplished with the aid of infra-red, closed-circuit television and video. On both of these occasions the female gave birth within a few hours of the white lights coming on.

Birth 1: Prior to "sunrise" at 00.15 much nesting behaviour was observed. This involved carrying pieces of twig, cherry stones and wood chips in and out of the nest-box and arranging and re-arranging the debris within the nest-box. No nest-like structure resulted from this activity. This kind of nesting behaviour was noted for the first time on the evening preceding that of the actual birth.

The imminence of birth was characterised by the female's inability to settle down and sleep after the white lights came on. After a period of furious nesting activity the female would appear to settle down only to change positions, or indulge in further nesting or grooming activity. Self grooming was very intense and the female often transferred her grooming activity to the nest-box itself, vigorously licking its floor and walls. Conversely very little attention was paid to the genitalia.

Contractions were observed shortly after the lights came on and became increasingly frequent as the birth became imminent (fig.23).

The first birth occurred two hours after the daylights came on. To deliver the infant, the female sat with her legs widely spread and leaned forward to lick the infant as it emerged (fig.24). As the foetal head emerged the mother took hold of it with both hands. There is no evidence to indicate that this behaviour aided actual delivery.

Delivery was very rapid and took only a few seconds. After the infant had emerged the mother bit through the umbilical cord and continued to groom and manipulate the infant, paying particular attention to its genitals.

The infant was surprisingly active at this stage; it could move quite well, grip the mother's fur and was suckling within twelve minutes. The mother was in labour again 15 minutes after the first birth and was observed to hold the first-born infant aside with one hand as she groomed her own genitals. Twenty

Figure 23

Labour

This is a photograph from the videotape recording of the birth.

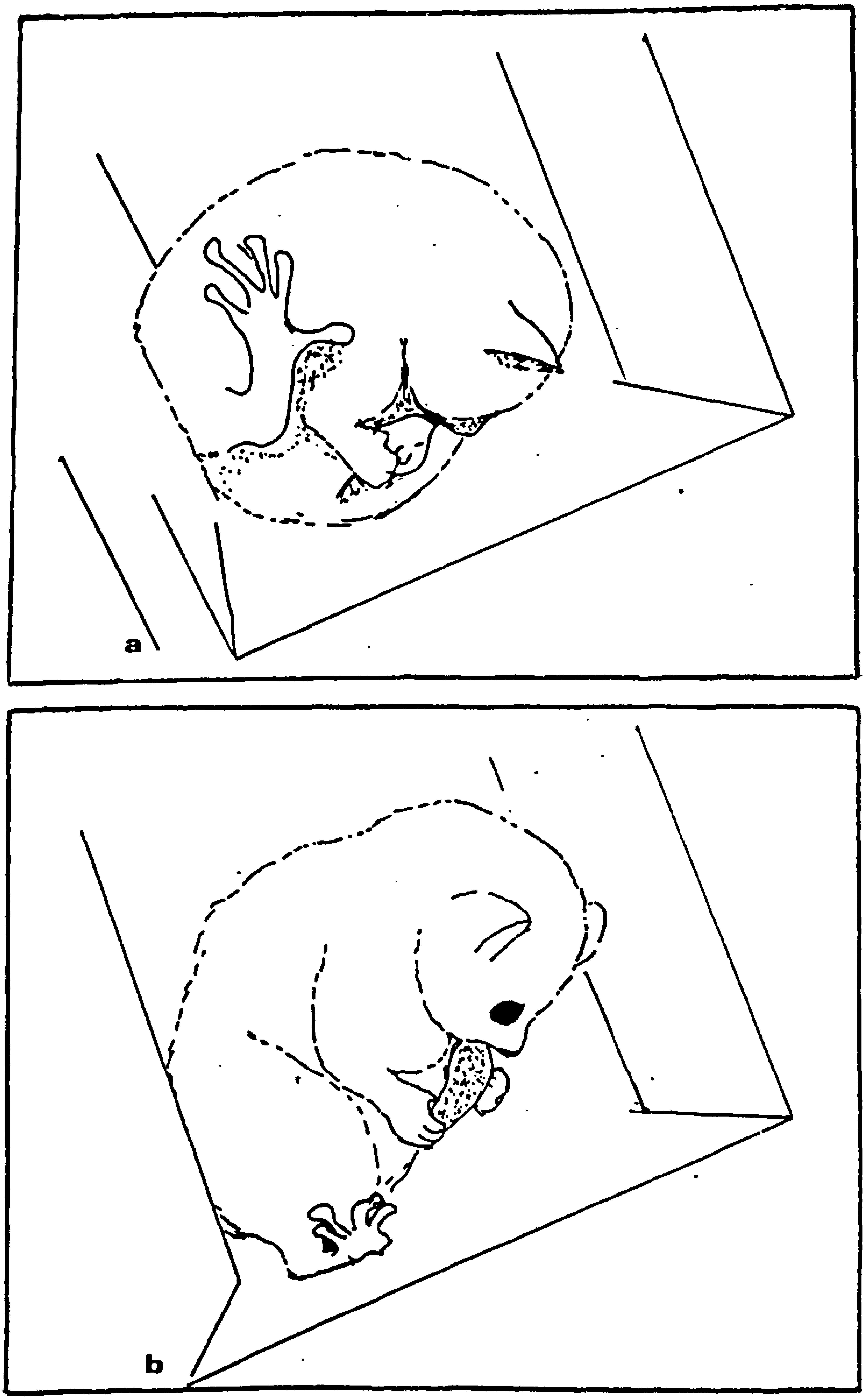


Figure 24

Delivery of an infant (a) and eating the placenta (b)

Both drawings taken from the videotape recording.

minutes later the second infant was delivered in a similar manner to the first.

Five minutes later, at 2.55, the mother was in labour again and a third infant was born four minutes later. This infant was very small and not fully developed. The mother's behaviour towards this infant did not follow the previous pattern. This will be discussed further in Chapter VIII, section 2.5.2.1.

Twenty-five minutes later the mother adopted the labour posture again and began to groom her genitals. This continued for abour 20 minutes until, at 3.45, the three,apparently fused placentae were delivered and consumed (fig.24). The entire process from first birth to delivery of the placenta took 1 hour 45 minutes.

Birth 2: This birth was rather more protracted than that described above and involved a possible breech delivery and still birth.

During the second birth a similar pattern of early activity was observed (fig.25), characterised by nesting, grooming and inability to settle. The first contractions were observed at 00.25, ten minutes after the daylights came on, and continued for 3 hours 25 minutes until the first infant was delivered. However, during the course of this labour the female ceased to adopt the normal labour position and assumed a posture on all fours with her head drooping; she pushed back with her abdominal muscles and then collapsed on the floor. It was noted that the vulva was stretched very wide at this time and this lead to the assumption that this was a breech delivery. The same delivery and parturient behaviour were observed as described for Birth 1.

Ten minutes after the birth of the first infant, labour was again observed and a second infant was born six minutes later at 4.06. This infant appeared to be lifeless; although the mother groomed it and severed the umbilical cord the lack of response from the infant caused her to turn her attention back

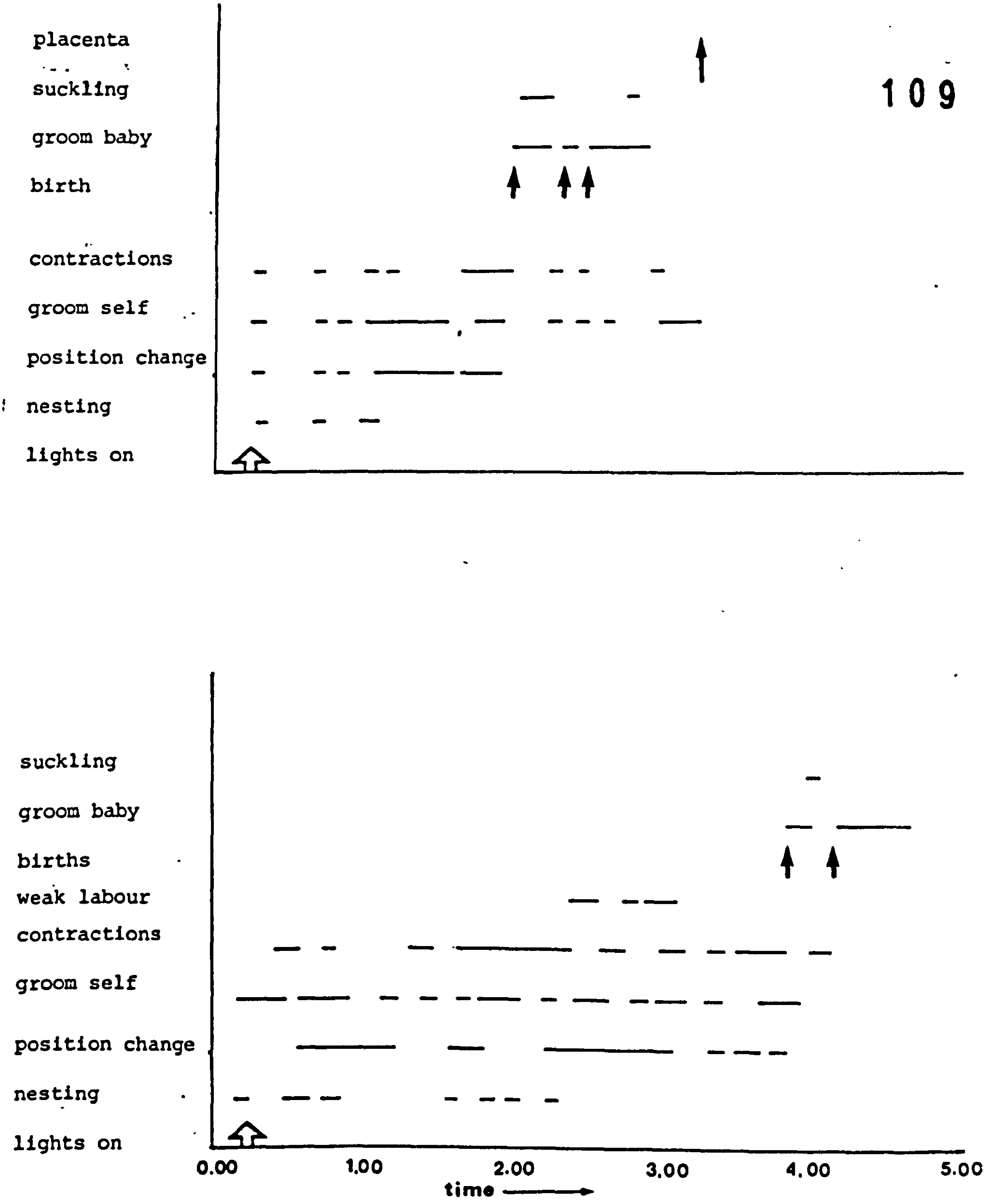


Figure 25

Schedule of activities leading to birth 1 (a) and birth 2 (b)

"placenta"means delivery of the placenta;"weak labour"indicates the occurrence of contractions unaccompanied by the typical labour position.

to the first infant.

When the mother and infants were checked later the same day the still-born infant had disappeared from the nest but the first-born infant was alive and well.

3.3. Maternal Care: Nest-Box Stage

During the first three weeks of the infant's life it remains in the nest the whole time. This was observed in the cases of 10 out of 11 successfully reared infants of three mothers over two breeding seasons.

All maternal care during these early weeks takes place in the nest and the amount of time that the mother spends in the nest is a good reflection of the efficacy of maternal care.

During the first day after birth the mother is rarely seen outside the nest-box. She emerges only for very brief periods to feed, drink, urinate or defaecate. The percentage of time which she spends with her young in the nest gradually decreases over the three week period that the young are continuously in the nest. Four mother-infant units were observed over this critical three week period and regression analysis indicates that the decrease in the amount of time that the mother spends with her young occurs in a linear fashion. If a comparison is made of the regression lines produced for these four mother-infant units it can be seen that the four regression lines do not differ significantly at the level of probability accepted in this thesis ($p \leq 0.05$). Thus one regression line has been produced to show the relationship between the amount of time a mother spends with her young as a function of the number of days after birth (see table 15, fig.26).

During these early weeks, when the mother is away from the nest-box, involved in some prolonged activity, she will return to the nest-box at intervals for a brief visit. During these visits the only behavour to be observed was the "nosing" behaviour which usually occurs when the mother returns to the nest-box; on entering

Table 15

Comparison of the four regression lines representing the rate of change over time of the amount of time a mother spends in the nest-box with her young

Regression lines

Mother/infant Unit	n	Slope	intercept	F	Probability
MF9	21	-0.867	83.17	1.78	$\leq$ 0.20
MF7 (yr1)	26	-1.77	101.07	51.13	$\leq$ 0.001
MF7 (yr2)	18	-2.03	97.22	7.91	$\leq$ 0.05
Bonnie	31	-2.37	92.70	11.87	$\leq$ 0.01
Combined regression line for all 4 units	96	-2.02	94.68	41.64	$\leq$ 0.001

Do the regression lines representing individual units differ significantly from the combined line.

$$F = \frac{(SStot - \Sigma SSunit) \div 6}{(SStot) \div (\Sigma n_i - 2)}$$

Where SStot is the sum of squares of the error of the combined line. And SSunit is the sum of squares of the error of the individual line.

$$F = \frac{39.50}{17.45} = 2.26$$

with 6 and 94 degrees of freedom.

This indicates no significant difference between the lines at the level of probability accepted in this thesis (p = 0.05.).

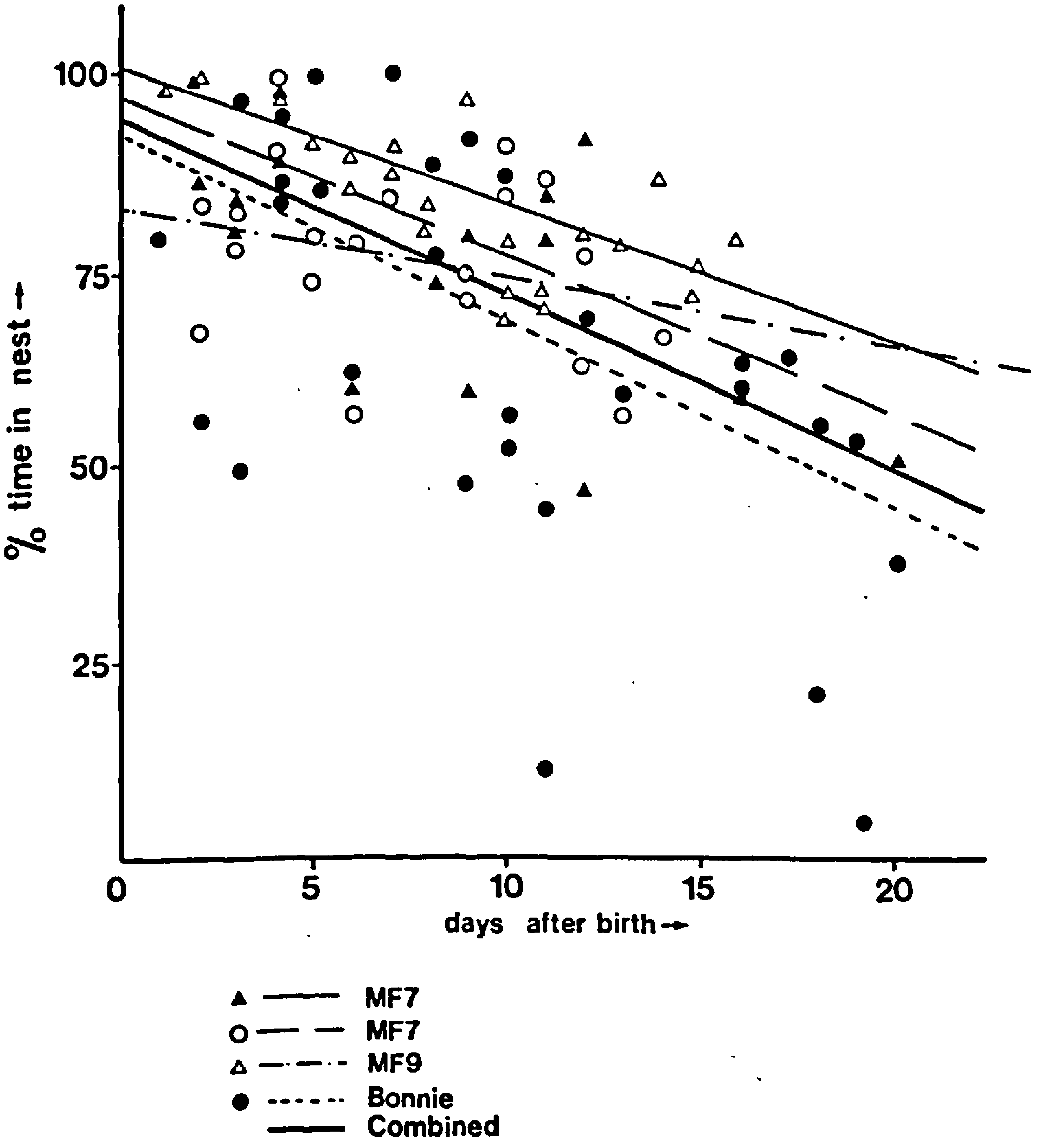


Figure 26

Amount of time mothers spend in the nest-box with their young

Lines representing individual females and combined lines are shown.

the nest-box the mother pushes her snout under one or both of her infants and pushes the infant back over her head. In the case of visiting behaviour she then leaves the nest-box again.

With the aid of infra-red equipment it proved possible to make a detailed study of early maternal behaviour. In particular data were collected on the frequency of occurrence and duration of suckling and cleaning behaviour. The schedule of these observations was described in the methods chapter (Chapter II, section 3.3.2.3.). However, in addition to the three basic 30 minutes observation periods, ten minute checks were made at half hourly intervals over nine of the fourteen "daylight" hours. These checks were implemented on three occasions during the first three weeks of the infants' lives. The hours observed during the light period were the three following "lights on" and six preceding "lights off".

No regular pattern emerged for the change in the percentage of time spent in suckling behaviour over time (fig.27). This was due to the fact that most suckling bouts last 20 minutes or longer, with considerable resting periods between succeeding bouts. Thus these bouts were very long in relation to the observation period (30 minutes). This resulted in very high or very low scores being obtained for this behaviour. Another problem encountered was the difficulty in determining when suckling was actually occurring. This problem was particularly marked when the infants were small (less than one week old) and was due to the absence of accommodating movements from the mother. These apparently were not necessary when suckling small infants. As the infants grow the mother exhibits several accommodating postures during suckling (fig.28). In addition to these the suckling posture described for Galago senegalensis (Doyle et al., 1967), with the infant lying on its back to suckle, has also been observed.

Suckling is observed at intervals during the latter part of the sleeping period (i.e., during the six hours preceding "lights off"), although none was observed in the first three hours after

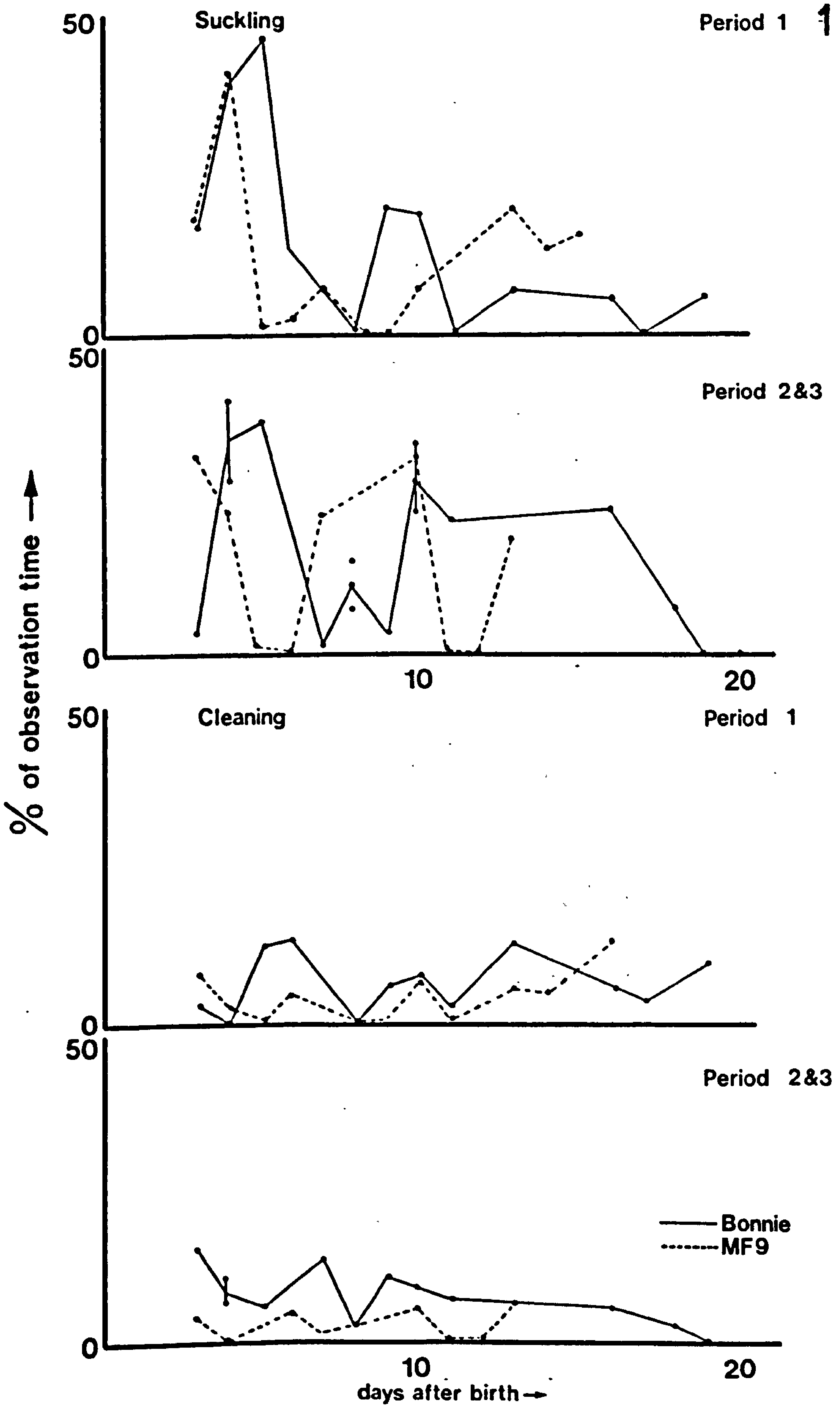


Figure 27

Suckling and cleaning frequency

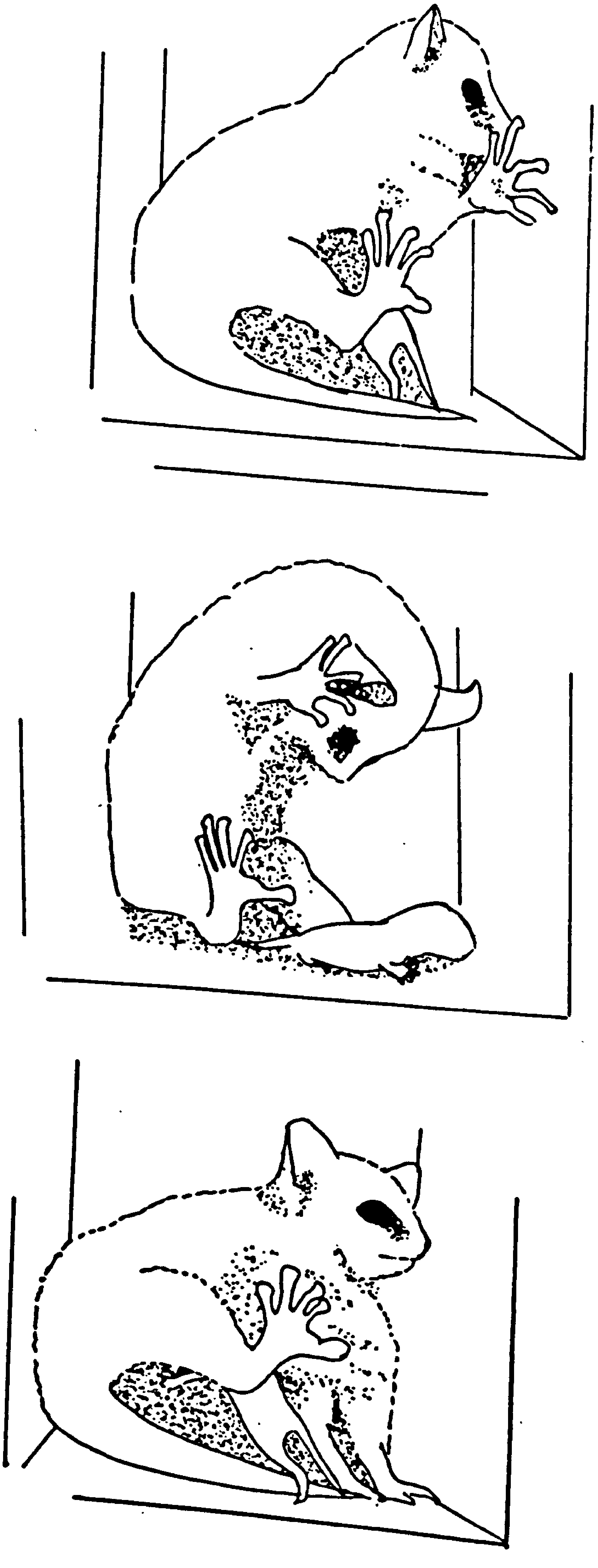


Figure 28

Suckling accommodation postures

the daylights came on. These suckling bouts cause minimal disturbance to the mother, she would fall asleep immediately the bout was terminated.

Data obtained on the frequency and duration of cleaning behaviour also indicate an erratic occurrence (fig.27). Infant cleaning usually occurs in association with suckling behaviour or with the mother's entry into the nest-box. The duration of cleaning bouts is rather shorter than that of suckling bouts, lasting five minutes or less. In addition no cleaning behaviour was observed during the sleeping period (between white lights on and red lights on).

3.4. Later Maternal Behaviour

A series of observations on mother-infant interactions were implemented on four mother-infant units during the period 30-90 days after birth. These ages were chosen because by 30 days the infants spend much of their time out of the nest-box and by 90 days they are more or less independent of their mother.

The proportion of interactions initiated and terminated by the mother was examined and they were found in general to decrease over time (figure 29). In most cases the linear regression of the proportion of initiations or terminations over time was significant ($p \leq 0.05$, table 16). However, although in the case of terminations the four individual regression lines did not differ significantly from the combined regression line, in the case of initiations they did differ from the combined regression line at the level of probability accepted in this study (table 16). This lack of agreement between regression lines seems to be attributable to the proportion of initiations made by the female Bonnie in Year II. In this case the mother was only interacting with one juvenile. Thus, it is possible that in the absence of siblings the juvenile directed all play interactions towards its mother and thus initiated more interactions with her than would

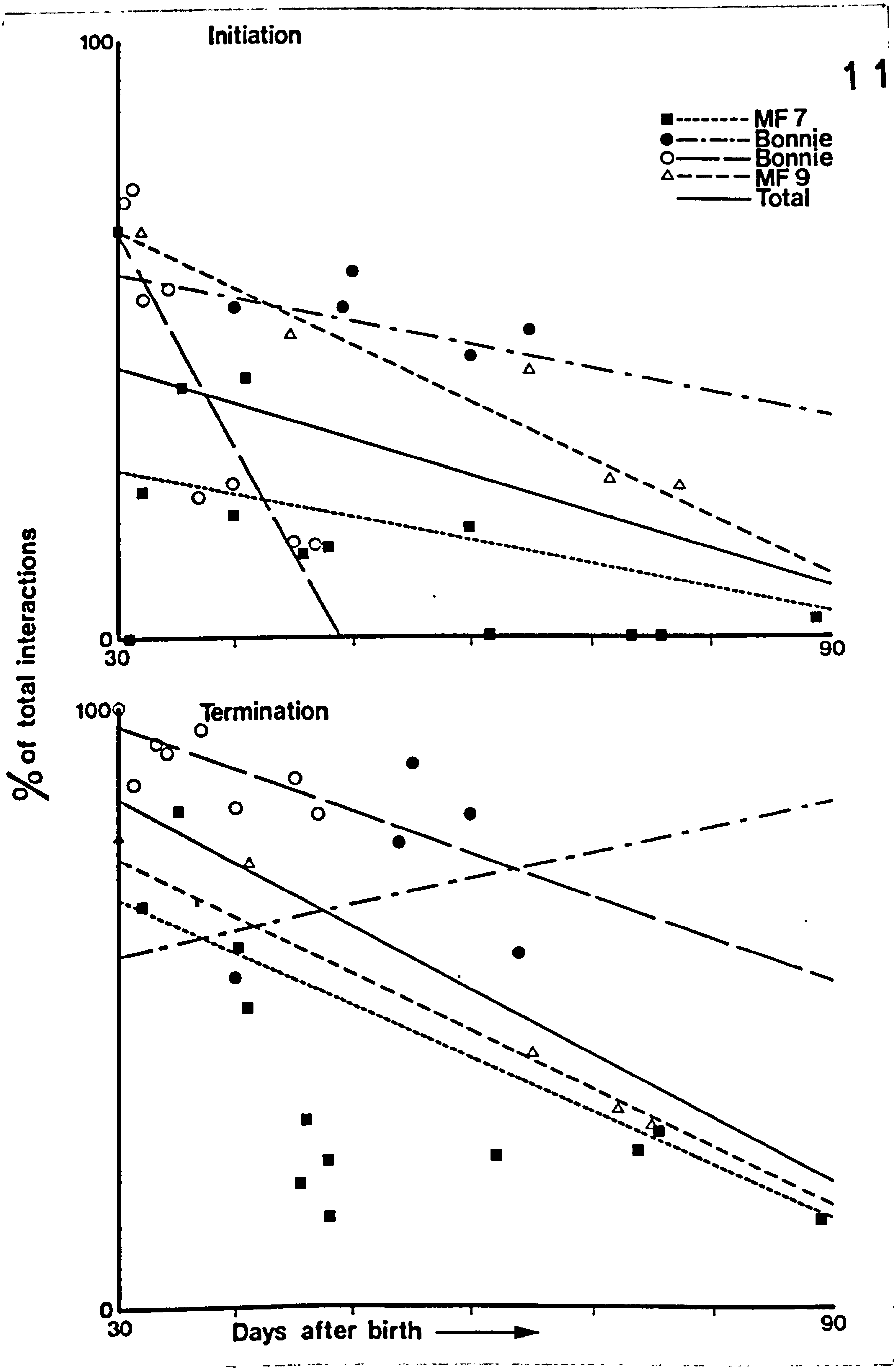


Figure 29

Frequency of maternal initiation and termination of social interactions

Lines representing individual females and combined line shown.

Table 16

Comparison of the four regression lines representing rate of change over time of the number of mother/infant interactions initiated by the mother and of those representing the rate of change of maternal terminations

Initiations

Mother/infant Unit	n	Slope	intercept	F	probability
MF7	15	-0.4403	39.64	5.60	$\leqslant 0.05$
Bonnie	8	-3.68	178.55	37.27	< 0.001
	5	-0.38	73.07	2.19	-
MF9	5	-0.971	97.51	0.95	-
Combined	33	-0.627	63.35	9.35	$\leqslant 0.01$

Do the regression lines representing individual units differ significantly from the combined line.

$$F = \frac{(SStot - \Sigma SSunit) \div 6}{(SStot) \div (\Sigma n_i - 2)}$$

Where SStot is the sum of squares of the error of the combined line and SSunit is the sum of squares of the error of the individual line.

$$F = \frac{1620.17}{426.64} = 3.797$$

With 6 and 31 degrees of freedom, this indicates a significant difference between the lines at the level of probability accepted in this thesis, ($p = 0.05$).

Table 16 cont'd

TERMINATIONS

Mother/infant Unit	n	Slope	Intercept	F	Probability
MF7	15	-0.858	95.49	11.06	$\leqslant 0.01$
Bonnie	8	-0.727	117.61	6.39	$\leqslant 0.05$
Bonnie	5	0.5676	42.01	0.32	-
MF9	5	-0.970	104.47	29.59	< 0.01
Combined	33	1.076	116.99	29.49	$\leqslant 0.001$

Do the regression lines representing individual units differ significantly from the combined line

$$F = \frac{(SStot - \Sigma SSunit) \div 6}{(SStot) \div (\Sigma n-2)}$$

where SStot is the sum of squares of the error of the combined line, and SSunit is the sum of squares of the error of the individual lines

$$F = \frac{918.74}{383.92} = 2.393$$

With 6 and 31 degrees of freedom there is no significant difference between the individual lines and the combined line at the level of significance accepted in this thesis, (p=0.05).

have been the case had siblings been present. In this way the maternal contribution towards the initiation of interaction was greatly reduced in relative terms;particularly as the juvenile got older and thus the slope of this particular regression line is much steeper than in those cases where two infants were present. This situation apparently did not affect the number of terminations attributable to the mother.

In addition, an investigation was undertaken on one pair of twins to determine whether there was any difference in the pattern of initiation and termination between mother/daughter and mother/son interaction. Such observations were only possible for one set of twins as only two sets of male/female twins were observed and of these it was only possible to distinguish them easily in one case. Although the number of mother/male and mother/female interactions were virtually the same, 87 and 85 respectively, some differences in the pattern of initiations emerged. These were not very marked and can be expressed as follows:- of the 28 interactions initiated by the mother 60 per cent were directed to the daughter, 40 per cent to the son; of the total of 172 observed interactions 6 per cent were initiated by the mother to the son, 10 per cent were directed to her daughter, 44 per cent were directed from son to mother and 40 per cent from daughter to mother. In addition, 53 per cent of the male/mother interactions and 47 per cent of the female/mother interactions were terminated by the mother.

The behaviours most commonly observed to occur between mothers and juveniles were as follows (the figures in brackets indicate the mean occurrence of that behaviour per 50 minute observation period, n=33):- association (5.8±2.0), allogrooming (2.2±0.7), nest-box sharing (1.8±0.6), food-taking (1.0±0.7) and play (2.0±0.4). A description of all these behaviours can be found in Appendix I. Play was generally passive on the part of the mother and included follow-my-leader games, in which the young pursued their mother, and pouncing, in which the young would jump on their mother's tail or hands.

4. Infants

4.1. Litter Size and Sex Ratio

Data have been collected from eleven litters produced during the course of the study. These data have been combined with those available in the literature (Martin, 1972a, Petter-Rousseax, 1964) to produce the following table (17):-

Table 17

Litter Size and Sex Ratio

Source of data	No. of Litters	Singletons	Twins	Triplets	Sex Ratio
The Author	11	1	9	2	1.4:1.0
Martin (1972a)	27	5	19	3	1.1:1.0
Petter-Rousseaux (1964)	12	3	8	1	1.3:1.0
Total	50	9	36	6	1.2:1.0

From this table it can be seen that the most frequently occurring litter size is two and that the sex ratio is slightly in favour of the males. In the current study the sex ratio increased to 1.8:1.0 by the time the animals were weaned.

4.2. Birth Weight

Weights on the day of birth have been recorded for 11 infants. These varied between 3.5 and 7.5 grams. Extrapolation back from weights obtained later in life for a further 10 infants confirms this range of weights (see table 18). However, all infants of

Table 18

Infant Weights

Weight in grams on day 1

Males	Females
7.5	7.0
7.0	3.5
6.5	3.75
6.0	3.6
6.75	
7.5	
4.0	

Weights in grams of older infants

Age (in days)	Males	Females
2	7.0; 7.5	-
3	11.5	-
4	13.0; 7.0	10.0; 14.5
5	13.0; 12.0	15.0

between 3.5 and 4.0 grams failed to survive longer than three weeks.

4.3. Growth Rate

The weights of five hand-reared and six mother-reared grey infants have been followed on a regular basis throughout their development. Figure 30 shows the typical growth curves for both groups. Growth rates obtained for study colony infants compare favourably with those given in the literature (Petter-Rousseaux, 1964) (figure 31).

4.4. Development

The infants are born with their eyes closed. The dorsal area is covered with downy grey fur in both the murinus and rufus infants and a dorsal stripe of darker fur runs from nose to tail (fig.32). The abdomen is not furred and the skin is transparent.

The infants' eyes open between 2 and 4 days after birth. It is often the eyes of small underdeveloped infants which open first. Eye opening on day 4 is most common for normal healthy infants. On one occasion eye opening was observed to be retarded until 6 days after birth. In this instance there were no obvious ill effects on the animal.

At birth the male infants' testes are inguinal. They have usually descended to the scrotal position by 4 or 5 days after birth.

The infants are born with the ability to cling and to support their own weight when suspended. However, at this stage when placed ventrally on a substrate they cannot support their body weight on their legs. This ability is not generally acquired until some six or eight days after birth. After this age the infants begin to crawl and climb and may be observed peering out of the nest-box at eight days. However, actual emergence does not usually occur before the infants are three weeks old. Observations made of the undisturbed infant inside the nest-box indicate that grooming behaviour both directed towards itself and towards other individuals

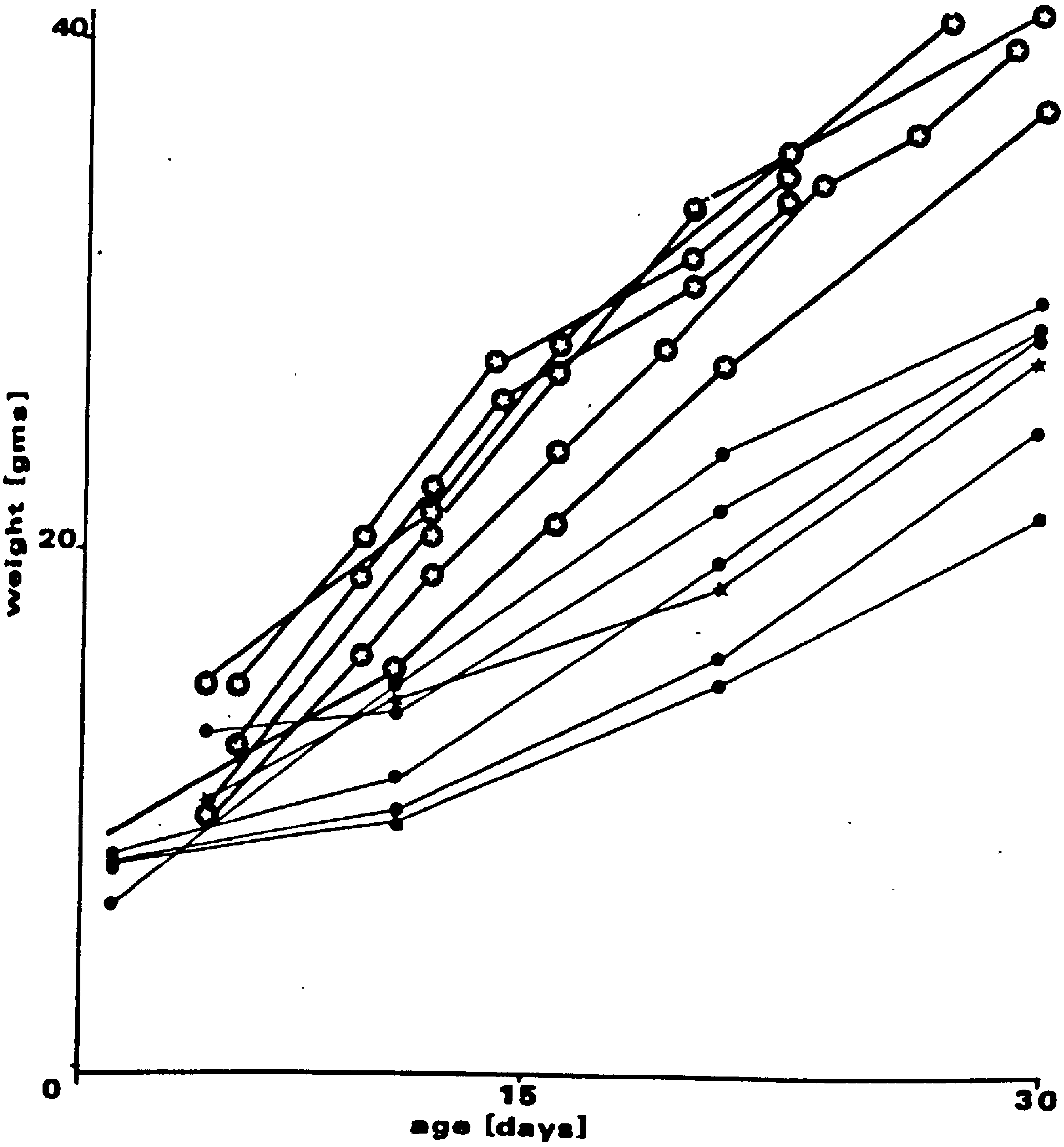


Figure 30

Infant weight gain

Heavy lines represent mother-reared infants, and thin lines represent hand-reared infants. Thin line with stars represents hand-reared *rufus* infant.

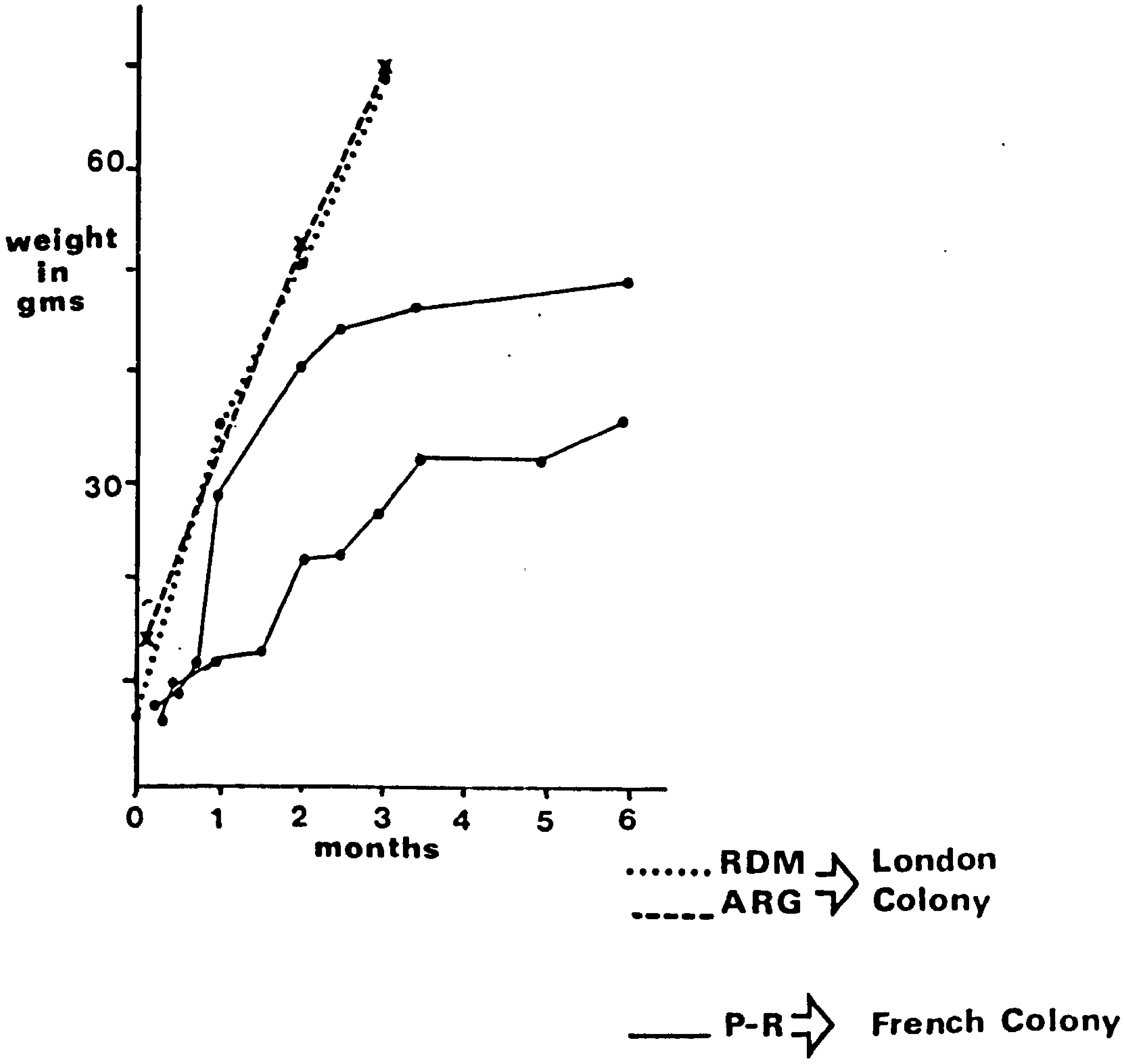


Figure 31

Infant weight gain: comparison of study colony and data from the literature

Two typical examples of weight gain of the study colony infants are shown. The fastest and slowest growing representatives of the French colony are illustrated.

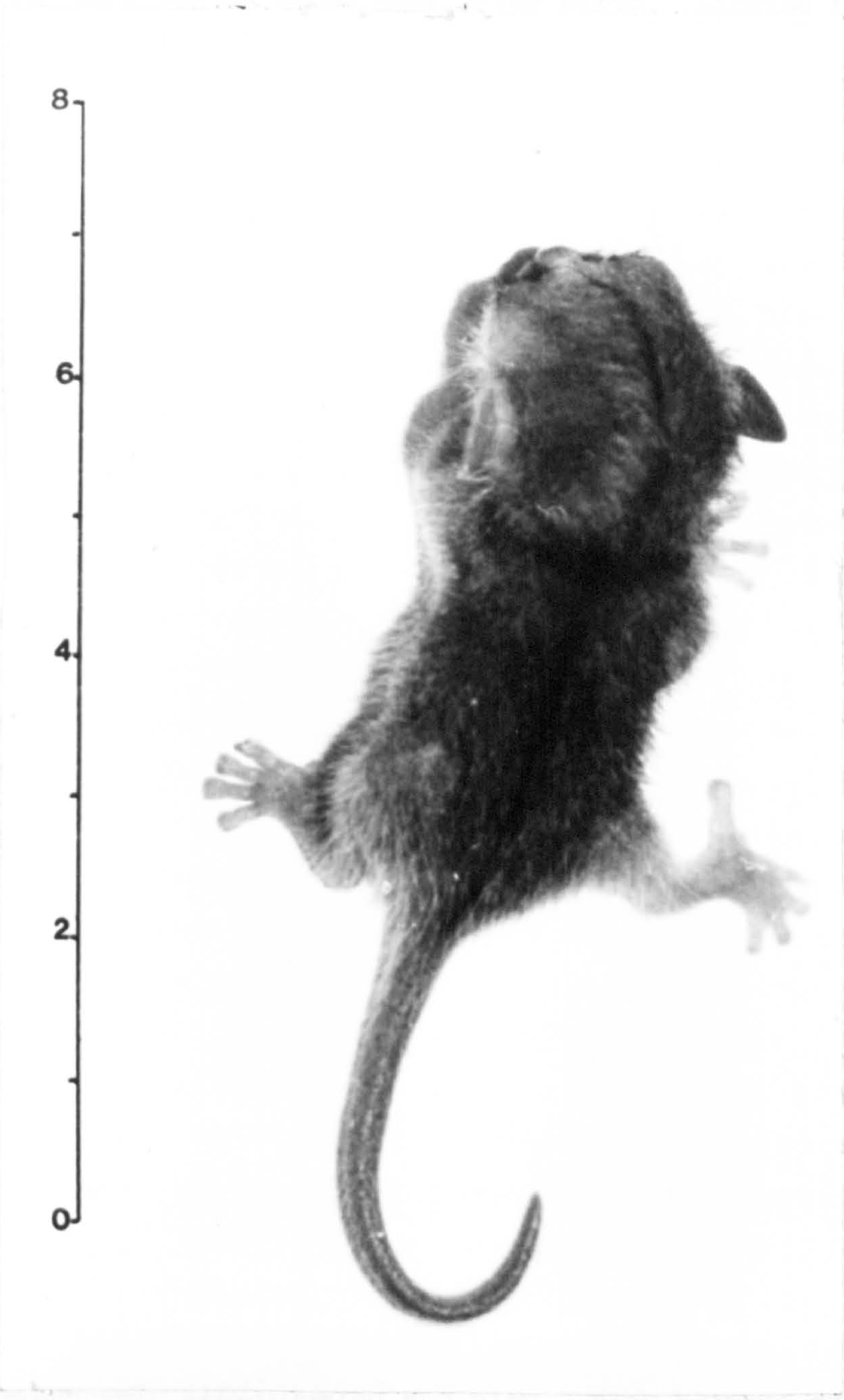


Figure 32

One-day old infant

Scale indicated centimetres.

is first seen six or seven days after birth. Licking the nest-box is also observed to occur at this age. The first parallax side to side head movements are seen between days ten and thirteen. These movements are associated with fixating distant or unfamiliar objects. Play is also observed for the first time at about this age. Play behaviour as observed in the nest-box includes manipulating objects within the nest-box such as twigs or cherry stones, locomotor play, during which the infant bounces up and down on its hind legs and pounces on various objects, and play in the form of wrestling is directed towards its siblings. In addition, it is also about this age when the infant is observed to adopt the adult sleeping position, partially curled up on its side with its tail over its snout. Prior to this age the infants lie flat on their bellies while asleep.

After emergence from the nest-box the infants become very active and inquisitive and are observed to start their first tentative jumps. Weaning, as demonstrated by an interest in solid food, occurs at about 20-25 days. (It must be noted at this point that the mother was never seen to give food to her young or show them where food could be found). Concurrent with this event is the development of the reflective tapetum of the eye. Tapetal development was assessed when the animals were handled for weighing by observing the reflection of the "day" lights in the eye.

Even after the age of three weeks the infants still spend a considerable proportion of their time in the nest-box, although this gradually decreases over time until by day 40 they are out most of the time. The most common interaction between siblings are association, allogrooming and play. Play between siblings is similar to that directed towards the mother except that wrestling is also observed.

The communicative behaviour of the infants in the form of vocalisation is discussed later (see Chapter VI, section 3.1.). All forms of marking behaviour are absent in the infant with the exception of urine-washing which is first seen at about 40 days after birth. (See Appendix I for a description of urine-washing).

4.3.1. Comparison of Mother-reared and Hand-reared Infants

The rate of growth of hand-reared infants is much slower than that of mother-reared (fig.30). This lower growth rate of the hand-reared infants is reflected in the later onset of the development of various characters; for example the acquisition of the ability to hold the body off the substrate and the onset of jumping and weaning occur later in hand-reared infants. (At 14, 28 and 29-32 days respectively). Other characteristics such as eye opening, grooming and urine-washing develop at the same age in mother-reared and hand-reared infants.

4.4. Sexual Maturity

Young mouse lemurs of both sexes are sexually active during the breeding season succeeding that in which they were born. Of the eight surviving males born during the first two years of the study, all exhibited normal testicular development during their first breeding season (see table 9, page 86). In addition, one of these males, Sam, fathered young during his first breeding season at an age of eleven months. The three surviving females which were born during the first two years of the study all exhibited normal oestrous patterns during their first season. In addition, one of these females, Bonnie, gave birth during her first breeding season at an age of thirteen months. The reason why most of these young animals failed to reproduce, although they were sexually active, is probably related to the overall problem of reproductive suppression which is discussed in more detail later. (See Chapter VIII).

5. Discussion

This chapter sets out to give a complete picture of reproduction of the lesser mouse lemur in as far as such information lies within the scope of a captive study of finite duration. The chapter itself can be divided into three sections: reproductive physiology, reproductive behaviour, and infant development; and for the purposes of discussion these divisions will be maintained for reasons of clarity. However, before continuing to the main part of the discussion, it is felt necessary to emphasise the role that captive studies play in obtaining data on reproduction. Much of the data presented in this chapter would be extremely difficult to obtain in the field; this is equally true of both the physiological and behavioural data. As Perry (1971) has pointed out, all data on oestrous cycles, for example, have come from captive studies. In the case of behavioural studies one has but to consider the difficulties which beset the observation of mating in a small bodied nocturnal species such as the mouse lemur under natural conditions, to realise the potential advantages of captive observation. Indeed observations on birth and early maternal care; both of which occur within the nest, would be extremely difficult if not impossible in the wild. Thus it is in the field of reproduction where the captive study comes into its own and unique data of both academic and practical interest can be gathered.

If the physiological data gathered in this chapter are considered first, it must be noted that information on these aspects of reproduction already exists in the literature. The annual fertility cycle of the male has previously been described by Spülher (1935), Petter-Rousseaux (1962, 1964) and Perret (1974, 1977). Various aspects of the reproductive physiology of the female mouse lemur have been described by Petter-Rousseaux (1962, 1964) and Andriantsiferana et al. (1974). As such the data presented may be considered as confirming existing knowledge. However, it must be remembered the data presented in the literature, with the exception of that of Andriantsiferana et al. (1974), tend to be based on a very small number of cases. Thus, the data presented here are, in many cases, the most comprehensive available. The advantages of having a large quantity of data available are that one can give greater credence to the parameters established and that one can reject with greater certitude the anomalous findings of other authors. One example of such anomalous data can be found in the paper of Andriantsiferana et al. (1974). In this paper gestation lengths of 48 and 77 days are given. These data are clearly at odds with the approximately 60 days gestation found in the study colony and reported by Petter-Rousseaux (1962, 1964). The discovery of such anomalies in publications must be taken as an indication of a lack of reliability in the data presented by the authors.

In most cases, unless otherwise stated, the data presented here are in agreement with those available in the literature cited above. Thus, only those results which do not agree with the general consensus of opinion or those which are felt to be of particular interest or relevance will be discussed in this section. The results presented in this chapter which are not consistent with information from other sources derive from the observations on oestrus and on weight gain during pregnancy.

In the former case, although the duration of the swelling phase at oestrus agrees with the 4-6 days reported by Petter-Rousseaux (1962, 1964) and the 1-8 days (mean 5 days, n=43) reported by Martin (unpublished), the range of results obtained for the duration of swelling phase is much greater than in either of these two studies. Furthermore, the modal value of 3 days lies outside the range given by Petter-Rousseaux and, as it does not agree with the mean (5.4 days),it indicates a skewed distribution, unlike the apparently normal distribution obtained by Martin. No reason is apparent for this discrepancy but it is possible that the situation would be clarified with more data. In addition, variation exists between the duration of the flattening phase given in this chapter of 1-6 days and that given in the other two studies of 5-9 and 8-10 days respectively. This discrepancy may have been due to diffent determination of the period to be measured. In the current study this was taken to be from the day following the observation of an open, white swelling until the vaginal opening was sealed. However, the determination of that period may have been different in the other two cases. A second possibility might arise from whether mated or non-mated females were used for the determination. During the course of the study it was noted that very long periods of vaginal opening occurred in the observation room females and in other unmated females. Therefore, the prolonged flattening periods observed by Petter-Rousseaux and Martin may have involved non-mated females.

Few data are available in the literature on weight gain during pregnancy. However, Petter-Rousseaux (1962, 1964) gives a figure of 6-7 grams during the first 45 days of pregnancy. The data presented in this chapter would tend to indicate a weight gain somewhat higher than this. However, the low figure of Petter-Rousseaux may be associated with the production of very small infants. The birth weights given by Petter-Rousseaux, presumably for the infants carried in these pregnancies, are also somewhat on the low side (see later in this discussion).

Four factors mentioned in the preceding chapter are felt to merit discussion here. These are the efficacy for the visual method for the estimation of scrotal size; synchrony of oestrus; the prolonged duration of the inter-oestrus periods; the problems encountered during the attempted estimation of oestrogen levels in mouse lemur urine. The first of these topics only requires a brief mention, but it is felt worth noting how useful this method of assessing scrotal size has proved. It is particularly so in non-handling situations, such as the Observation Room and Jersey Zoo and it also eliminates the stress of scrotal measurement using callipers in those animals which are handled. In view of both its ease of employment and its reliability and accuracy (see Chapter II, section 3.1.), it is felt that this method could easily be adapted to the field situation where it could be a very useful technique.

Synchrony of oestrus has also been reported by Andriantsiferana _et al._ (1974), but in this case the females were housed together. In the case of the study colony, synchrony was observed between females housed in separate cages. Both these results accord with the observations made on wild mouse lemurs by Martin (1972a, 1973). Martin reported that synchrony of oestrus occurred not only in those females which nested together (this parallels the situation of Andriantsiferana _et al._), but also in the population as a whole (this parallels the study colony situation). However, synchrony was tightest within the nesting groups. Unfortunately, sufficient results do not exist for the Observation Room females to test whether synchrony is in fact tighter in females caged together. As the females in the other rooms could neither touch each other, or necessarily see each other, some olfactory or vocal cue must be responsible for mediating reproductive synchrony. As no specific vocalisations were recorded in association with the onset of oestrus it is suggested that the female mouse lemurs were responding to an olfactory cue. In most mammals where reproductive synchrony is reported olfactory cues are cited as the probable mediating factor; for example in the mouse (Bronson, 1971, 1976) _Lemur catta_ (Jolly 1966, 1967) and _Lemur fulvus_ (Harringron, 1975).

One particularly interesting fact which is apparent from the data presented in this chapter, and which has not been remarked on by previous authors, is the relatively long length of the inter-oestrus period (approximately 50 days) in this species. This is particularly apparent when it is remembered that the gestation length of the mouse lemur is only approximately 60 days. The inter-oestrus period of the mouse lemur is also relatively long, both with respect to other prosimians and to primates in general: Butler (1974) reports that the primate oestrous cycle is typically around 30 days; Vincent (1969) reports that of _Galago demidovii_ as 38 days and that of _Galago allenii_ as 47 days; Manley (1966) gives the oestrous cycle of _Galago senegalensis_ as

31.7 days and Ioannou (1966) gives that of Perodicticus potto as 37-39 days. Lisk (1978) has indicated that in most mammals, with the exception of a few primates, the period of sexual heat is restricted to a period of no more than 15 per cent of the cycle. Thus in the mouse lemur, where oestrus represents only 2 per cent of the cycle, the ratio of oestrus to inter-oestrus period can be considered very low.

One of the few species known in which the inter-oestrus period approximates to gestation is the domestic dog, Canis familiaris (Perry, 1971) postulates that this might result from the fact that the reproductive physiology of the dog is intermediate between the prolonged period of heat and mono-oestrus habit of the carnivora and that of the typical polyoestrous species. This may well be the case in the mouse lemur. Butler (1974) postulates that the trend in primate sex cycles is away from the situation in insectivores and primitive rodents, where year round polyoestrus, short cycles and short periods of sexual receptivity unaccompanied by changes in the external genitalia are the norm, towards seasonal reproduction and longer cycles and longer periods of receptivity.

In the dog, this prolongation of the inter-oestrus period is due to the fact that the unmated bitch enters a period of anoestrus after the luteal phase, which is of similar duration to pregnancy, and this again could be the case in the mouse lemur. This system might have an added benefit for the mouse lemur in that it would allow the second oestrus of the season of unmated females to be synchronised with the postpartum oestrus of their nest mates. In an hypothetical situation, an inter-oestrus period of 50-51 days would allow females which did not conceive during the first oestrus to be mated for a second time just prior to the second oestrus of those females which failed to carry their young to term. The females which successfully give birth could then have a post-partum oestrus 69 days after the first conception. This would limit the second oestrus period of the groups to a 16-21 day period between 50 and 70 days after the first oestrous period. The third oestrous period of the season would likewise be limited to a three-week period (figure 33).

Results of hormone assays undertaken on mouse lemur urine have not previously been published. Even though the results obtained in the study were negative they still allow for some interpretation of the picture of hormonal activity in the mouse lemur. However, it is felt that future work on the measurement of excreted progesterone is necessary before a complete understanding is possible. At this stage it can be said that at present the assay of urinary oestrogens or gonadotrophins cannot be used as a method of pregnancy determination in this species. Before going on to discuss these negative results it is necessary to see whether they are accurate reflections of the hormones present or artifacts caused by the techniques or the samples used.

The lack of positive results is to be anticipated from the HCG immunoassay technique. Protein hormones, such as the gonadotrophins, vary considerably in their structure between species. Thus, it is extremely unlikely that mouse lemur gonadotrophins would have the same structure as those of the human. This structural difference would severely constrain any cross-reaction between the mouse lemur chorionic gonadotrophin or LH and the HCG antibody and thus render the assay ineffective.

The absence of positive results from the oestrogen assay is more difficult to explain in these terms. Steroid hormones, such as oestrogen, are unlike protein hormones in that they have the same structure in all mammals. Thus, the absence of positive results in the oestrogen assay cannot be attributed to the mouse lemur oestrogens being structurally incompatible with the antibody used in the assay and therefore not cross-reacting. Nor can the absence of positive results be attributed to the age of the samples, as oestrogen does not deteriorate when deep frozen, and it is unlikely that the creatinine levels, on which the oestrogen concentrations are based, would deteriorate when deep frozen. In addition, all samples older than one year were discounted from the assay.

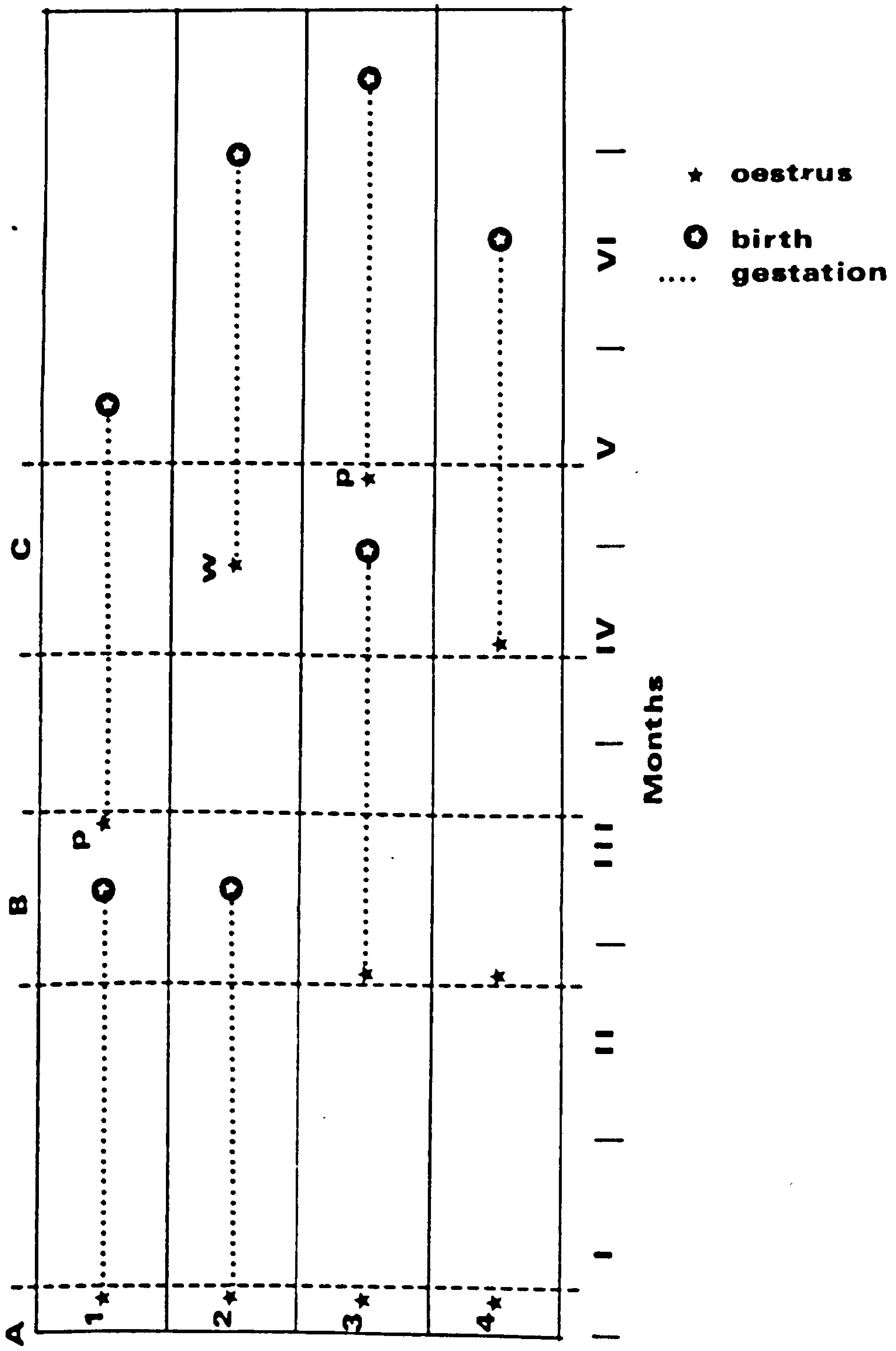


Figure 33

Oestrus synchrony: action of long inter-oestrus periods

Taking an inter-oestrus period of 50 days the figure illustrates four possibilities; (1)gestation following first oestrus followed by a post-partum oestrus (p) followed by gestation,(2)gestation following first oestrus followed by a post-weaning oestrus (w) followed by pregnancy,(3) pregnancy at second oestrus, post-partum oestrus, pregnancy and(4)pregnancy at third oestrus. It can be seen that oestrus is thereby limited to discrete periods A,B & C.

However, there are two sources of error which could account for the inability of the assay to detect any changes in oestrogen levels in association with oestrus and pregnancy: the paucity of samples and the method of analysis employed. In the first case it must be noted that only incomplete series of samples were available from individual females. Therefore the comparisons of oestrus/non-oestrus and pregnant/non-pregnant had to be made between individuals. Thus, large individual variation in oestrogen production could negate definite trends. In the second case it must be noted that the assay technique used was not equally sensitive to all three oestrogens, the cross-reactivity with oestriol was only 15 per cent (Martin et al., 1975). Therefore, if mouse lemurs produce a high proportion of oestriol this assay technique would not prove very accurate.

In the case of oestrus, the classical picture of hormonal control envisages the developing follicle, under the influence of FSH, secreting oestrogen which in turn causes changes in the reproductive tract and induces behavioural oestrus. Concurrent changes changes in vaginal cytology and oestrogen concentration have been reported in many primate species: bonnet macaques, Macaca radiata (McArthur et al., 1972), the greater bushbaby, Galago crassicaudatus (Eaton et al., 1973) and the rhesus monkey, Macaca mulatta (Parakkal & Gregoir, 1972) (all cited by Reynolds & van Horn, 1977). Reynolds & van Horn (1977) also show a relationship between exogenous oestrogen application, copulatory behaviour and vaginal cornification in intact ringtail lemurs, Lemur catta, undergoing photoinhibition of oestrous cycles.

A rise in oestrogen production in association with pregnancy is also known in many species and it is thought that this oestrogen counteracts the progesterone block of myometrial activity and thus brings about birth (Austin & Short, 1972). However, in the human female, where oestrogen production during pregnancy is particularly high (Ryan & Hopper, 1974), labour can still be induced without recourse to oestrogen therapy.

Oestrogens do not have a similar importance in the termination of pregnancy in all species and there is much variation between species in its secretion. Even within the primate group there is great variation between species in both the concentration and pattern of secretion of oestrogen during pregnancy (Lanman, 1977). The rise in oestrogen levels during pregnancy in the human is particularly marked but this pattern is not necessarily repeated in other primates; for example the oestrogen rise during pregnancy is much lower in the chimpanzee, Pan paniscus (Reyes et al., 1975) and the common marmoset, Callithrix jacchus (Hearn & Lunn, 1975), and the rhesus macaque, Macaca mulatta produces almost no oestrogen at all during pregnancy (Bosu et al., 1973). Thus the absence of any marked rise in oestrogen levels during pregnancy in the mouse lemur is not unexpected in view of the variability of oestrogen secretion in the primate group as a whole.

However, this is not the case with the oestrogen levels at oestrus. One would anticipate that the levels of oestrogens would be higher in the breeding season than the non-breeding season and higher still around the time of oestrus. Should the results obtained give a true picture of the patterns of oestrogen secretion and not be attributable to artifacts, then one could establish that the hormonal control of oestrus in the mouse lemur was unusual and differed from that in any other primate so far studied.

All the behavioural data presented in this chapter are unique as very little information on reproductive behaviour exists in the literature. Petter-Rousseaux (1962, 1964) provides a little basic information on mating behaviour and birth, and Martin (1972a, 1973) gives a brief description of the mating behaviour of wild mouse lemurs but beyond this, at the present time, there is nothing.

One of the most notable characteristics which emerges from the behavioural data is the similarity that exists between all aspects of mouse lemur reproductive behaviour and that of Galago senegalensis, as reported by Doyle et al. (1967, 1969).

These similarities in reproductive behaviour reflect only part of the overall similarity in behavioural repertoire between the two groups; for example,in modes of visual communication (see Chapter VI, section 1.). This affinity between Cheirogaleinae and Galaginae has been noted by Charles-Dominique (1977) and by Charles-Dominique & Martin (1970), and they have suggested that those behaviours which are common to the two groups are probably those which were also exhibited by the ancestral primate. Thus, in view of this interesting phylogenetic angle it has been decided to couch the discussion of reproductive behaviour in terms of such similarities, both to the galagos and to the primate group as a whole.

The timing of birth in the mouse lemur, within a few hours of "lights on" or dawn, agrees with the observations of Manley (1966) and Petter-Rousseaux (1962) and conforms to the general tendency amongst the nocturnal primates to give birth during the daylight hours. There seems to be a general tendency amongst primates to give birth during their sleeping period. Diurnal primates give birth at night; this is true of squirrel monkeys, *Saimiri sciureus* (Bowder *et al.*, 1967, cited by Brandt & Mitchell, 1971), the common marmoset, *Callithrix jacchus* (Lucas *et al.*, 1973, cited by Brandt & Mitchell, 1971) and the proboscis monkey, *Nasalis larvatus orientalis* (Pournell, 1966 cited by Brandt & Mitchell, 1971). Women also show a marked tendency to give birth in the early hours of the morning (Lincoln & Porter, 1976, Jolly, 1972b).

The timing of birth is of great importance to species survival and,as Lincoln & Porter (1976) have suggested, this is a result of selection to ensure maximal survival of mother and offspring. Obviously in the case of the mouse lemur it is better to give birth during the sleeping period when the mother is quiet in the nest and when she is not involved in collecting food. Environmental factors such as light, in the case of the mouse lemur, tides or predators, are of great importance in providing the cues which influence the final hour of birth (Lincoln & Porter, 1976).

The actual behaviours observed during parturition in the mouse lemur are similar to those reported for *Galago senegalensis* (Doyle *et al*., 1967). In addition, parturition, as in the galago, can be divided into three distinct phases: the nesting phase which involves intensive nesting behaviour on the part of the female concerned but with no apparent nest being constructed as a result of her efforts; the restless phase, which is characterised by the inability of the female to settle to sleep and involves much grooming behaviour, and finally the delivery phase when actual parturition occurs. The only differences that emerge between the parturient behaviour of the mouse lemur and that of the lesser bushbaby are in the facts that the mouse lemur does not apparently groom its genitals prior to parturition and that the galago apparently does not manipulate its young during birth. This seems unusual as manual manipulations of the young as they emerge are common feature of parturition in primates. For example it has been reported in the squirrel monkey, *Saimiri sciureus* (Bowden *et al*., 1967, cited by Brandt & Mitchell, 1971), the common marmoset, *Callithrix jacchus* (Stevenson, 1976), and in many of the old world monkeys (Brandt & Mitchell, 1971). However, manipulation of the infant does not appear to be a feature of puerperal behaviour of the lesser galago, *Galago senegalensis* (Doyle *et al.*, 1967).

It must be noted at this point that the observations on parturient behaviour in the lesser bushbaby were made in a group situation whereas those of the mouse lemurs were not. In this group situation, the galago mother did not apparently isolate herself from the group. However, observations conducted on mouse lemurs in Rotterdam Zoo since the termination of the study in London, have indicated that mouse lemur mothers will isolate themselves from the group prior to birth. Two mothers housed together in a group of six animals (2 males and 4 females) behaved in this fashion. In addition, during the last week prior to parturition, aggression became so marked in this previously amicable group that one female, the most subordinate, had to be removed from the group altogether.

During the two to three weeks following the births this situation persisted. After this time the mother with the older infants formed an "alliance" with the third non-parturient female. From that time these two females nested together along with the infants and the second female was observed to participate occasionally in the care of the young, grooming them, carrying them to new nests and defending the nest from the approach of males. Further work is planned on birth and maternal care in the group situation. These observations are far removed from the situation in the wild where several females and their young are observed to share the same nest (Martin, 1972a, 1973). Although similar observations have been made on groups of captive Galago demidovii (Vincent, 1969).

Early maternal care in the mouse lemur also shows many parallels with that reported in Galago senegalensis by Doyle et al. (1969). Similarities obviously exist due to the altrical condition of the young in both cases. This results in all maternal care occurring in the nest during the early weeks. However, the young galagos leave the nest at a younger age than do the mouse lemurs. Other similarities in early maternal behaviour exist in the accommodation postures adopted during suckling and in the somewhat erratic occurrence of both suckling and cleaning behaviour in the two species. However, it must be noted that more time is allocated to these behaviours in the galago.

To date there are no data available on later maternal care in the galago, thus no comparison is possible. However, an investigation into this phase of the mother/young relationship was deemed necessary in the case of the mouse lemur. The intention was to determine whether there was any significant difference in the frequency or pattern of instigation between mother/daughter and mother/son interactions. The reason for this was to determine whether a difference occurred in the apparent sociability of a mother towards her male and female offspring and whether this difference might be associated with the establishment of the nesting groups of females found in the field. Martin (1973a) reported that female mouse lemurs nested together in groups while the males generally nested together in pairs or alone and only rarely with non-oestrous females. Such nesting groups of females are presumed to be matriarchies. Thus one might anticipate that whilst the mother maintains social contact with her maturing daughter she does not do so with her maturing male offspring and that in fact she might actively discourage them from social interaction with her. In this way she could establish the normal female nesting group pattern. However, currently too few data are available for any conclusions to be drawn as to the accuracy of this hypothesis.

The final section of this chapter deals with the parameters of infant growth and development. Birth weights of mouse lemurs are not extensively dealt with in the literature, but those found in the study colony compare favourably with those reported for infants born to wild caught pregnant females in Madagascar (Martin, 1972a, 1973). This is a testimony to the efficacy of the husbandry techniques employed in the study colony. Data from Martin (1972a, 1973) also confirm the non-viability of low birth weight infants. He found that four out of five infants weighing between 3 and 4 grams failed to survive. These data would all seem to indicate that the mouse lemur birth weights of 3.5 and 4.3 grams reported by Petter-Rousseaux (1962, 1964) are somewhat low. These infants also failed to survive.

The data on litter size, development and sexual maturity agree with those of Petter-Rousseaux (1962, 1964). However, although she found that the males were physically sexually mature within their first year, she did not observe any successful matings occurring before their second year. The data presented in this chapter have shown that the males can father offspring in their first year.

The data collected on the various aspects of mouse lemur reproduction in captivity agree fairly well with what is known about this species in the wild, as reported by Martin (1972a, 1973). It has been noted that birth weights are of a similar order to those found in the wild, that synchrony of oestrus occurs in both situations and the observations of post-partum oestrus, both in this study and in that by Andriantsiferana et al. (1974), confirm the possibility suggested by Martin (1972a, 1973), that the mouse lemurs give birth to two litters per season in the wild.

As a final point of discussion some mention must be made on the difference in gestation length between red and grey mouse lemurs. This fact has not been noted previously in the literature and has obvious implications for arguments involving the taxonomic status of the two "subspecies" of mouse lemur. In consideration of the difference in duration of testicular development discussed in the previous chapter it would be interesting to establish whether the shorter breeding season and shorter gestation are possibly coupled with more rapid infant development. Unfortunately only one rufous infant was born during the course of the study and this infant was hand-reared. These are naturally somewhat limited data from which to draw any conclusions. However, it must be mentioned that although this infant did not gain weight any faster than its murinus counterparts some behaviours, notably jumping and weaning, did occur earlier in this infant (20 and 25 days respectively). The observations of shorter gestation length in the rufous mouse lemur do not accord with the theory of r and K selection in which one would expect the stable environment of the rain forest, which the rufous form inhabits, to encourage a K selection pattern with its concomitant slower reproductive turnover (Wilson, 1975, Martin, 1975). In addition, there are no data available which indicate whether the shorter gestation in the rufous form leads to a lower weight gain in pregnancy or the production of smaller infants. What data are available indicate that both weight gain during pregnancy (20 grams by day 37, see table 13) and infant birth weight (5.5 and 7.0 grams approximately) are the same in the red sub-species as in the grey.

Before concluding, one fact which has come to light during this study needs further emphasis because of the influence it could have on captive studies in the future. This is the apparent normality of the hand-reared specimens. Unlike the situation in many other primates in which hand-rearing effects the later sexual behaviour of the individual concerned, the hand-reared mouse lemur is sexually normal and in many cases the hand-reared specimens are far more successful in reproductive terms than their mother-reared counterparts. This is equally true for both males and females. One of the most successful mothers in the colony was hand-reared, as were the two breeding males, Spike and Sam. This is an obvious advantage in reproductive studies as the animals remain friendly into adulthood and thus can be easily handled for collection of samples and various measurements.

In conclusion, it must be repeated that while some of the data presented in this chapter are not new they are invaluable, particularly by virtue of the larger sample sizes available in this study, in that they confirm and expand upon the existing information, thus eliminating disparities. These data together with the new information provided in this chapter provide a comprehensive, broadly based approach to the topic of mouse lemur reproduction. This ensures that sufficient baseline data are available to make feasible a realistic attempt at assessing captive breeding problems.

SUMMARY

1) The testis of the male mouse lemur undergoes an annual cycle of growth and regression. Testis sizes are maximal during the breeding season. The testes of the prime breeding males exhibit a seven-fold increase in scrotal area at this time. Testis growth occurs in all males at this time but is generally less spectacular in the other males. The males with the smaller testes may not necessarily be infertile although they do not usually mate with females. The testes of all the males are small during the non-breeding season and histological examination indicates that spermatogenesis does not occur at this time.

2) The female mouse lemurs are seasonally polyoestrous. Females which fail to conceive come into oestrus three times during a breeding season. The vagina in this species is imperforate except at oestrus or during parturition. The duration of vaginal opening at oestrus is generally 2-6 days and it is preceded by a period of vulval swelling and colouration. Peak female receptivity and vaginal oestrus (as determined from vaginal smears) occurs 2 or 3 days after vaginal opening. The length of time between successive periods of oestrus is approximately 50 days and gestation lasts approximately 60 days.

3) Hormone assay techniques undertaken on mouse lemur urine have as yet failed to provide a successful means of pregnancy determination in this species.

4) Observations of reproductive behaviour and parturient behaviour, indicate many similarities to those reported in Galago senegalensis in captivity. Birth normally occurs a few hours after "dawn". The imminence of birth is characterised by the inability of the female to settle to sleep.

5) Due to the altrical condition of the young, all early maternal care takes place in the nest. Thus, the amount of time that the mother spends in the nest with her young is a good measure of efficacy of maternal care.

6) The most common litter size is two although both singletons and triplets are produced and successfully reared. Infants generally weigh between 3.5 and 7.5 grams at birth. However, it has been noted that infants with a birth-weight of below 4.5 grams fail to survive. Infants are born with their eyes closed and are only sparsely furred. They are able to support their body-weight if suspended from a twig, but are unable to stand holding their bellies off the substrate. Despite this the infants are surprisingly active at birth and it is suggested that it is this activity which triggers maternal care. The infants grow rapidly and are fully independent by 60 days and sexually mature in the breeding season succeeding that of their birth.

7) When mature, hand-reared mouse lemurs do not differ significantly in their behaviour towards conspecifics from their mother-reared counterparts. They reproduce normally and due to their tractability can be useful additions to a study colony.

8) The *rufus* mouse lemurs would seem to have a significantly shorter gestation length than the *murinus* specimens.

9) The discussion focuses primarily on the long inter-oestrus periods exhibited by the mouse lemur, the inability of hormone assay techniques to detect oestrus or pregnancy in this species and the similarities between the reproductive behaviour of the mouse lemur and the lesser bushbaby, *Galago senegalensis*.

SECTION B

BEHAVIOUR IN CAPTIVITY

This section deals with the more commonly observed patterns of behaviour. While it is obvious that communication is a form of behaviour, it is such a large topic that it has been dealt with in a separate chapter. It is hoped therefore, that the purist will bear with the titles of the two chapters in this section. Although this study was conducted solely in captivity it is possible to extrapolate some of the findings to the field situation.

CHAPTER V

ACTIVITY PATTERNS AND SOCIAL BEHAVIOUR

In this chapter various aspects of the behaviour of the mouse lemur in captivity are investigated. To date no detailed information on the behaviour of the mouse lemur has been available in the literature. This is due to the fact that previous studies have tended either to be field studies or to concentrate on the physiology of this animal. Thus, although some behavioural data are included in the field studies, it is of necessity not very detailed. Detailed behavioural observations of this kind of animal (i.e., small bodied and nocturnal) are more readily conducted in captivity where the animals can be easily observed over prolonged periods.

As is indicated by the title, this chapter is divided into two sections, the first dealing with activity patterns exhibited in captivity and the second dealing with social behaviour. They are included in the same chapter because of the reciprocal influences they can have on each other, though it must be noted that it is activity which primarily influences social behaviour. Data on communication are not included in this chapter as this is deemed to be a large enough topic to merit a separate chapter (See Chapter VI).

It is hoped that the data presented in this chapter will be of relevance to future field studies of the mouse lemur, where detailed information on behaviour collected in the laboratory can both supplement and aid in the interpretation of incomplete observations made in the field. However, the prime objective of this chapter is to provide behavioural data which can be employed to improve captive conditions for mouse lemurs. An increase in the availability of knowledge concerning a particular species increases the possibilities of designing the optimal captive environment for that species. Data on activity patterns can be employed to aid in the efficient management of a colony. These data together with those on social behaviour can be used for planning the best time of the year for establishing the animals in groups, and also indicate the best group structure and cage design to be used. Finally data on behaviour have a diagnostic value as changes from normal behavioural patterns may be indicative of illness or stress in the animal concerned.

1. Activity Patterns

The study of activity patterns was initially undertaken in order to ascertain which times of the day and year would be most suitable for collecting detailed information on social behaviour. However, as we have seen earlier (Chapter III, section 3.) the measurement of activity levels, particularly that of feeding behaviour, would seem to provide an indication of adrenal activity. Thus the benefits of this study are two-fold.

As discussed earlier (Chapter III, introductory section) under natural conditions it is the level of illumination which controls the onset and cessation of activity. Thus cloud cover and foliage density could cause variability in activity patterns. Pariente (1974) has reported that sportive lemurs, Lepilemur mustelinus, which inhabit forests (i.e., areas of dense foliage cover), emerge from their nests earlier in the evening and return to them later in the morning than their conspecifics which inhabit the bush (i.e., areas of less foliage density). This observation indicates a relationship between foliage density and activity patterns. Climatic factors may also affect activity. Kavanau & Peters (1976a, b) have indicated that both cool, rainy weather and clear, exceptionally hot weather will delay the onset of evening activity in both the slow loris, Nycticebus coucang and the owl monkey, Aotus trivirgatus in captivity. Therefore, it is only in the indoor conditions of captivity that the influence of daylength on activity can properly be investigated, as in the field these other factors (climatic conditions and habitat type) which affect activity come into play.

1.1. Nocturnality

In common with the galagos, lorises and other cheirogaleine species the mouse lemur is strictly nocturnal under natural conditions (Hill, 1953). However, in captivity, mouse lemurs are often found to be active 2-3 hours prior to "sunset". This occurrence is particularly prevalent during the long daylength period, "summer".

1.2. Variations in total activity levels

As can be seen from the graphs (fig. 34), there is considerable variation in the level of activity over the day, over the year and between individuals. These differences were analysed using a three-way analysis of variance and the results are presented in table 19. As can be seen, the levels of activity in both the London and Jersey groups varied significantly with all three variables. In all cases the level of probability exceeds that accepted in this thesis ($p = 0.05$). However, due to the presence of empty cells in the analysis matrix (the reason for which is given in Chapter II) no interactions were computed. To overcome this problem, the results from the London group were re-analysed in two separate sections so as to eliminate the empty cells. The cells which were empty were those of times 4 and 5 in month block I. Thus the analysis was rerun in one instance with all times but minus month block I data and in the second instance with all months but minus the data from times 4 and 5. From these data (table 20) it can be seen that the two-way interactions between time and month, time and animal and month and animal are all significant with the exception of the time/month interaction in the analysis where the data collected in times 4 and 5 were not included. However, in both cases the three-way interaction of time, month and subject on activity was not significant. Assuming that the lack of two-way time/month interaction in the analysis which excluded the data from times 4 and 5 is an artifact (explanation given below) these results can be interpreted as follows:

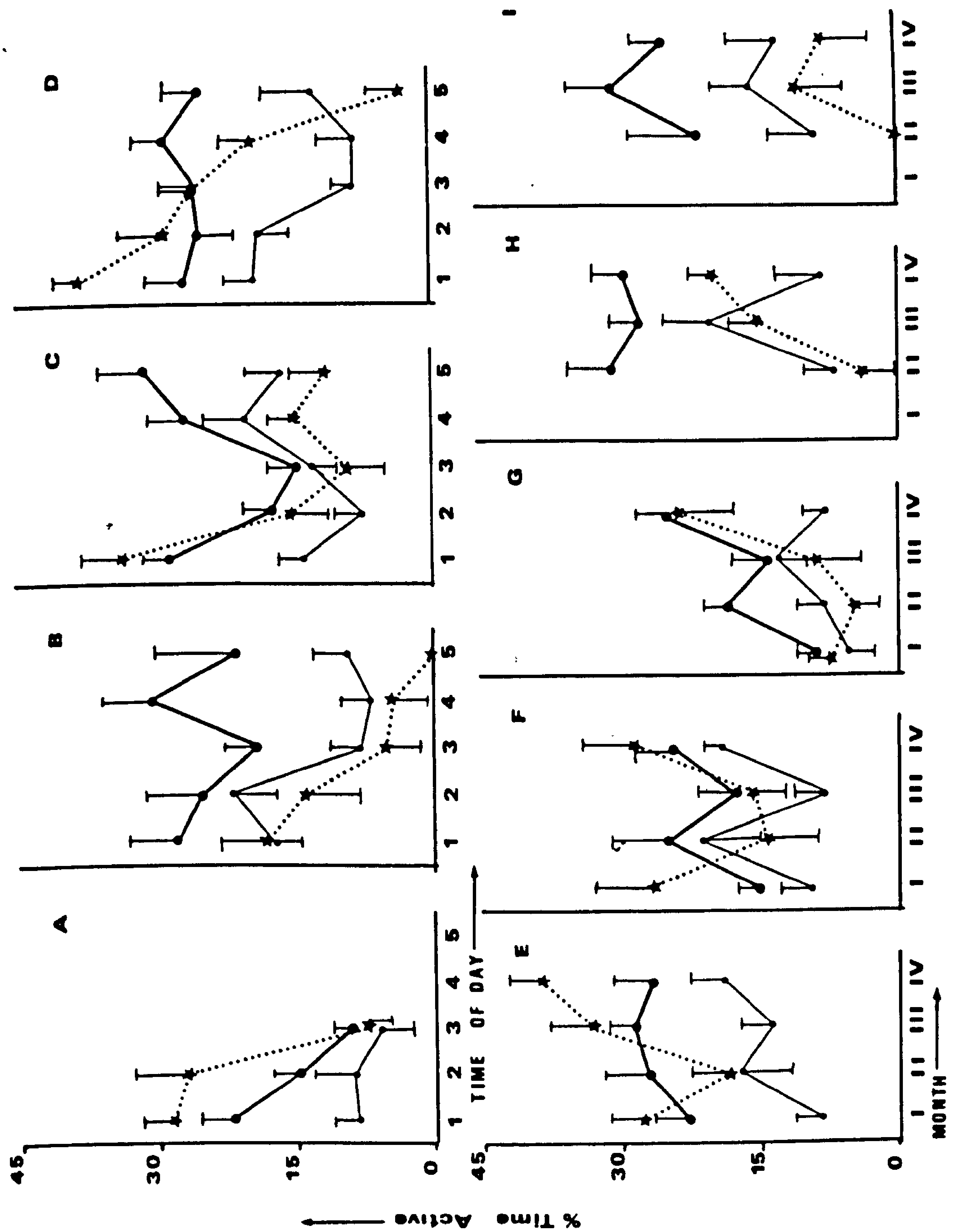


Figure 34

Annual and daily activity patterns

Figs. A-D show the daily activity patterns for months I-IV, figs. E-I show annual variation in activity at times 1-5. Dotted line represents the male and the heavy and light lines represent the dominant and subordinate females respectively.

Table 19

Three-way Analysis of Variance on Activity in the London and Jersey Groups of Mouse Lemurs

LONDON

Source of variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Main effects	17935.328	9	1992.814	11.167	0.000
Time	6915.793	4	1728.948	9.689	0.000
Month	3932.695	3	1310.898	7.346	0.000
Animal	8459.895	2	4229.945	23.704	0.000
Explained	17935.375	9	1992.819	11.167	0.000
Residual	77446.563	434	178.448		
Total	95381.938	443	215.309		

JERSEY

Source of variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Main effects	14495.734	9	1610.637	11.752	0.000
Time	2174.725	4	543.681	3.967	0.005
Month	5667.324	3	1889.108	13.784	0.000
Animal	7592.625	2	3796.313	27.700	0.000
Explained	14495.734	9	1610.637	11.752	0.000
Residual	17542.574	128	137.051		
Total	32038.309	137	233.856		

Table 20

Three-way Analysis of Variance of Activity of London Group of Mouse Lemurs minus Empty Cells

Minus Times 4 and 5

Source of variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Main effects	15253.500	7	2179.071	13.731	0.000
Time	5530.770	2	2765.385	17.426	0.000
Month	4437.375	3	1479.125	9.320	0.000
Animal	4842.121	2	2421.061	15.256	0.000
Two-way interactions	5853.598	16	365.850	2.305	0.003
Time Month	1131.109	6	188.518	1.188	0.313
Time Animal	1433.171	4	358.293	2.258	0.063
Month Animal	3556.921	6	592.820	3.736	0.001
Three-way interactions	1816.418	11	165.129	1.041	0.411
Time Month Animal	1816.418	11	165.129	1.041	0.411
Explained	22923.516	34	674.221	4.244	0.000
Residual	44435.172	280	158.697		
Total	67358.688	314	214.518		

Minus Batch Observation Month I

Source of variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Main effects	15511.629	8	1938.954	11.682	0.000
Time	4962.121	4	1240.530	7.474	0.000
Month	2428.823	2	1214.411	7.317	0.001
Animal	8020.645	2	4010.322	24.161	0.000
Two-way interactions	10991.547	20	549.577	3.311	0.000
Time Month	2655.285	8	331.911	2.000	0.046
Time Animal	4742.895	8	592.862	3.572	0.001
Month Animal	3643.069	4	910.767	5.487	0.000
Three-way interactions	2055.758	16	128.485	0.774	0.715
Time Month Animal	2055.758	16	128.485	0.774	0.715
Explained	28558.934	44	549.067	3.911	0.000
Residual	55603.379	335	165.980		
Total	84162.313	379	222.064		

(1) The level of activity observed at different times varies with the month of observation; (2) the level of activity at a given time of day varies with the subject; and (3) the level of activity in a given month also varies with the subject. The converse of all the above also applies. However, due to the absence of three-way interaction the level of activity does not vary in a significant fashion with all three parameters combined. This is important, as it allows the data for one parameter to be pooled in order to investigate the effects of the other two parameters. Thus it is possible to group the data for all three subjects and consider the effects of time and month, to group the data from all five times to consider the effects of month and subject and to group the data for all four months to consider the effects of time and subject.

1.2.1. Variation in total activity over the year

The variation in total activity levels over the course of the year has been discussed earlier (Chapter III, section 6) and, as is mentioned in section 1. of this chapter, analysis of variance indicates that this variation is significant. In general terms the animals are most active in observation month IV (just after the longest day) and least so in observation month I (just before the shortest day), whilst the levels for the remaining two observation months are intermediate. The differences in activity exhibited in months IV and I are significant. This is true if one considers the total activity per month of each of the subject animals or if one considers the total activity of all three subjects combined at each of the five separate observation times (see table 21).

1.2.2. Variation in total activity over the day

The variation in total activity over the course of the day, as discussed in section 1.of this chapter, is indicated to be significant according to the analysis performed. From the activity graphs

Table 21

Variation of Total Activity over the Year Taking Observation Times 1-3 Together

	I	II	III	IV
MF6	7.67 (24)	15.67 (27)	11.70 (27)	15.33 (30)
MF8	15.67 (24)	23.56 (27)	20.15 (27)	20.73 (30)
MM3	20.5 (24)	12.65 (27)	19.33 (27)	31.07 (30)

Figures in brackets are numbers of observations in each cell.

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t-test comparing months I and IV

Using residual mean square deviation from computer output 158.60

	IV - I	Standard Deviation	t
MF6	7.66	3.45	2.22
MF8	5.06	3.45	1.47
MM3	10.57	3.45	3.06

With 280 degrees of freedom these differences are significant.

Taking all three subjects together

	I	II	III	IV
1	19.83 (24)	20.89 (27)	25.40 (27)	27.87 (30)
2	16.83 (24)	20.19 (27)	13.48 (27)	24.63 (30)
3	7.17 (24)	10.67 (27)	12.30 (27)	19.73 (30)
4		14.07 (27)	21.03 (27)	19.00 (24)
5		15.20 (15)	19.62 (21)	16.20 (15)

Figures in brackets are number of observations in each cell.

Table 21 (cont'd)

t-test comparing months I and IV

Using residual mean square deviation from computer output 158.70.

	IV - I	Standard Deviation	t
Time 1	8.04	3.45	2.33
Time 2	7.7	3.45	2.23
Time 3	12.66	3.45	3.64

With 280 degrees of freedom these differences are significant.

(fig.34) it can be seen that there are basically two activity patterns: a bimodal distribution of activity in which there is a peak of activity shortly after "sunset" and a second some time prior to "sunrise"; and a pattern of activity in which the subjects are most active some time shortly after "sunset" after which time activity levels decline progressively. These patterns are not constant but vary with subject and over the year. In the case of the subject, only MF8 exhibits a significantly bimodal pattern (table 22) in her total activity levels. MF6 shows a tendency to bimodality, but this is not significant and the male exhibits no tendency to bimodality in activity, but rather his activity declines over the course of the night.

Time of year also has its affect on the daily activity pattern. Activity in general terms has the greatest tendency to bimodality in observation month III and the least in observation month IV. This can be demonstrated by comparing the levels of activity at times 1 and 3 and times 3 and 4 for each observation month. Month IV is clearly not bimodal; there is a tendency to bimodality in month II which is not significant, but bimodality is significantly apparent in the breeding season (just before the longest day), month III (table 22).

1.2.3. Variation in total activity patterns between subjects

As discussed in section 1 of this chapter, analysis of variance has indicated that there is a significant variation in activity pattern between subjects. Although in all cases the activity in month I is the lowest, the levels of activity in the other months vary between individuals (fig.34). Daily activity pattern also varies between subjects (see section 1.2.2. above). When these differences between individuals are examined in detail it can be seen that the differences in activity patterns between male and females are structured whereas those between the females are random. In table 23 it can be seen that the difference in levels

Table 22

Variation in Activity over the Day

Considering the subjects separately and excluding month I

	TIME				
	1	2	3	4	5
MF6	12.78 (28)	16.14 (28)	9.78 (28)	11.85 (26)	12.8 (17)
MF8	27.32 (28)	22.47 (28)	19.65 (28)	29.07 (26)	25.68 (17)
MM3	30.06 (28)	19.78 (28)	13.27 (28)	13.18 (26)	6.47 (17)

Figures in brackets are number of observations per cell

t-test comparing times 1 and 3 and 4 and 3

Using residual mean square deviation from computer output 165.98.

	1 - 3	st. deviation	t	4 - 3	st. deviation	t
MF6	3	3.44	0.89	2.07	3.51	0.59
MF8	7.67	3.44	2.27*	9.72	3.51	2.68*
MM3	16.79	3.44	4.89*	-0.09	3.51	0.00

With 335 degrees of freedom. * indicates t-values, which are significant at $p = 0.05$

Table 22 (cont'd)

Considering Subjects Together

MONTH

	II	III	IV
1	20.89 (27)	25.40 (27)	27.87 (30)
2	20.19 (27)	13.48 (27)	24.53 (30)
3	10.67 (27)	12.30 (27)	19.73 (30)
4	14.07 (27)	21.03 (27)	19.00 (24)
5	15.20 (15)	19.62 (21)	15.20 (15)

---

t-test comparing times 1 and 3 and 4 and 3

Using residual mean square deviation from computer output 165.98.

	1 - 3	st.deviation	t	4 - 3	st.deviation	t
II	10.22	3.50	2.92*	3.4	3.50	0.97
III	13.1	3.50	3.74*	8.73	3.50	2.49*
IV	8.14	3.33	2.44*	-0.73	3.53	0.00

With 335 degrees of freedom. * indicates t-values which are significant, $p = 0.05$.

Table 23

Comparing Activity of the Females to that of the Male

Difference between mean female activity and male activity

		MONTHS			
		I	II	III	IV
Time	1	-12.62	4.00	-12.22	-15.80
	2	-14.62	9.28	- 3.11	- 7.00
	3	0.62	8.01	5.11	8.80
	4	-	14.45	8.89	- 1.50
	5	-	15.20	12.29	10.80

Rankings to show time-animal interaction and coefficient of concordance (W)

		MONTHS		
		II	III	IV
Times	1	1	1	1
	2	3	2	3
	3	2	3	2
	4	4	4	4
	5	5	5	5

W = 0.956 Significant, p = 0.0009

Rankings to show month-animal interaction and coefficient of concordance (

	MONTHS		
	II	III	IV
1	3	2	1
2	3	2	1
3	3	2	1
4	3	2	1
5	3	2	1

W = 1.0 Significant, p = 0.0008

---

of activity between male and females increases over the course of the day. The coefficient of concordance indicates that this trend is significant (p = 0.0009), whereas in table 24 it can be seen that no such trend emerges between the females. In table 23 it can also be seen that the difference between male and female activity is greatest in month II and that it decreases through months III and IV respectively. The coefficient of concordance indicates that this trend is significant (p = 0.0008). In table 24 it can be seen that no such trend emerges between the females.

1.3. Variations in specific activities

Specific activities which were recorded in the course of the investigation into activity patterns, being a constituent part of the total activity levels discussed in section 1.2. above, also show variation over the day, over the year and between individuals.

1.3.1. Variations in specific activities over the year

It is obvious that there is considerable variation in the daily pattern of the occurrence of the various behaviours over the year. This will not be taken into consideration here. Thus, the occurrence of each behaviour at each of the five observation times will be summed to give the mean occurrence of the behaviour for each of the three subjects for each month.

The annual variation of feeding frequency was discussed earlier (Chapter III, section 3.). The pattern of annual variation for each of the other behaviours; marking, self-grooming, allogrooming,, moving, in the nest-box and in proximity, are shown in figure 35. From this figure it can be seen that proximity, nest-box and social grooming scores tend to be lower in the long daylength months III and IV. The other behaviours tend to occur at an almost constant level except there appears to be a slight elevation in marking frequency during the breeding season (observation month III). Locomotion scores seem to show the greatest individual

Table 24

Comparing Activity Levels of Two Females

Difference in activity between MF8 and MF6

		MONTHS			
		I	II	III	IV
Time	1	14.25	9.78	14.22	7.60
	2	6.50	3.22	9.78	6.00
	3	3.25	10.67	1.34	17.00
	4		23.56	7.11	21.00
	5		11.20	15.43	12.00

Rankings to show time-animal interactions and coefficient of concordance (W)

		MONTH		
		II	III	IV
Time	1	2	4	2
	2	1	3	1
	3	3	1	4
	4	5	2	5
	5	4	5	3

W = 0.40 Not significant, p= 0.213

Rankings to show month-animal interaction and coefficient of concordance (W)

		MONTH		
		II	III	IV
Time	1	2	3	1
	2	1	3	2
	3	2	1	3
	4	3	1	2
	5	1	3	2

W = 0.04 Not significant, p = 0.367

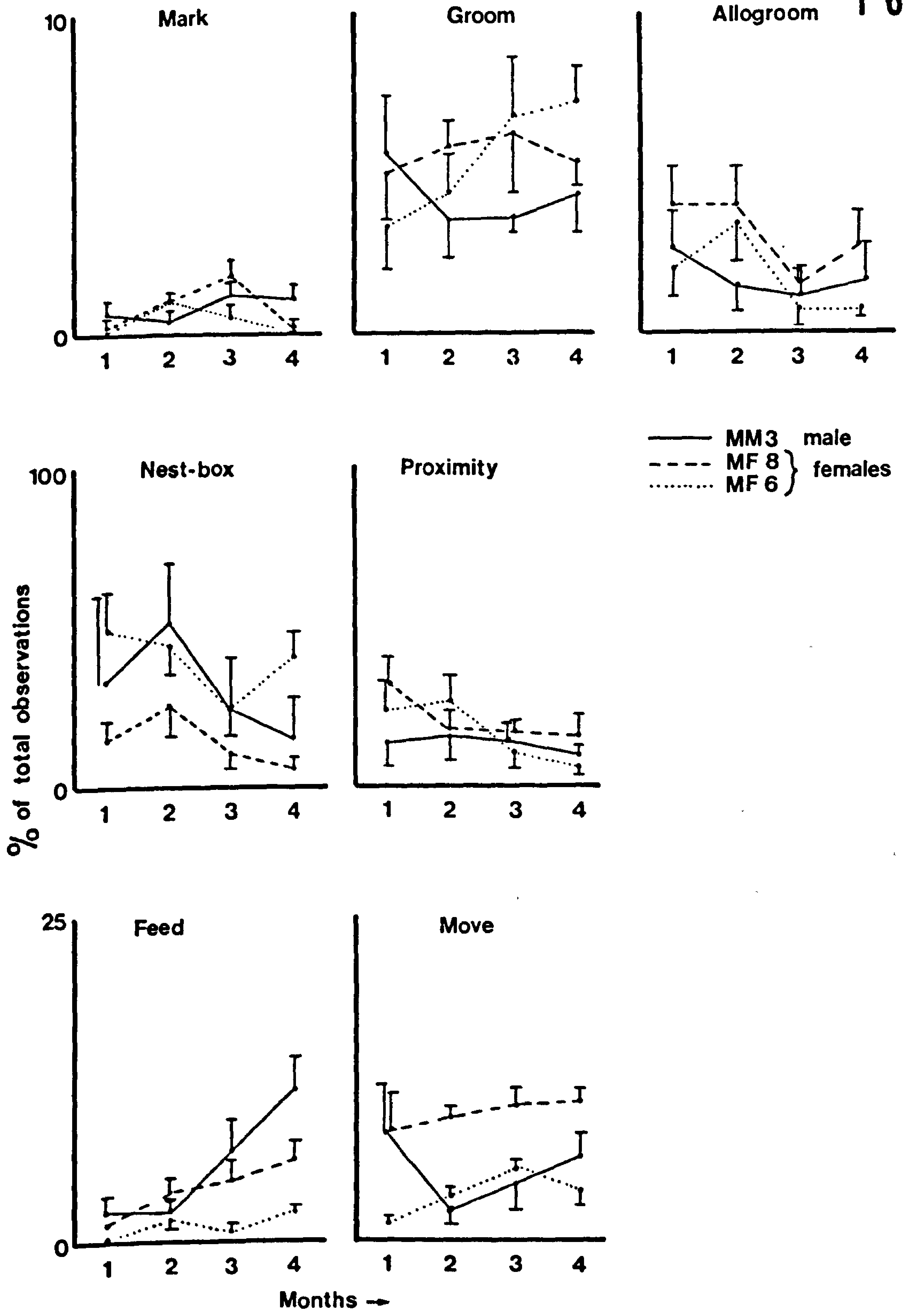


Figure 35

Mean annual variation in specific activities difference. The females show similar patterns although the level of locomotor activity exhibited by the dominant female is much higher than that of the subordinate female. However, the annual variation in locomotor activity in the male can be seen to be entirely different.

1.3.2. Variation in specific activities over the day

As indicated in the previous section (1.3.1.) it is obvious that the time of year has considerable influence on daily patterns of various activities. This is not taken into consideration. It is the overall daily activity pattern of each individual subject for the whole year which will be examined. From the graphs of these daily patterns (fig. 36) it can be seen that marking occurs most frequently in the first hours after waking and then persists at a more or less constant level throughout the active period with the exception of the dominant female which exhibits a second peak of marking in the last hour prior to "lights on". Self-grooming occurs at a constant level throughout the active period, with both females exhibiting a peak of grooming behaviour during the last hour prior to "lights on". The male does not exhibit elevated levels of grooming behaviour at the end of the active period. Allogrooming patterns are very similar for all three subjects, with all showing a peak of this behaviour in the second and third observation times. The pattern of time spent in the nest-box is very similar for both females but the subordinate female spends considerably more time there than the dominant female. The pattern of nest-box use in the male is completely different. Feeding behaviour also varies considerably over the day with the male exhibiting a pattern very different from that of the females. In the male most feeding occurs in the hour after waking and decreases thereafter, whereas the females both exhibit a second increase in feeding behaviour in the latter half of the night. Locomotor patterns show the most individual variation. Those of

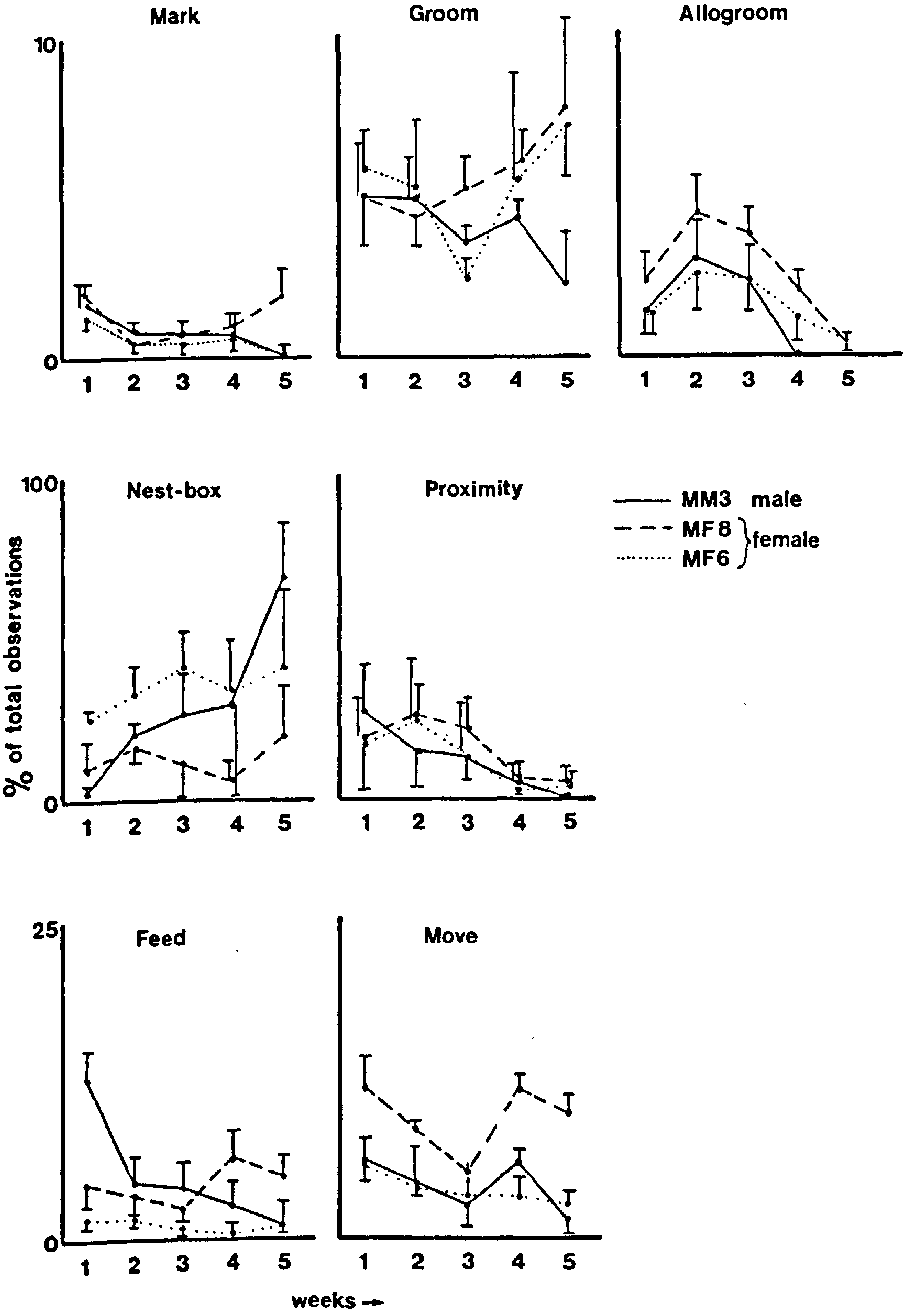


Figure 36

Mean daily variation in specific activities the dominant female and the male seem to be biphasic, while that of the subordinate female is not so. In addition the level of locomotor activity exhibited by the dominant female is much higher than that of her two cage mates throughout the whole of the active period.

1.3.3. Variation in specific activity levels between subjects

The most notable differences in specific activity patterns between individuals are primarily in locomotor activity as reflected in the different monthly pattern of activity in the male and the elevated levels of activity exhibited by the dominant female throughout the day and the year. The other markedly different pattern is apparent in the occurrence of nest-box use. The pattern exhibited by the male for time in the nest-box is very different from that of the two females, as is his daily pattern of feeding behaviour. The annual pattern of feeding behaviour discussed earlier (Chapter III, section 3.) is similar for all individuals. However, the variation in feeding over the year is most marked in the male.

1.4. Comparison of different groups

As mentioned in section 1.2. above, in both the London and Jersey groups all of the three variables, subject, time and month, contributed significantly to the variation in activity recorded. However, due to certain difficulties with the Jersey observations, such as the removal and death of one of the subject animals, it was impossible to adjust the data so as to omit empty cells in the analysis matrix, thus it was not possible to obtain information on interactions. However, it can be seen from the graphs of activity patterns of the two animals which were present throughout the observations (see fig. 37), certain similarities as well as certain differences emerge. Most important is the high level of activity in month IV and the low level of activity in month I

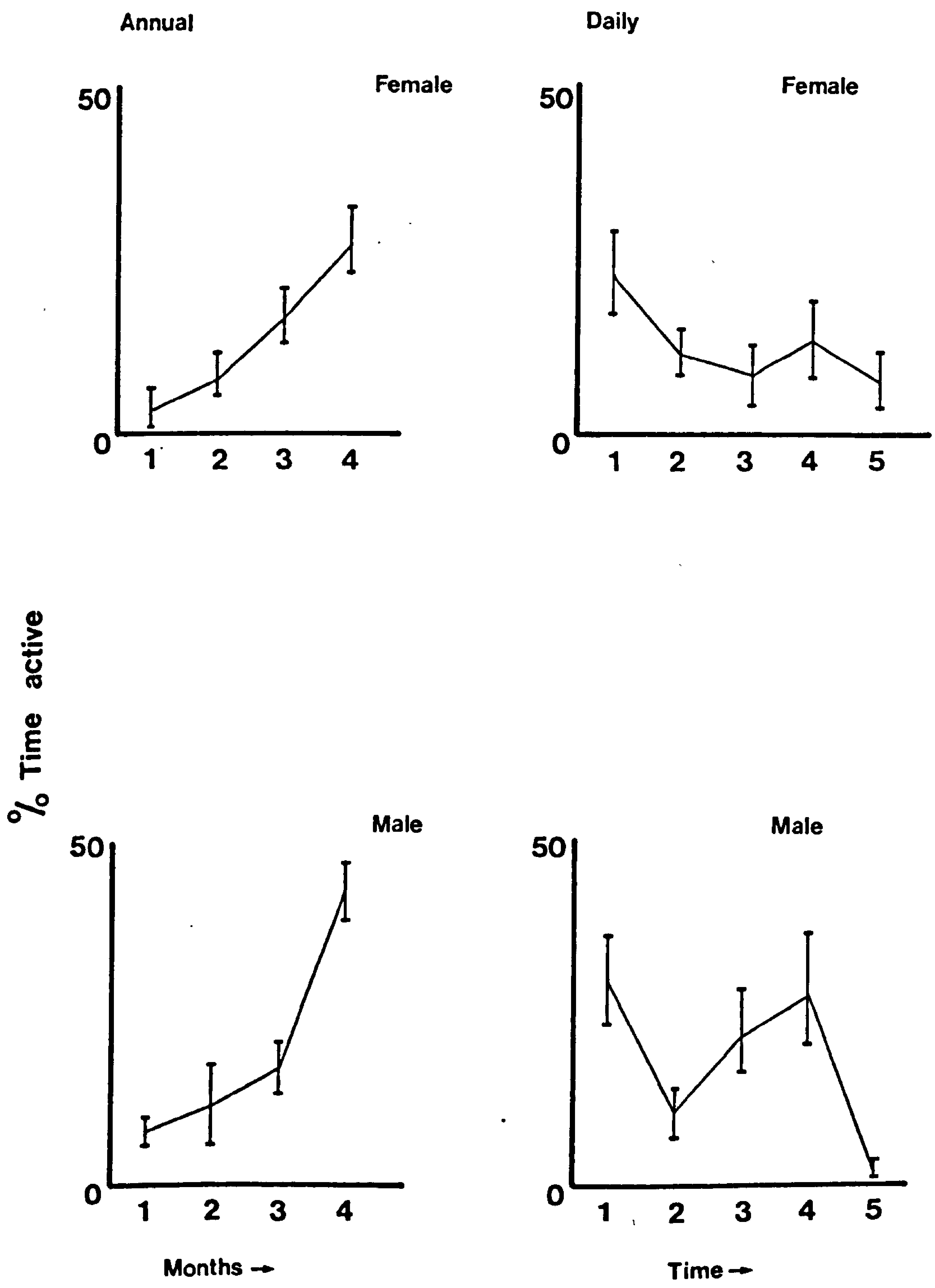


Figure 37

Mean annual and daily activity patterns of the Jersey group is apparent in both groups. However the bimodal pattern of activity persists throughout the year in the Jersey group and is most apparent in the male. If the method of presentation of the results is altered somewhat so that the data for the various months and the various subjects are pooled it is possible to compare the results obtained from this study with some already available in the literature (Pinto et al.,1974)(fig. 38). From this it can be seen that the differences in activity patterns obtained in the two different studies far exceed those within the same study and in addition the tendency to a bimodal activity pattern is particularly marked in Pinto's group.

Further discussion on differences existing in the occurrence of different behaviours between the London and Jersey groups is not included due to the paucity of the Jersey results in some months. However, one difference is felt to be worth noting as it probably reflects a difference in the environmental conditions of the two groups. This is the pattern of nest-box use over the night. In the Jersey group there is a period late in the night when the animals spend a considerable amount of time out of the nest-box. This observation is not as apparent in the observations of the London group.

2. Social Behaviour

The social behaviour in the mouse lemur was studied in two ways. The first was through a series of introduction experiments during which the various inhabitants of the harem cage were allowed access to each other and the second was by direct observation of the interactions occurring in two groups of mouse lemurs, one kept in London and the other on display in the Jersey Zoo. The London group comprised two females and one male, as did the Jersey group initially. However, two months after the commencement of observations, one of the Jersey group females was removed for hospital treatment and was never returned to the group. The

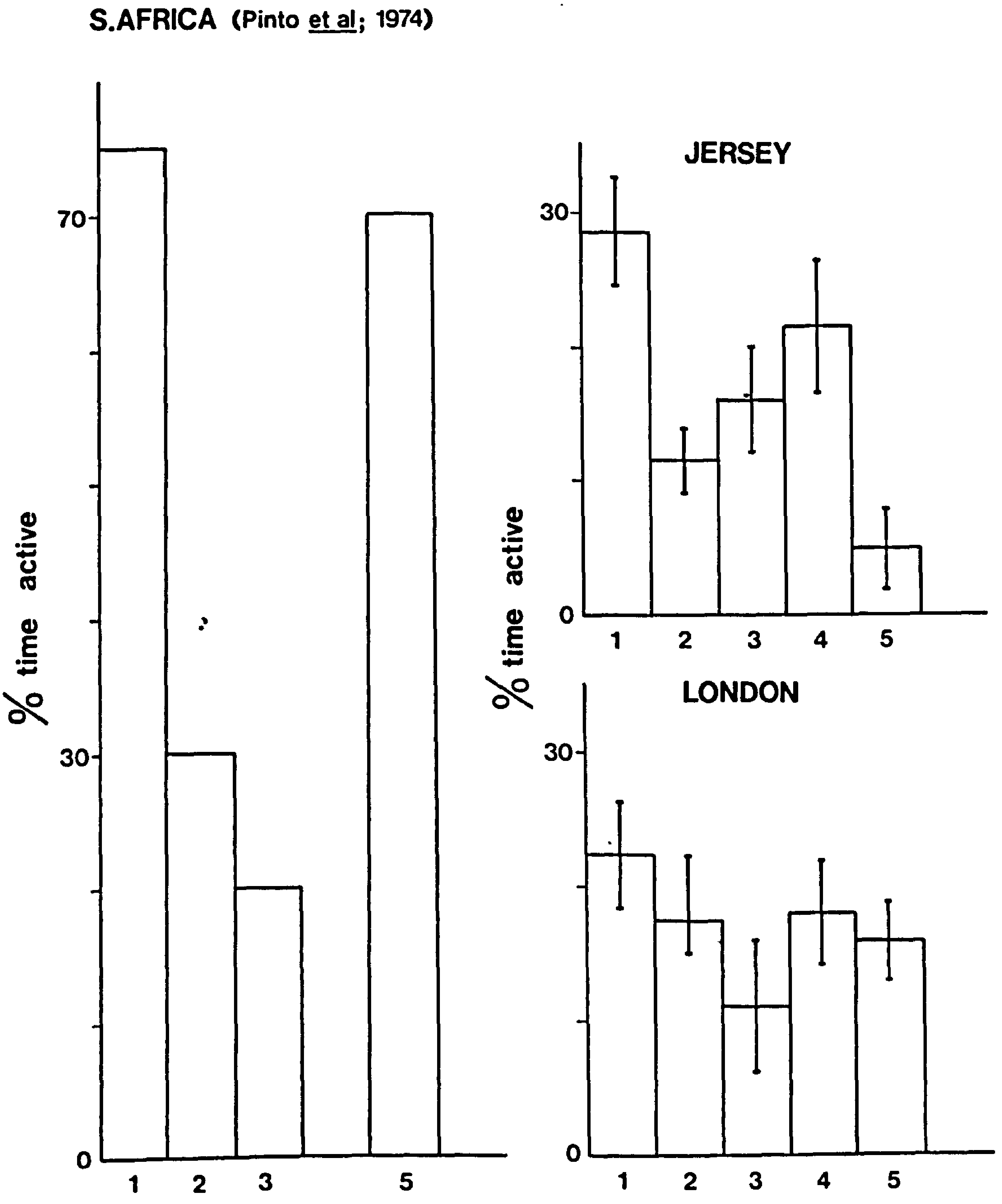


Figure 38

Variation in daily activity pattern between groups

Jersey group had been living together for several years and had been transferred to new accommodation six months prior to the start of the observations. The London group, on the other hand had been introduced to each other and to new accommodation simultaneously one year before observations commenced. One other factor which must be considered in connection with the observations is the fact that at the time of the observations the Jersey group contained a potential breeding male while the London group did not. However, no breeding success was recorded in either group.

The advantages of direct observation of social behaviour in the group situation are apparent. The hierarchical structure of the group and the occurrence of aggression can be recorded and related to reproductive success which was the reason behind instigating the observations on the two groups. Introduction experiments, on the other hand, also give an indication of the relative dominance status of the individuals involved and, in addition, aid in the interpretation of interactions observed in the group situation. In the future they could also help in the interpretation of the behaviour of mouse lemurs in the field. Bearder & Doyle (1969) have reported that behaviour observed during laboratory introductions of Galago senegalensis was similar to that observed during natural encounters in the field.

Before continuing with this chapter a definition of the term "social behaviour" must be established. Two views on the definition of social behaviour persist in the literature. One restricts the term "social" to behaviours exhibited in naturally formed groups containing more than two adults (e.g., the view formerly held by Martin, 1968). This opinion holds that the term "solitary" is the antithesis of "social" rather than "gregarious", a point made by Charles-Dominique (1974, 1977a). Thus, normally, solitary animals are not thought of as forming a community of any kind, an interpretation which ignores the observations that these solitary prosimians sleep together in groups and are apparently familiar with their neighbours (Charles-Dominique, 1974).

The view adopted in this thesis will be that of Charles-Dominique (1977a), that the term "solitary" is the opposite of "gregarious". Thus, the communicative behaviours observed in these animals can be termed "social" behaviours. It must also be stressed that the main reason various animals have been termed solitary seems to be, as Leyhausen (1965) said, "that they can only be shot one at a time". However, there does seem to be a relationship between relative solitariness and nocturnal habits in mammals, a fact noted by many authors (for example, Crook & Gartlan, 1966).

2.1. Introduction Experiments

These introductions involved only two animals in each instance and were implemented during the period of increasing daylength over the course of two years. The females, with the exception of two individuals, were previously unaquainted but the male involved had mated with all the females in previous years.

A total of 22 female/female and 53 male/female introductions were implemented of which 28 of the male/female were quantitative. Fewer all-female introductions were performed as it proved difficult in many instances to make the females leave their own cages. This problem did not arise with the male as he was a tame animal and could be placed in the female's cage to initiate interaction. In addition, the all-female introductions involved so few interactions that it was felt that a descriptive approach was of more value than a quantitative one.

2.1.1. Female-Female Introductions

During most of the female-female introductions performed the more dominant female would enter the cage of the subordinate female. The cage owner would retire to her nest-box or some other part of the cage normally used for resting. The dominant female would then explore the cage thoroughly, sniffing and mouth-wiping (see Chapter VI , section 2.2.) frequently.

At intervals during these introductions, the dominant female would approach the other and sniff her face or nose. If the subordinate female responded to this by giving a threat call, the dominant female would leave immediately. Otherwise a period of allogrooming or nest-box sharing would follow. These bouts were also usually terminated by the subordinate female giving a threat call.

These introductions were usually terminated by the dominant female returning to her cage at her own volition.

Dominance relations between these females did not remain constant throughout the year. Oestrus in the mouse lemurs seems to be associated with an increase in both dominance and aggressiveness (two observations).

Serious fighting between females has only been observed on two occasions. On both of these it occurred in the "neutral territory" of the inter-cage connecting tunnel and involved an oestrous female. The actual fight was preceded by a brief naso-nasal sniff after which the two antagonists leapt at each other and grappled, rolling over on the ground biting and kicking at each other. No vocalisations were given during the course of the fighting, which in one of these cases was so violent that the animals had to be separated to avoid the possibility of any injury.

From the above data it can be seen that considerable variation of response occurs in these introductions.

Observations made during this study indicate that the bond between female mouse lemurs is very durable. On reintroducing one pair of females which had previously been cage mates, recognition was very rapid. On contact there was a brief period of naso-nasal sniffing followed by a long bout of allogrooming. These bouts of grooming could last 30 minutes or longer. The females would then separate to explore each other's cage, meeting occasionally for further bouts of allogrooming or nest-box sharing.

Female companions appeared to recognise each other after a separation of five months and similar recognition has been observed on reintroduction of mother to daughter after a similar period; recognition is here defined not only as the absence of any antagonistic interactions but also as the occurrence of very positive amicable behaviour (30 minutes of social grooming) not observed in other introductions. This indicates the existence of a durable social bond between the females.

2.1.2. Male-Female Introductions (non-oestrous)

The male-female introductions are very different in character to the female-female introductions described above. In all cases it is the female which enters and occupies the male's cage. The male may briefly enter the female's cage during the period of access, but is rapidly chased away by the female in question. On entry, the female proceeds to explore the male's cage in a similar manner to that seen during female-female introductions. However, she frequently ceases her exploratory behaviour to attack and chase the male until he assumes a "cringing" posture on the floor. The female then continues her exploration, but will attack or threaten the male if he moves or gives the male "trill" call (see Chapter VII, section 3.2.).

Occasionally the male will threaten the female or a brief fight may occur, but serious fighting of the kind observed between females was not observed in this situation.

The method of approach of the dominant animal was one of the most obvious differences in behaviour between male-female and female-female introductions. In the all-female situation approaches towards the other animal were slow and cautious, whereas in the male-female situation they were rapid and confident. Figures 39 and 40 sum up the behaviour of males and females in this situation.

2.1.3. Effect of Familiarity

The levels of various behaviours were measured during a series of male-female introductions. The male was then allowed free visual access to all the females for a period of a month - this was accomplished by allowing the male use of the inter-cage connecting tunnel from where he could see and smell the females through wire

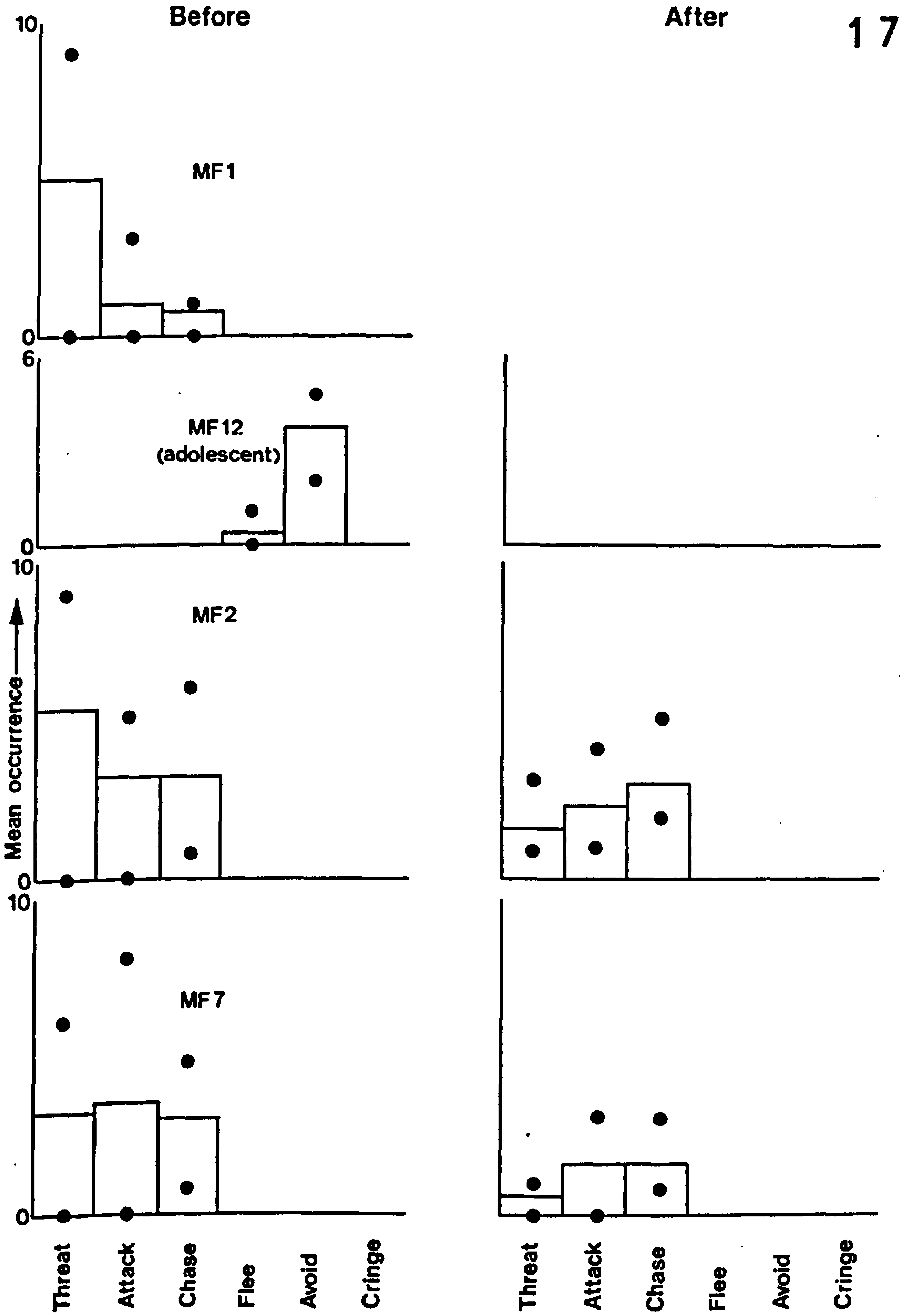


Figure 39

Relative frequency of behaviours in the females during introduction to the male. before and after a period of familiarisation

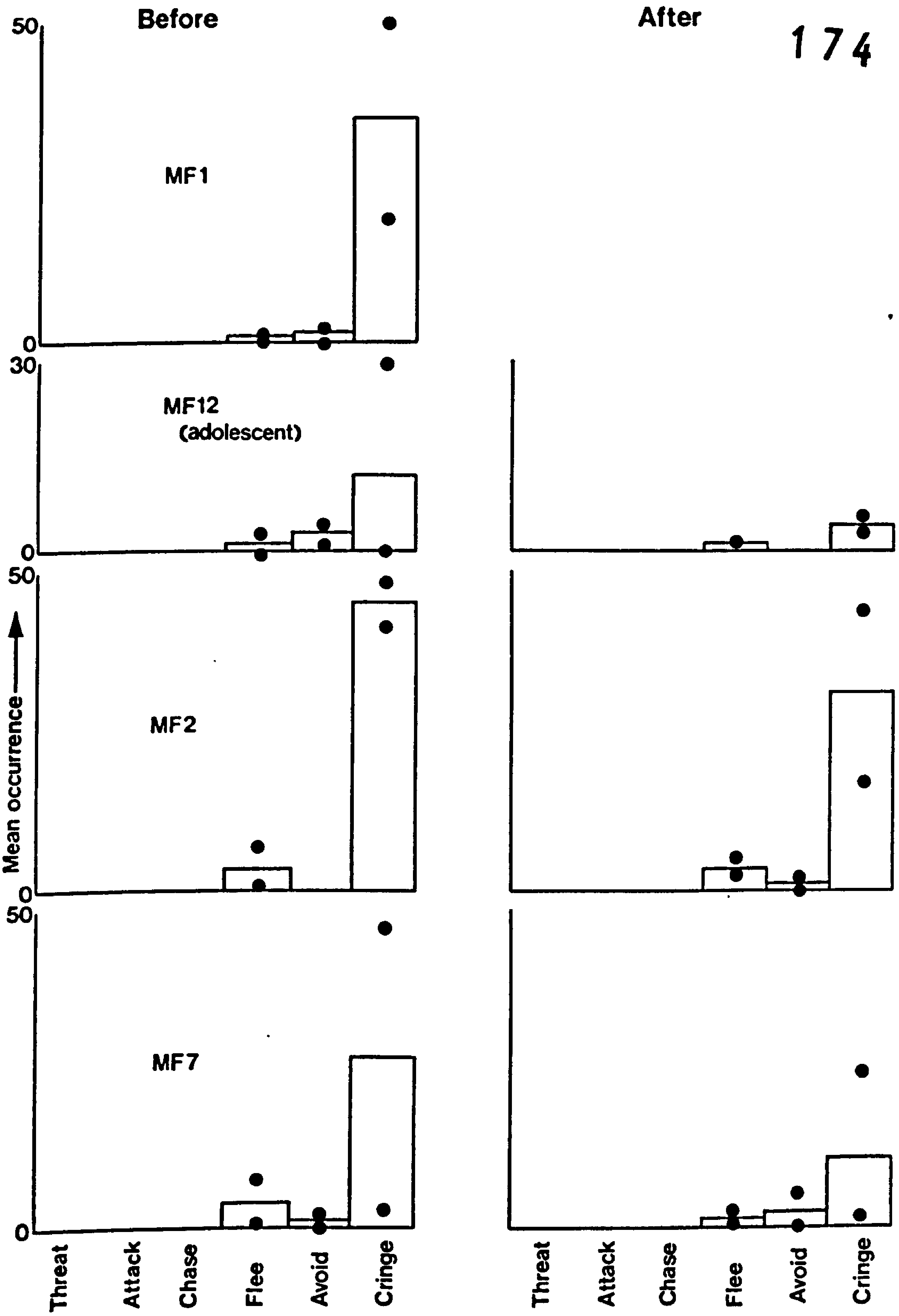


Figure 40

Relative frequency of behaviours in the male during introduction to the females before and after a period of familiarisation grids - after which a second series of introductions were performed. Unfortunately only a few results were obtained, owing to the need to complete the observations during a time when the animals were active (see section 2.1.4. below), but before the onset of the breeding season could affect the results. In addition one of the subject females died before the second series of introductions were implemented. As such the results can only be taken as an indication of the influence of familiarity. Figures 39 & 40 show the occurrence of various aggressive behaviours on the part of the female before and after familiarisation and the occurrence of cringing behaviour in the male. Of the three aggressive behaviours measured in the females, threat, attack and chase, none were reduced by the period of familiarisation (Mann-Whitney U test). However, cringing behaviour in the male was reduced (Mann-Whitney U test, $p = 0.05$).

The decline in cringing behaviour in the male after familiarisation should have stimulated female aggression whereas female aggressive behaviour remained constant or declined. This would indicate that the overall decline in female aggressive tendencies may be greater than is apparent from the data at first sight.

2.1.4. Effect of Daylength Variation

During the course of the study it was noted that introductions were more difficult to effect during the non-breeding season than during the breeding season. In the breeding season females would leave their cages and explore as soon as the exit was opened and would often sit by the divider into the male's cage if this was closed. During the non-breeding season, however, females could rarely be tempted to leave their cages. In 11 out of the 17 introductions attempted in the short daylength period, females would not leave their cages, even to chase an intruding male.

2.1.5. Introduction to an Adolescent Female

When introductions were effected between the male and one young female prior to her first breeding season, the introduction was characterised by none of the "chase" and "cringing" behaviour typical of adult male-female introductions. While the male showed caution towards the female, avoiding her frequently, she only exhibited curiosity and amicable behaviour towards the male. Some periods of allogrooming were observed during these introductions. Generally, the female would dart towards the male and then flee from him or sniff him. However, she spent much of the time peering at the male from other parts of the cage.

When a second series of introductions were implemented a month later much of this behaviour was no longer evident, but had been replaced by the more typical female pattern (fig. 39).

Unfortunately, only one series of introductions has been made involving an adolescent female. This is due to the fact that only one cage set-up was suitable for introductions, the "harem" cage. This unit housed four females of which only one was adolescent at the time of the experiments.

2.2. Group Behaviour

The social interactions occurring in the London and Jersey groups of mouse lemurs were followed throughout the course of one year. The type of behaviours observed in each group and the pattern of their occurrence were then compared in order to obtain a more comprehensive picture of the social behaviour of the mouse lemur in captivity.

2.2.1. Comparison of the groups: similarities

The two groups were similar both in the types of interaction observed and in the number of interactions occurring in each 45 minute observation period. Interaction is defined here as being the sequence of events occurring between the approach of one individual

towards a social companion and the termination of contact by the departure of one of the individuals. The interactions observed in both groups were:-
allogroom, associate, nest-box sharing, sniffing, food-taking, approach-retreat, following, avoidance, threat, and chase.

The number of interactions recorded in the London group was 24.24 ± 7.73 (n = 26) per 45 minute observation period. In Jersey 24.75 ± 1.77 (n = 7) interactions occurred per 45 minute period when three individuals were present in the group. After the removal of the subordinate female the number of interactions dropped to 16.00 ± 4.89 (n = 19). Hence it would seem that a mean interaction rate of 8 interactions / individual / 45-minute period was maintained. The frequency of interaction increases markedly when the females are in oestrus. At the time of oestrus the number of interactions rose to 80 per 45-minute period in the London group and to 42 in the Jersey group (all three animals were present in the latter group at this time). This difference could be due to the absence of tussle interactions in the Jersey group. Tussles are pseudomale interactions only observed in the Observation Room females. The occurrence of tussles increased markedly at oestrus.

Both groups contained a dominant female, which was the heaviest animal in the group, although whether this was the cause or result of dominance is unclear. This female instigated more interactions than the subordinate female and, in the London group, more than the male (table 25). In almost all cases oestrus increased the number of interactions instigated, except in the case of the subordinate female in the Jersey group. This may have been due to the fact that this female was injured and spent most of her time in the nest-box.

After the removal of the subordinate female from the Jersey group, the dominant female initiated more interactions with respect to the male than she had done previously. This is a reflection fo the changing dominance relations between them (see this Chapter, section 2.2.2.).

Table 25

Number of Interactions Instigated by Each Group Member

	Dominant Female	Subordinate Female	Male
London	13.2±3.7(n=26)	7.2±2.85(n=26)	3.8±2.0(n=28)
Both females in oestrus	47.0(n=1)	22.0(n=1)	11.0(n=1)
Jersey	11.9±2.95(n=7)	2.35±1.9(n=7)	10.5±0.7(n=7)
Dominant female in oestrus	18.0(n=1)	3.0(n=1)	20.0(n=1)
Jersey with two animals only (no oestrus)	9.7±2.85(n=19)	-	6.3±2.2(n=19)

The difference in the pattern of instigations between the groups will be discussed in the following section.

2.2.2. Comparison of the Groups: Differences

The most obvious behavioural differences between the groups were in the frequency of certain interactions, the pattern of instigations, the organisation of behaviour as well as individual differences in behavioural repertoire.

The greatest difference in the frequency of various interactions between the two groups was seen in occurrence of aggressive behaviours, allogrooming and nest-box sharing. These differences are illustrated in the relative frequency histograms (fig. 41, 42, 43). The level of aggression can be seen to be much higher in the Jersey group, as is illustrated particularly well by the elevated levels of fighting seen in this group. In addition, the dominant female in the Jersey group participated in more chases than her counterpart in the London group and the subordinate female in the Jersey group demonstrated a greater proportion of submissive behaviours than did her equivalent in London.

The relative frequency of social grooming was lower in the Jersey group than in the London group (fig. 41, 42, 43) and it must also be noted that it appears to be a more predominant behaviour within the males' repertoire than within that of the females. Conversely, the occurrence of nest-box sharing was greater in the Jersey group. All group members slept together during the "day" and nest-box sharing can be seen from the relative frequency histograms (fig.41, 42, 43) to be the most frequent category of amicable behaviour. On the other hand, none of the individuals in the London group slept together during the light phase and throughout the "night" this activity was of both infrequent occurrence and brief duration.

A further difference in interaction observed between the two groups was in the occurrence of food taking. Food taking was a pattern of social behaviour seen quite frequently in the London

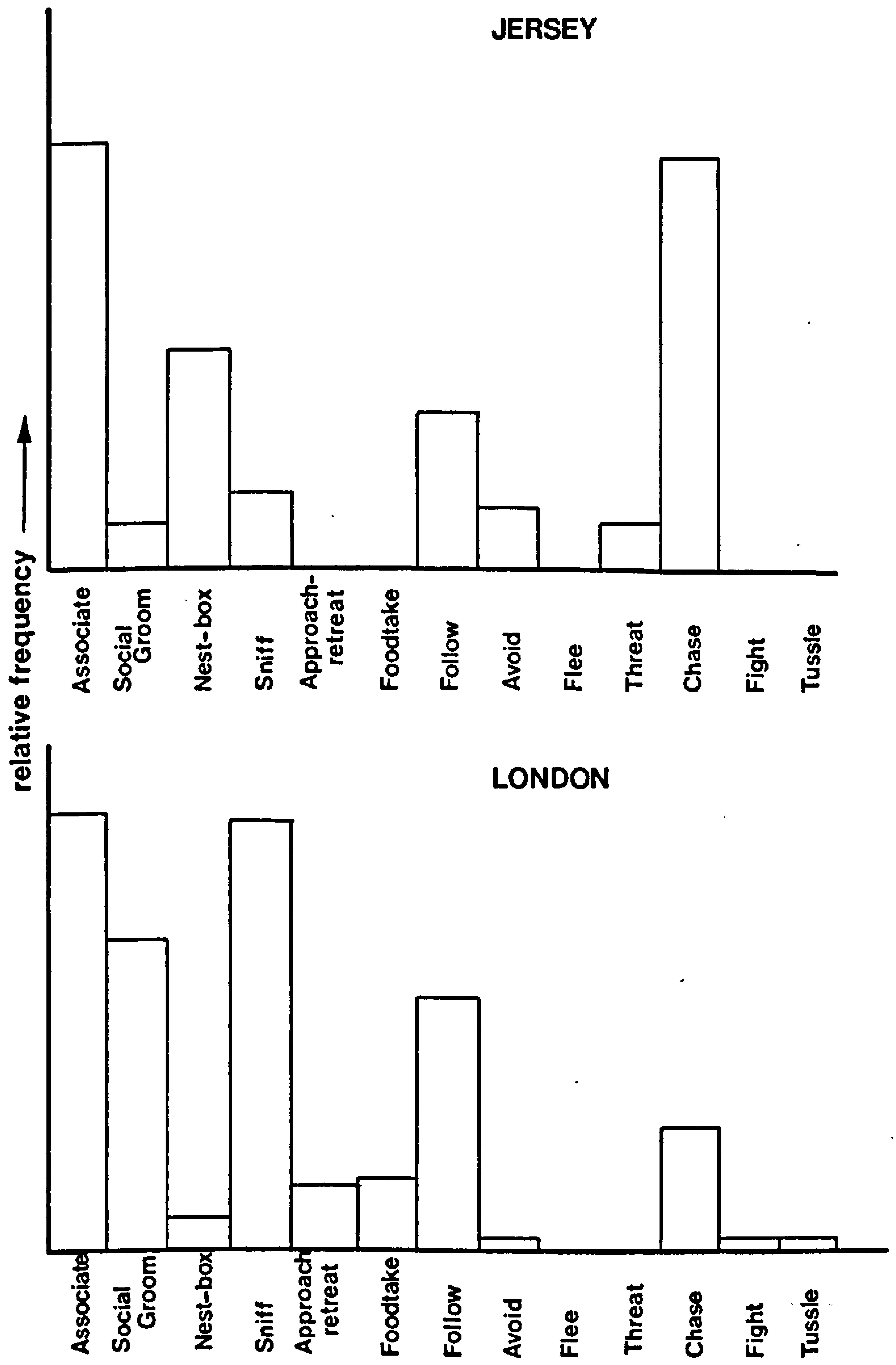


Figure 41

Relative frequency of behaviours in dominant females

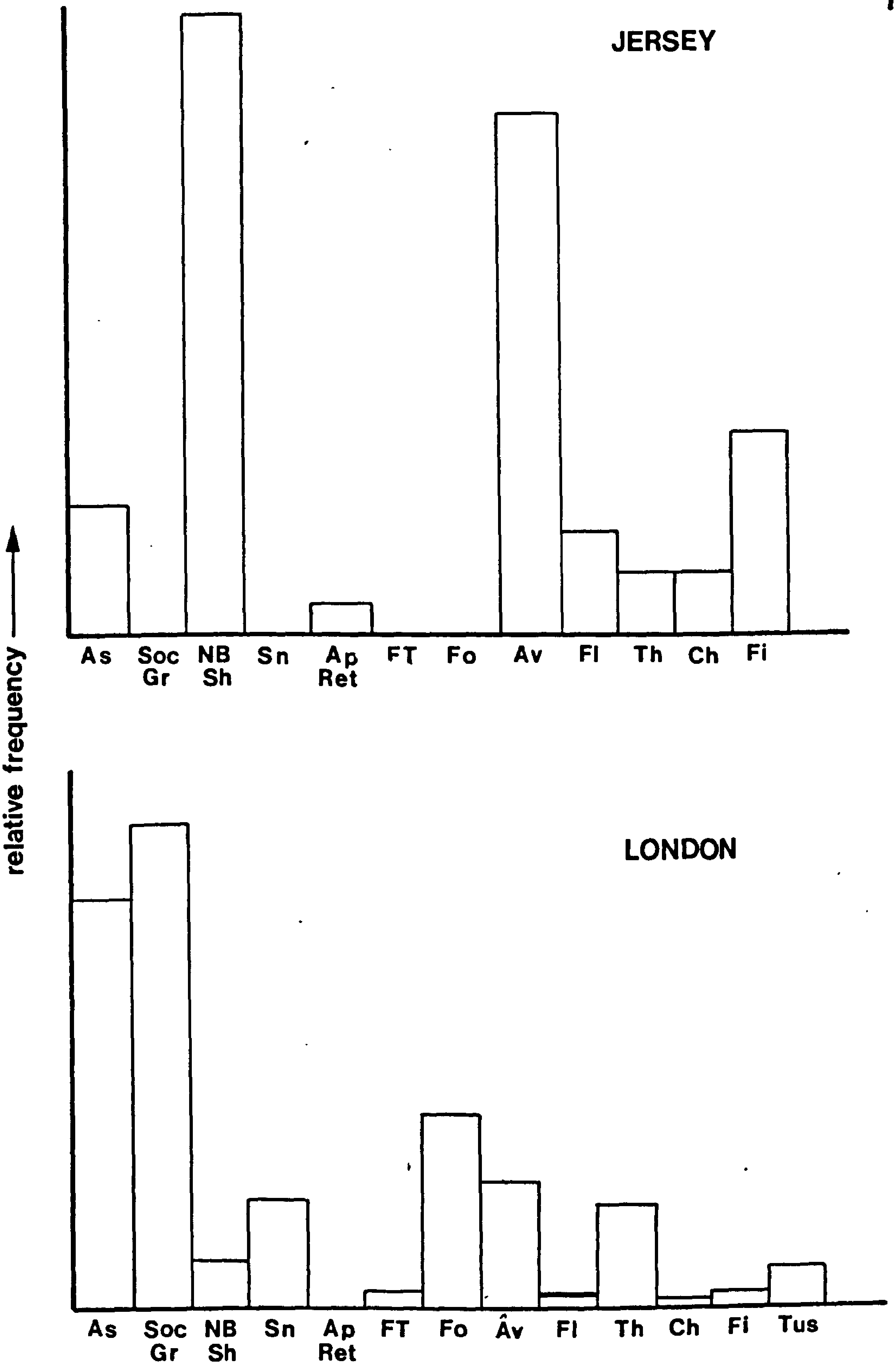


Figure 42

Relative frequency of behaviours in subordinate females

Abbreviations, see figure 41.

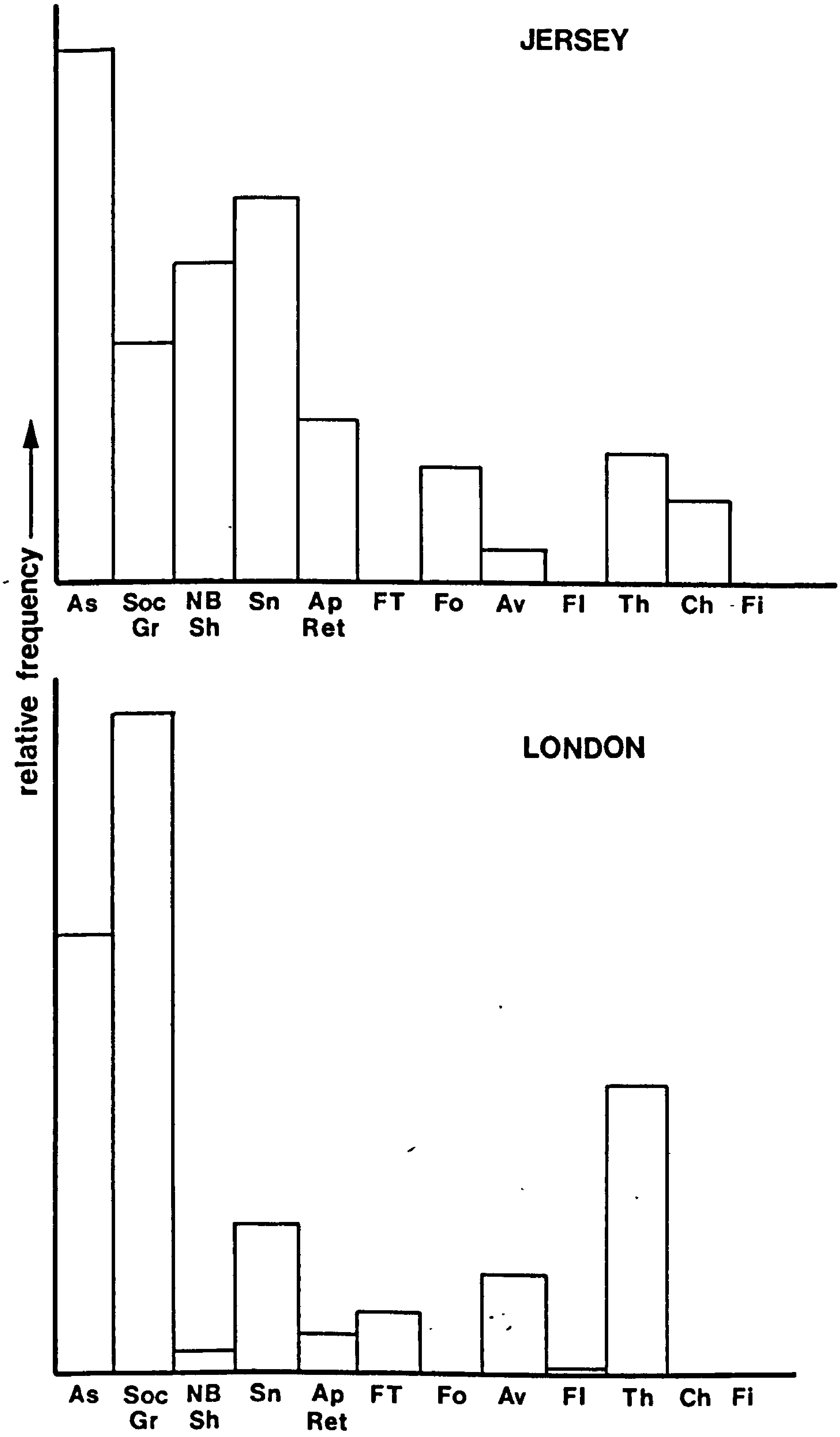


Figure 43

Relative frequency of behaviours in the males

Abbreviations, see figure 41.

group but which was rarely observed in the Jersey Group prior to the removal of the subordinate female (fig. 41, 42, 43, 44).

From table 25 in the previous section it can be seen that there is a difference in the pattern of instigation of interaction between the two groups. Whilst in both groups it is the dominant female which instigated most interactions, the proportion of interactions instigated by the remaining group members varies between the two groups. In the London group it was the male which instigated the least interactions whereas in the Jersey group it was the subordinate female.

The relationship between sequences of behaviour in the two groups was investigated using single link cluster analysis. From the dendrogram of behaviour in the London group (fig. 45) it can be seen that one cluster of behaviours dominates the whole pattern and that is the follow, sniff, nest-box share, avoid, associate, chase group. These behaviours together with tussling (see below) form the basis of the pseudomale interactions of the females in this group. A period of sniffing and following could be followed by tussling and/or a period of association or nest-box sharing. However, when the dominant female tried to avoid indulging in this behaviour a chase would ensue or the behaviour pattern would be diverted to a period of association.

In the Jersey group (fig. 46) there are four clusters of associated behaviour patterns: avoid and fight which represent the most aggressive behaviour pattern; sniff, associate and mount, which represent the sexual behaviour pattern; food-take and follow, which is a usual food-taking pattern, and chase and nest-box share which represents a frequently observed behavioural pattern between dominant and subordinate animals in which the dominant individual chases the subordinate into a nest-box and then proceeds to share the nest-box with it.

A further dendrogram was made for the behaviour of the Jersey Group after the removal of the subordinate female. As can be seen from figure 47, the removal of this animal had

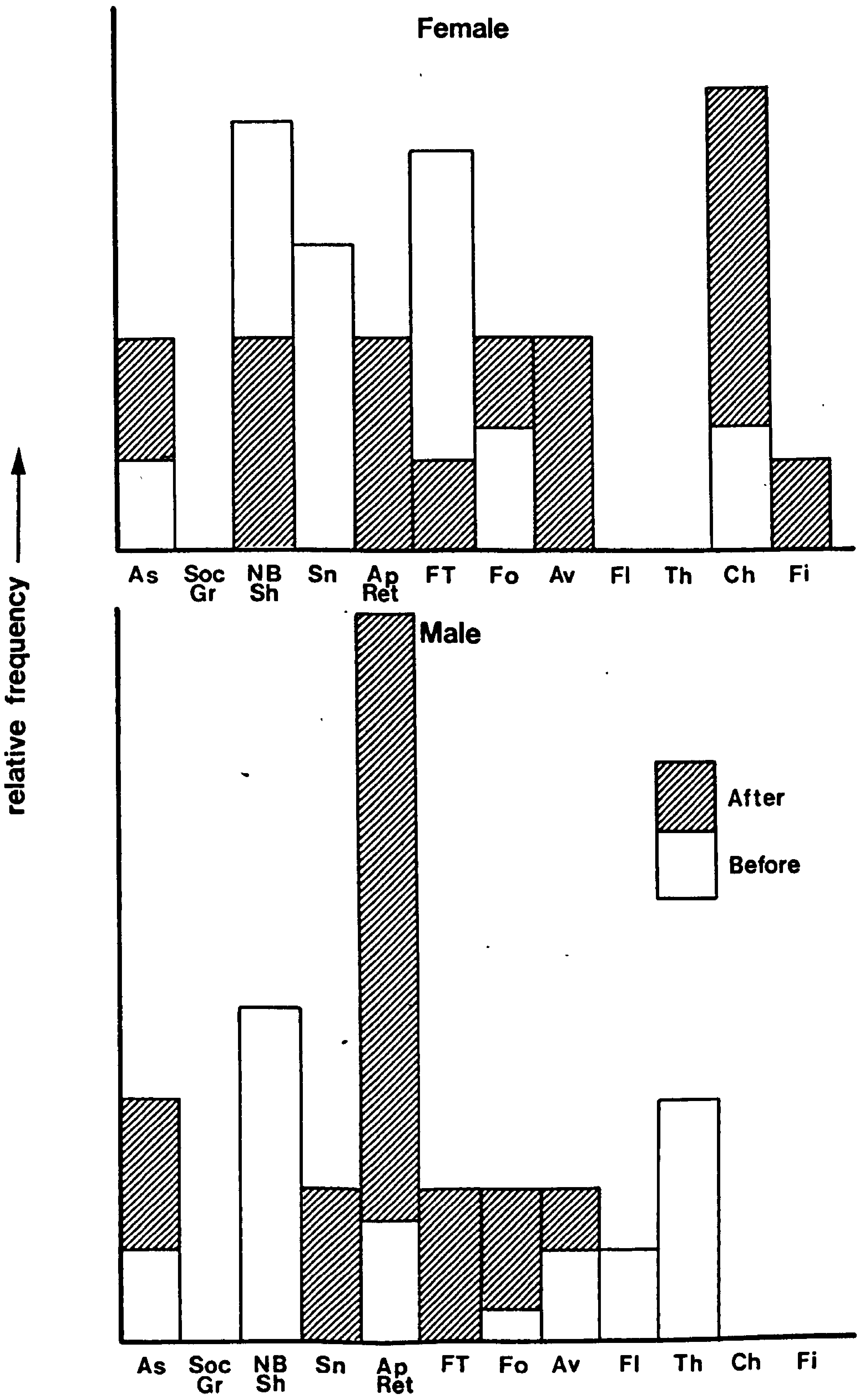


Figure 44

Change in relative frequency of behaviours in the dominant female and the male after the removal of the subordinate female

Abbreviations, see figure 41.

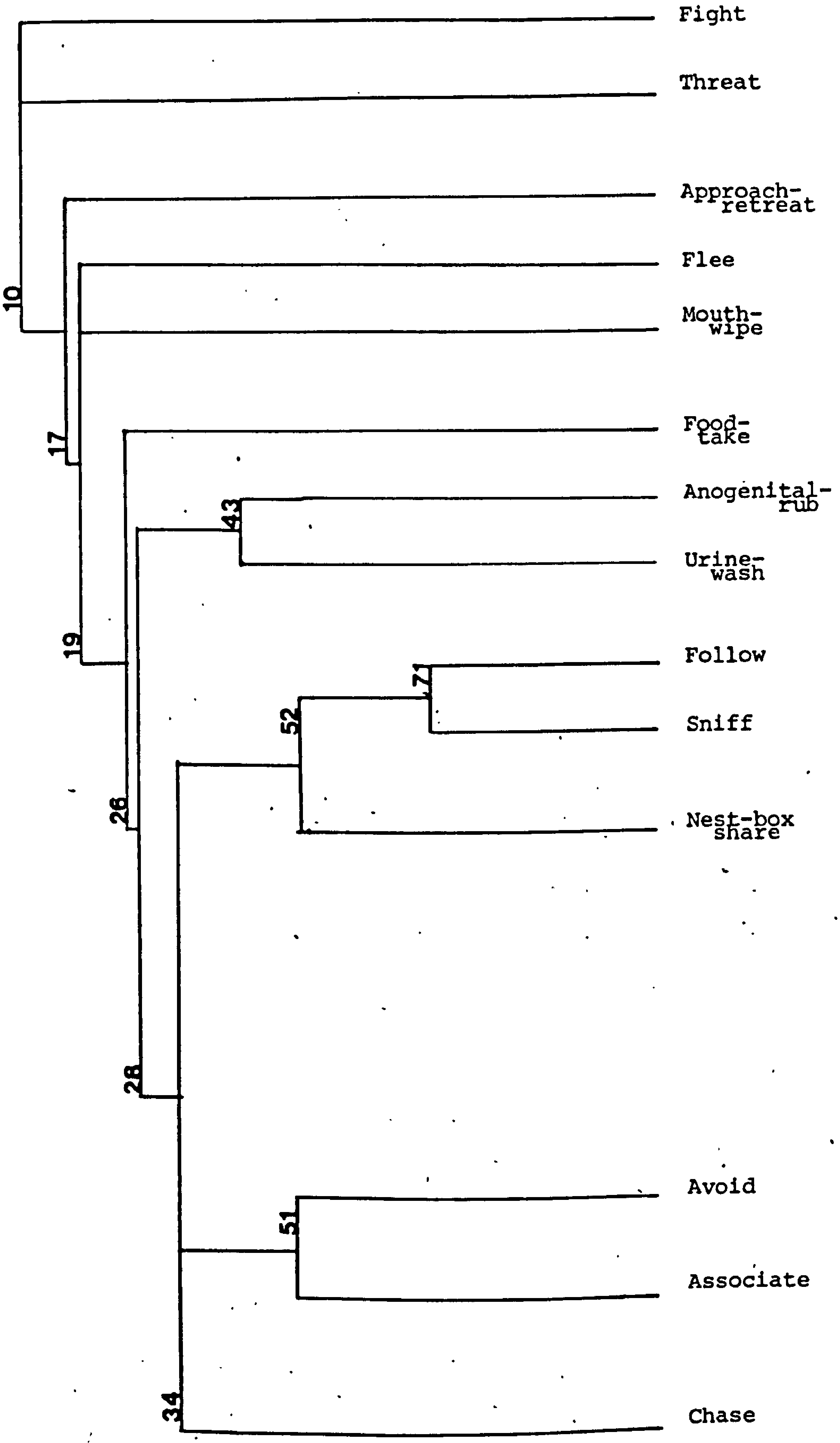


Figure 45

Dendrogram of social behaviour in the Observation Room group

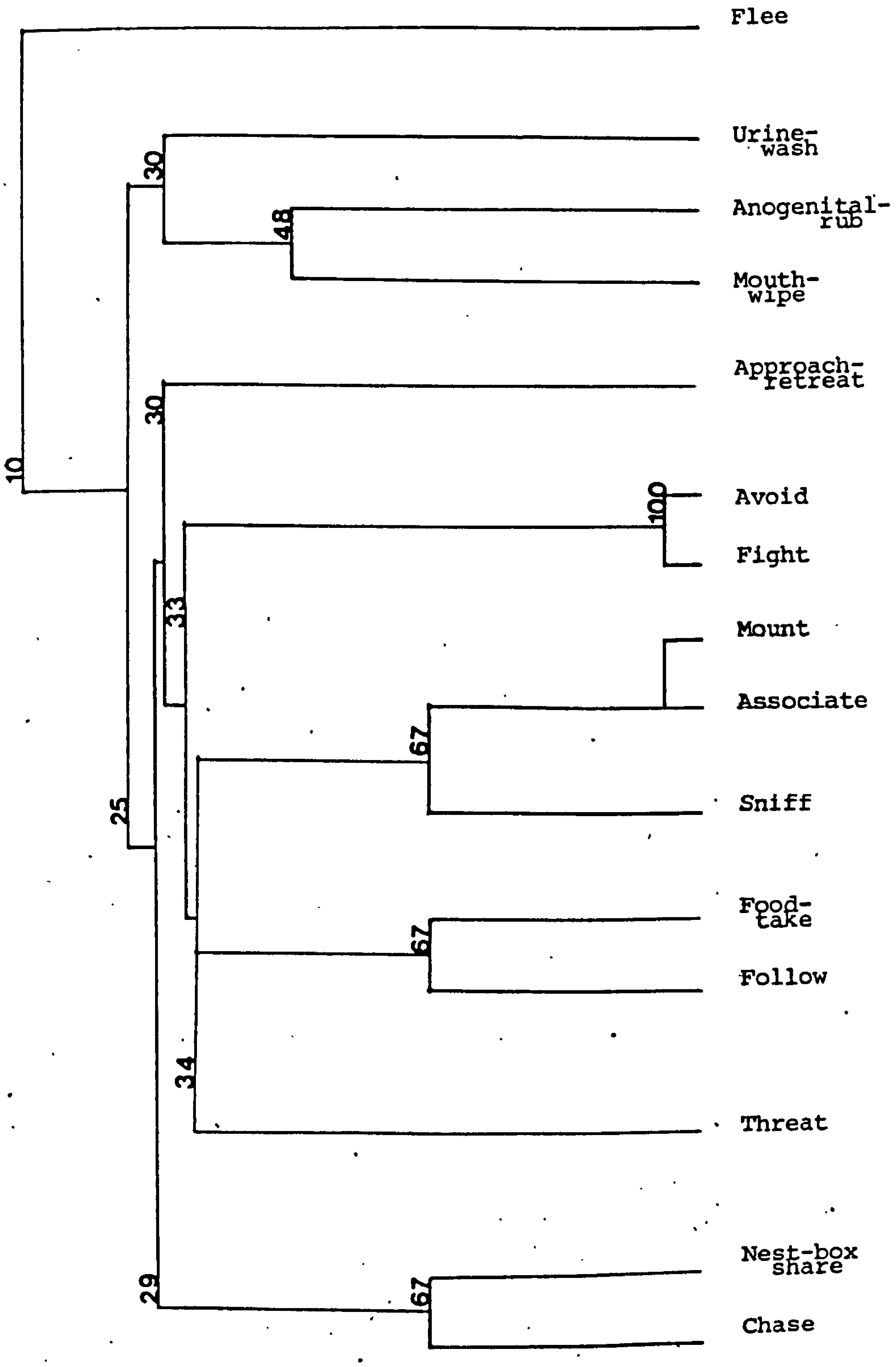


Figure 46

Dendrogram of social behaviour in the Jersey group before the removal of MFA

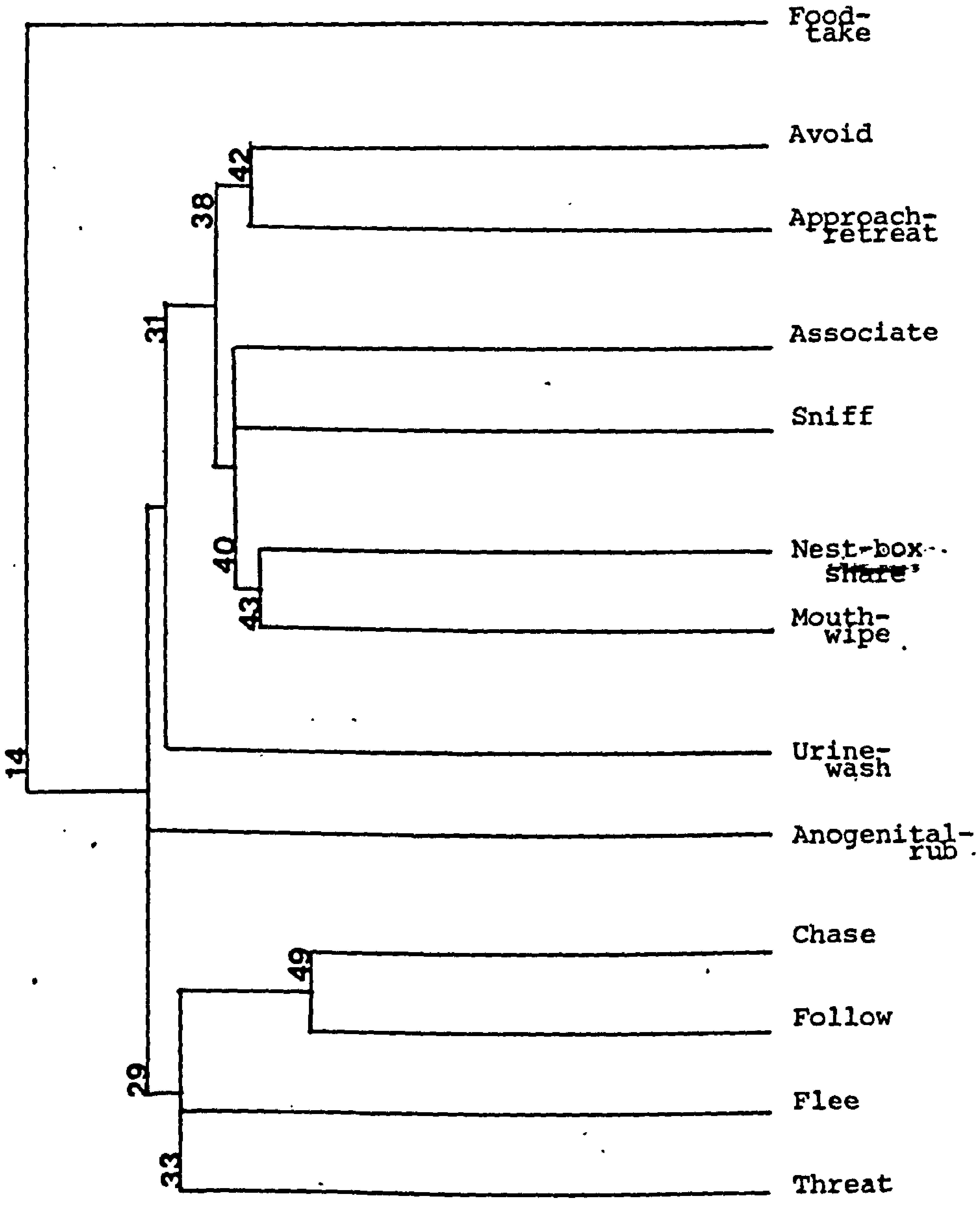


Figure 47

Dendrogram of social behaviour in the Jersey group after the removal of MFA repercussions on the pattern of interactions. The clusters are not as tightly linked which indicates a greater flexibility in behavioural sequences. Two clusters of behaviours are apparent: one aggressive, consisting of threat, flee, follow and chase, and the other amicable,consisting of associate, sniff, nest-box share and mouth-wiping. (Marking behaviours were included in these analyses to see how they linked to other behaviour patterns).

The matrices, and thus the above dendrograms, were then compared mathematically using the method devised by Jenkinson (1975). Comparison of the behavioural organisation of the mouse lemur groups using this method indicates that there is no significant continuity in the organisation of behaviour in the different situations (table 26).

The most notable difference in individual behavioural repertoire was apparent in the pseudomale behaviour observed in the London group, particularly in the occurrence of tussle behaviour which was not observed elsewhere. In addition to this there were differences in the behavioural repertoire of the two males. The London male was involved in a greater proportion of social grooming activities than the Jersey male and also threatened other individuals more frequently (fig. 43). The Jersey male was involved in more approach-retreats, sniffs and follows and relatively more associations than the London male; sniff and association can be seen from the dendrogram (fig. 46) to be closely linked to sexual behaviour (mount links to associate at 100%). Also a great disparity in marking behaviours was noted between the males. This will be discussed in the following chapter (Chapter VI, section 2.5.).

2.2.3. Behavioural Changes within the Group

After the removal of the subordinate female from the Jersey group, the relationship between the two remaining animals changed. The female became more dominant relative to the male, initiating most of the interactions (see table 26) and frequently chasing the male.

Table 26

Comparison of the dendrograms of the Observation Room and Jersey

Observation Room Ranked Matrix

	Sn	As	Nb	Ft	Fo	Ch	Th	Fl	Av	Ap	C	M	U	A
Sniff		6.5	3	9	1	6.5	13	10.5	6.5	12	3	10.5	8	8
Assoc.			6.5	9	6.5	6.5	13	10.5	4	12	6.5	10.5	8	8
Nest sh.				9	3	6.5	13	10.5	6.5	12	2	10.5	8	8
Food Take					9	9	13	10.5	9	12	9	10.5	9	9
Follow						6.5	13	10.5	6.5	12	3	10.5	8	8
Chase							13	10.5	6.5	12	6.5	10.5	8	8
Threat								13	13	13	13	13	13	13
Flee									10.5	12	10.5	10.5	10.5	10.5
Avoid										12	6.5	10.5	8	8
Ap.-Ret.											12	12	12	12
Call												10.5	8	8
M.Wipe													10.5	10.5
U.Wash.														5
A.Rub.														

Jersey Ranked Matrix

	Sn	As	Nb	Ft	Fo	Ch	Th	Fl	Av	Ap	C	M	U	A
Sniff		2	11	6.5	6.5	11	6.5	13	8	9.5	6.5	12	12	12
Assoc.			11	6.5	6.5	11	6.5	13	8	9.5	6.5	12	12	12
Nest sh.				11	11	2	11	13	11	11	11	12	12	12
Food Take					2	11	6.5	13	8	9.5	6.5	12	12	12
Follow						11	6.5	13	8	9.5	6.5	12	12	12
Chase							11	13	11	11	11	12	12	12
Threat								13	8	9.5	5	12	12	12
Flee									13	13	13	13	13	13
Avoid										9.5	8	12	12	12
Ap.-Ret.											9.5	12	12	12
Call												12	12	12
M.Wipe													9.5	4
U.Wash.														9.5
A.Rub.														

$$d' = \frac{283}{1040} = 0.272$$

This indicates that the dendrograms are not significantly similar, ($p \leqslant 0.05$).

Table 26 Cont'd

Comparing Dendrograms of the Jersey Group before and after the Removal of MFA (Subordinate Female)

Matrix with MFA

	Sn	As	Nb	Ft	Fo	Ch	Th	Fl	Av	Ap	M	U	A
Sniff		2	10	5.5	5.5	10	5.5	10	7	8.5	11	11	11
Assoc.			10	5.5	5.5	10	5.5	10	7	8.5	11	11	11
Nest sh.				10	10	2	10	12	10	10	11	11	11
Food Take					2	10	5.5	12	7	8.5	11	11	11
Follow						10	5.5	12	7	8.5	11	11	11
Chase							10	12	10	10	11	11	11
Threat								12	7	8.5	11	11	11
Flee									12	12	12	12	12
Avoid										8.5	11	11	11
Ap.-Ret.											11	11	11
M.Wipe												8.5	4
U.Wash.													8.5
A.Rub.													

Matrix without MFA

	Sn	As	Nb	Ft	Fo	Ch	Th	Fl	Av	Ap	M	U	A
Sniff		2	4.5	12	10.5	10.5	10.5	10.5	6	6	6	4.5	10.5
Assoc.			4.5	12	10.5	10.5	10.5	10.5	6	6	6	4.5	10.5
Nest Sh.				12	10.5	10.5	10.5	10.5	6	6	6	4.5	10.5
Food Take					12	12	12	12	12	12	12	12	12
Follow						1	7.5	7.5	10.5	10.5	10.5	10.5	10.5
Chase							7.5	7.5	10.5	10.5	10.5	10.5	10.5
Threat								7.5	10.5	10.5	10.5	10.5	10.5
Flee									10.5	10.5	10.5	10.5	10.5
Avoid										3	6	9	10.5
Ap.-Ret.											6	9	10.5
M.Wipe												9	10.5
U.Wash.													10.5
A.Rub.													

$$d' = \frac{210.5}{819} = 0.26$$

This similarity is not significant ($p < 0.05$).

The male in turn exhibited a higher frequency of submissive activities (fig. 44).

During the course of the study there was an apparent change in the behaviour of the animals in the London group. This change involved an apparent increase in aggressive behaviour and an associated decline in amicable behaviour (see fig. 48). Although these changes were not found to be significant at the level of probability accepted in this thesis (Mann-Whitney U test), they were self-evident to the observer. One of the causes of the change appeared to be the decreased inclination on the part of MF6 to engage in genital sniff and tussle behaviours (see 2.2.2. above and Chapter VIII, section 2.6.). When the dominant female tried to solicit these behaviours, chase and threat behaviours would ensue.

2.3. Discussion

The behaviour of the mouse lemur in captivity is an integral part of this study. As mentioned in the introduction to this chapter, very little has been published about the behaviour of this species. As such this work is of use both in future field studies of the mouse lemur and in considering the optimum captive environment of these animals. Beyond this, behavioural data may be used in diagnosing problems in the animals or, alternatively be linked to the causes of some of these problems. In the former case, behavioural changes may be important in diagnosing sickness,and the role which observation of feeding frequency may play in the recognition of adrenal activation and hence stress has been indicated earlier (Chapter III, section 3.). However, as mentioned above social behaviour may also play a role in suppressing reproduction and in the aetiology of disease. The occurrence of aggressive behaviour in the group situation,and the social status of each individual in either group,or individually-housed conditions is particularly important in this respect. However, as activity patterns have

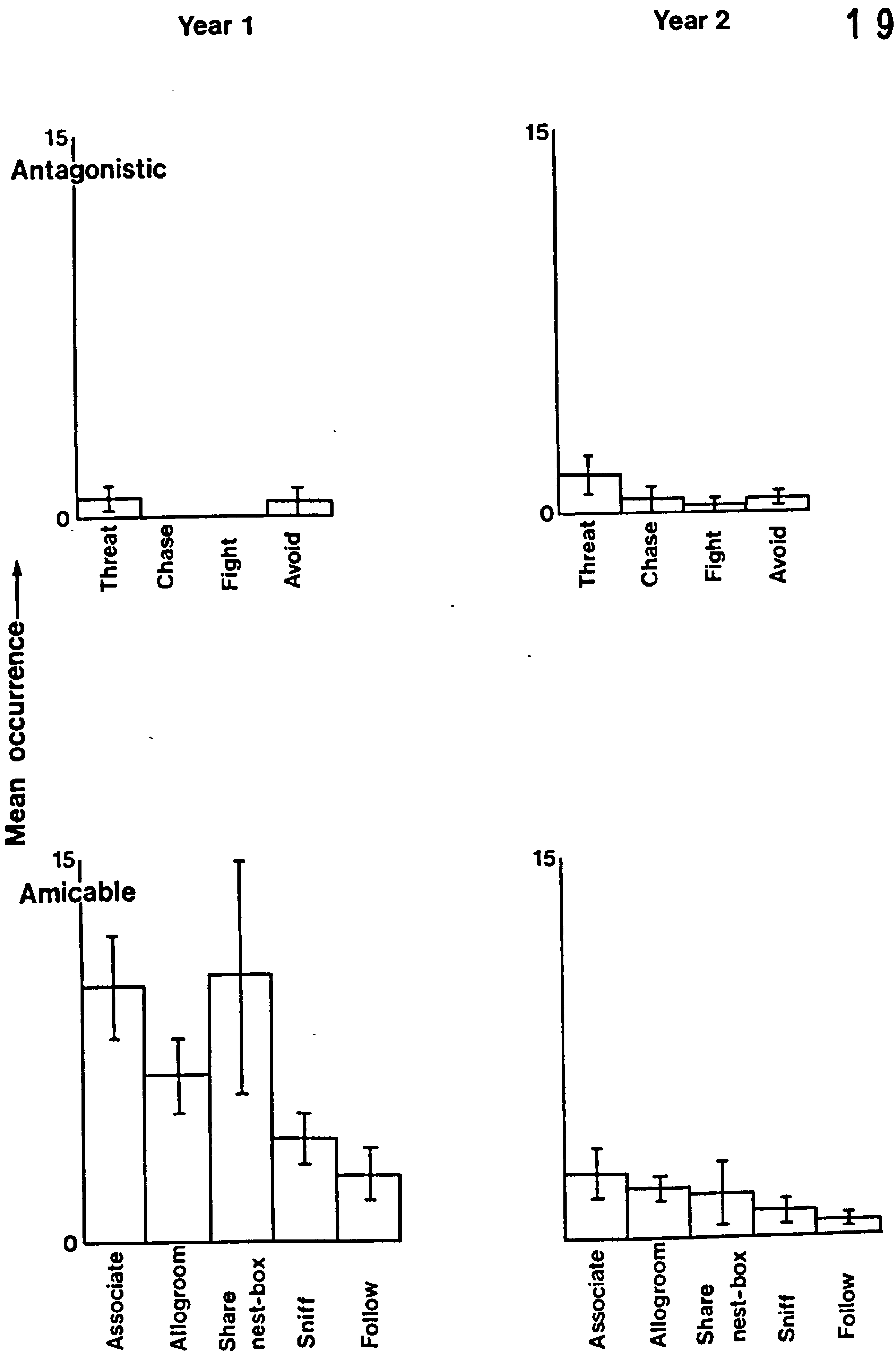


Figure 48

Changes in behaviour of the Observation Room animals considerable influence on the occurrence of social behaviour, the effects of time of day or season must be fully understood before any inferences can be drawn from the social behaviour observations.

The results presented in this chapter have been divided into two sections; activity patterns and social behaviour. For ease of presentation this division will be maintained in this section. Before moving on to the consideration of activity patterns in the mouse lemur some mention must be made of the daylight activity observed in these animals. Similar observations have been made in other nocturnal species and Charles-Dominique (1977a) suggested that daytime activities in captivity, at least in the case of *Galago demidovii* and *Arctocebus calabarensis*, are due to a lack of insect food in their diet. Andriantsiferana & Rahandra (1973a) report an increased intake of insect food in the diet of captive *Microcebus* in Madagascar during the summer months. Therefore, the observation of increased daylight activity of the study mouse lemurs in the summer may be due to an inadequate supply of insect food at this time of year. Bearder & Doyle (1969) report observations of daylight activity in *Galago senegalensis* in the field, but these observations were made in the winter months and the animals were apparently seeking warmer sleeping sites.

In the consideration of activity patterns there are four prime sources of variability: time of the day, month of the year, subject and the environment. A biphasic pattern of activity has been reported in several nocturnal prosimians: an intense period of activity is observed for one or two hours after sunset, this is followed by a rest period and a second burst of activity prior to settling to sleep at dawn. This pattern has been reported in *Galago demidovii* under natural conditions (Vincent, 1969, cited in Doyle, 1974) and in *Galago crassicaudatus* and *Microcebus murinus* in captivity (Pinto *et al.*, 1974). Conversely, in his field study of the mouse lemur, Martin (1972a) reported that there was no obvious indication of a biphasic activity pattern. The results obtained in this study show that the activity pattern of the mouse lemur in captivity does not conform to the simple biphasic one reported by Pinto nor do the field observations of Martin appear to be confirmed. Daily activity pattern varies over the course of the year and appears to be only truly biphasic during the breeding season. This situation is very reminiscent of that in the olympic marmot, Marmota olympus, in which there is also an annual variation in daily activity pattern with a biphasic one emerging only in the breeding season (Barasch, 1973).

In addition to its influence on daily activity pattern, photoperiod, as represented by the influence of the month observation, also influences the total level of activity of the individual. All individuals are most active in the month after the longest day where increased activity is in part a reflection of the increased levels of feeding behaviour recorded at this time (Chapter III, section 3.). It might be argued that this increase in activity is not in fact real but rather is a function of the difference in duration of the "night". During the "summer" the night and hence the active period of the mouse lemur is shorter. One might therefore assume that the elevated levels of activity recorded at this time were due to the compression of the "winter" level of activity into the shorter summer night. However, this would not appear to be the case. The elevation in activity levels recorded are real. In addition the activity recorded in month IV is higher than that in month III although the duration of the red-light period is the same in both cases, 11 hours.

Individual variation in activity pattern was also very marked. In addition to intrinsic differences between individuals, some variation in activity could be related to sex or to social status. In both the London and Jersey groups for example, activity was lower in the subordinate female. There is also a difference in activity pattern between males and females. Although there are obviously differences between the activity patterns of the females, which are in part a reflection of social status, there is a distinct difference between these patterns and those of the male, both over the course of the day and the year. The difference in daily pattern is related to the fact that the propensity toward a bimodal activity pattern is greatest in the female. Thus, the male-female difference is greatest towards the end of the active period (times 4 and 5). In the case of the annual activity pattern the difference is greatest in the non-breeding season (month II) and is probably a reflection of the marked inactivity observed in the fat, adult females at this time (Chapter III, section 3.). Conversely, the difference is least marked in month IV, when all individuals are active feeding (Chapter III, section 3.).

Various environmental factors also have an influence on activity as reflected in the differences in activity observed in the different groups. In the Jersey group for example, there is a greater tendency towards a bimodal activity pattern than in the group housed in the study colony. This is probably related to the Zoo environment where, during the first three observation times (1 - 3), the animals are exposed to disturbance from the general public and from diurnal animals housed in the vicinity. This may have led to the observed increase in activity in the undisturbed periods, times 4 and 5.

Temperature and humidity are also important environmental factors to be considered in this respect. Pinto et al.(1974) reported that high temperature and humidity effectively lower the level of activity and Petter (1962, 1965) has reported that low temperature can induce torpor in the mouse lemur. In addition, Martin (1972a) has cited a paper by Starmühlner (1960) which indicates that this torpor can be reversed by warmth. Daily fluctuation in temperature is another pertinent factor. Perret (1975) has reported that mouse lemurs maintained for any length of time at constant temperature are prone to develop hypothyroidism (see Chapter IX, section 2.5.1.). This condition may well affect activity levels. Lowered activity levels are recorded in the mouse lemur during the short daylength period when a natural reduction in thyroid activity occurs (see Chapter III, section 3.).

The differences in total activity discussed above are obviously reflected in similar patterns and differences in specific activities as these are constituent parts of activity as a whole. As such, only variations in those behaviours which are of particular interest or which run contrary to the pattern as a whole will be included here.

Chief amongst these are the frequencies of social grooming and proximity. These two behaviours occur more frequently when total activity levels are lower, that is during the winter months (I and II) and during the lull of activity in the middle of the night (times 2 and 3). Social grooming also shows considerable variation between the groups. For example, the frequency recorded in the study colony group was much higher than that reported by Pinto et al.(1974), and Andrew (1964) has claimed never to have observed allogrooming in mouse lemurs. This difference in occurrence of social grooming is of importance when considering the level of aggressive behaviour observed in a group and thus will be discussed in greater detail later in this section.

The frequency of occurrence of both feeding and marking behaviour over the day is of interest. Both these behaviours were observed to occur most frequently within one hour of waking (that is during observation time 1). In the case of marking behaviour this is probably a reflection of the need on the part of the animal to urinate on waking which is translated into urine marking. This relationship between urine marking and the natural occurrence of urination has been noted by Andrew & Klopfer, (1974).

The daily pattern of feeding behaviour, which varies very little over the year despite the great variation in total levels of feeding over the year, agrees with that found by Pinto et al. (1974). A peak of feeding observed shortly after waking has been reported in other primates: for example, in *Galago senegalensis* and *Galago crassicaudatus* in captivity (Pinto et al., 1974) and in *Galago demidovii* (Charles-Dominique, 1977a), *Macaca mulatta* (Southwick *et al.*, 1965), *Pan troglodytes* (Goodall, 1965) and *Gorilla gorilla* (Schaller, 1965) in the field. Adrenal activity, as discussed earlier (Chapter III, section 3.), is associated with feeding level. This feeding activity shortly after waking is also probably associated with adrenal activity. Many species are known to have prewaking surges of ACTH and hence of adrenal activity; these include the rhesus monkey, *Macaca mulatta* (Setchell *et al.*, 1975) the rat (Feder *et al.*, 1971) and man (Wurtman, 1975).

The difficulty encountered in trying to effect introductions during the short daylength period is probably associated with the general lowered activity recorded at this time. Not only were the females usually asleep, which itself hindered introductions, but even when they were wakeful they were imperturbable. Even the introduction of the male into their home cage, an event which at other times would have elicited vigorous threats and chases, at this time evoked only one or two threats at most. This apathy on the part of the adult females is probably related to the low levels of TSH and ACTH found in mouse lemurs at this time. The low adrenal activity resulting from the lowered levels of ACTH probably led to this lack of aggression. Under natural conditions, where food is less available at this time, it would be a disadvantage to these animals to waste energy in unnecessary aggressive encounters.

Before going on to discuss the results presented in this chapter, a few words must be said on the natural social organisation of the mouse lemur. Mouse lemurs have been reported as being solitary under natural conditions. Petter (1965) reported that *Microcebus murinus*, *Phaner furcifer*, and *Cheirogaleus major* were generally encountered alone. However, Martin (1972a) reported that female mouse lemurs slept together in groups. Males were rarely found nesting with females but were found either sleeping alone or in pairs. On waking all groups of animals would disperse to feed separately. Similar social organisation is found in other nocturnal prosimians, e.g., Galago demidovii and Galago alleni (Charles-Dominique, 1977a).

Social behaviour was studied in introduction situations and group observations. The most notable fact emerging from these studies is the occurrence of female dominance. Dominance in these observations was determined from the ability of one individual to displace another, to steal its food and to enter the other's home cage with impunity. Competitive food tests were not used to assess dominance and have been reported as unreliable in another prosimian, Galago crassicaudatus (Drews, 1973), presumably because these animals do not compete for food under natural circumstances. Urine washing frequency in mouse lemurs is also not a good guide to dominance, for although it correlates with dominance in the group situation, in individually-housed animals other factors appear to be involved (see Chapter VI, section 2.5.).

Female dominance is not unknown in prosimians. Jolly (1966) reported it in Lemur catta and Lemur macaco. Pollock (1979) has reported it in Indri indri. Andersson (1969) has stated that in some cases female Galago senegalensis are dominant to the males and both Roberts (1971) and Drews (1973) found that in the group situation one female Galago crassicaudatus initiated and won most of the aggressive encounters. The phenomenon of female dominance will be discussed in greater detail in the final discussion (Chapter X). In the mouse lemur, female dominance was most apparent in the introductions and generally less marked in the groups. The absence of a clear hierarchical structure in the group situation will be discussed later.

Other important factors which have come to light as a result of the introduction experiments were: the immediate recognition of dominance in previously unacquainted individuals; the influence of daylength and familiarity on female aggression, the influence of puberty on female aggression, the variability of behaviour during female-female introductions and the durability of the female-female bond.

It can be clearly seen during introductions that dominance was recognised almost immediately even in the case of previously unacquainted individuals. Bearder & Doyle (1969) related dominance to four factors: age, sex, environment, and "character". Obviously in the male-female introductions sex is the overriding factor. The females are on average somewhat heavier than the males, which is certainly important in this situation. In the female-female introductions other factors come into play. As all the females involved were wild caught and had been more than eight years in captivity it is unlikely that age could have been important. The initial encounters were usually in the neutral territory of the inter-cage connecting tunnel and thus it would seem that environment was also of little importance. In fact it must be noted here that, even if the initial encounter was in the subordinate animal's home cage (a subordinate animal would never enter the cage of a more dominant individual), the cage owner would never attack the intruder and only gave threat calls if the intruder tried to join her in her nest-box. Another pertinent observation on the influence of environment on introduction behaviour is evident when unacquainted individuals are introduced to each other and a new environment simultaneously. For example when the group in the Observation Room was established and when a group cage of six individuals was set up in Rotterdam Zoo after the completion of the study in London, no aggression or obvious dominance were seen in the initial encounters. The one factor in the introduction experiments on which status was based was probably "character" and this was probably determined on the basis of scent prior to introduction. The structure of the harem cage was such as to prevent visual and tactile contact between individuals prior to introduction but neither scent nor sound could be impeded. It has been suggested previously (Epple,

1970) that "changes in hormonal equilibrium might cause chemical alterations in the nature of glandular secretions and/or urinary secretions which in turn provide information on the sexual and social status of the animal". In the situation of group establishment mentioned above, the absence of an immediate dominance hierarchy might be interpreted in light of the absence of prior olfactory contact. However, it is also possible that the shock of such a transfer, to both new individuals and new surroundings, overrides aggressive tendencies and allows the animals to settle together amicably.

The effect of familiarity in reducing female aggression is to be expected and that of daylength has been discussed above. The increase in aggression observed in a young female at puberty is probably the result of the increase in ACTH and adrenal activity which are associated with puberty (Ramaley, 1974, and Chapter VII, section 4.). The results of the female-female introductions, on the other hand give a considerable insight into the social behaviour of the females. Charles-Dominique (1974) reports a similar variability during introductions of female Galago demidovii, ranging from serious fighting to bouts of allogrooming. He also notes that in the field females bear fewer scars due to intraspecific fighting than do the males. Both these observations he attributes to the fact that females often share territories under natural conditions. This conclusion would also be appropriate to the situation in the mouse lemurs. The ability to recognise a former female cage-mate after separation indicates the durability of the bond which arises between females which share the same territory, even between those which are not mother-daughter pairs.

Unfortunately no male-male introductions were implemented in the course of the study. The harem cage was primarily designed as a breeding unit and not an experimental cage. Thus, as there were only five sections, these were occupied by one male and four females. Male encounters obviously occur in the field. Martin (1972a) has reported that males may sleep together in pairs, which indicates that males can coexist amicably. In addition, observations undertaken since the completion of the study on a proportion of the original colony, now housed in Rotterdam Zoo, have indicated that, in a group situation with ample nest-box space provided, males will preferentially sleep with other males while females prefer to sleep with other females.

Juvenile-juvenile introductions were not conducted for the same reasons. However, during the course of the study a young hand-reared male was introduced to a cage containing three other juveniles, two males and one female, with no apparent aggression. The results of the introductions between the male and the immature female would tend to confirm that, prior to puberty, aggressive tendencies are low.

The most important results which have emerged from the study of behaviour in the group situation involve the observations made on antagonistic behaviour, its relationship to the frequency of social grooming and the influence of environmental factors on both of these behaviours.

The level of antagonism was much lower in the group situation than during introductions: aggressive behaviour occurred infrequently and, although there was a dominant female in each group, the hierarchical structure of the group was not pronounced. The behavioural organisation within the mouse lemur groups was very similar to that reported in Galago crassicaudatus (Roberts, 1971, Drews, 1973) with one female initiating and winning most of the aggressive encounters but with very few of these being directed towards the male.

In the London group during the first year the dominance relationship was not a strict one. This situation contrasted with that observed in the Jersey group where the level of aggression was much higher. This elevated level of aggression was associated with a much lower frequency of social grooming. This inverse relationship between aggression and allogrooming has been noted in other species. Many authors point out that social contact is the main function of allogrooming in prosimians and that is equally as important as in the old world monkeys (Doyle, 1974). Jolly (1966) has reported that social grooming is more frequently observed in *Lemur catta* than in *Propithecus verreauxi*. As the former species is more aggressive and has a strict hierarchical social system, a high level of allogrooming is necessary to reduce intra-group tension. As Andrew (1964) has pointed out, allogrooming diverts the attention of the groomee to behaviour incompatible with agonistic behaviour and therefore reduces aggression.

In the mouse lemurs, social grooming is a much more important part of the behavioural repertoire of the males than of the females. This may be a reflection of the lower social status of the males or it may be associated with the formation of courtship bonds such as those reported in *Galago demidovii*, *Euoticus elegantulus* and *Perodicticus potto* (Charles-Dominique, 1977a). In the courtship behaviour of these species, the male visits the female regularly and gradually approaches her until he is allowed to lick her face and eventually groom her. He will then continue visiting and grooming her at regular intervals. This behaviour occurs whatever the age or reproductive status of the female.

The low levels of social grooming and associated high levels of aggressive behaviour seen in the Jersey Zoo mouse lemurs may be attributable to disturbance resulting from display to the general public and other factors of Zoo life. This possible association between stress and antagonistic behaviour may be related to the change in behaviour which was observed in the London group. This relationship may also be inferred from Leyhausen's (1965) findings when discussing dominance in domestic cats, another non-gregarious, nocturnal species. He noted that under normal captive conditions only, what he termed, "relative" dominance prevailed. However, as the number of individuals in a cage increased an "absolute" dominance hierarchy emerged. In other words as social stress increased the relative dominance situation was transformed to an absolute one.

From the observations made in this study and those made in the field (Martin, 1972a) it is apparent that in spite of being termed "solitary", the mouse lemur has a relatively complex social organisation based on female dominance and matriarchal lineages of females which have social bonds durable over prolonged periods of separation. Aggression is high and directed towards strange individuals but allogrooming is also frequent and lowers female-male aggression. This organisation of social behaviour should be noted and taken into consideration when planning housing for mouse lemurs in captivity. Facilities should be such as to allow for the development of natural matriarchal groups. If such a group is to be artificially established, care should be taken in the selection of female companions so that no absolute dominance hierarchy emerges between them. These groups of females should be housed in proximity to males so that male-female familiarity can act to reduce antagonism between the sexes. Obviously the housing of males and females apart, only to be introduced when the female is in oestrus, is not feasible in all research or zoo situations and in addition complicates routine husbandry procedures. Thus, further work is needed to establish ideal groupings. The group of one male and two females does not appear suitable, particularly given the lack of breeding success in both the groups studied here and of a group of similar structure reported by Andriantsiferana et al.(1974). Data from Andriantsiferana et al. (1974) would indicate that a grouping of three females and two males was successful.

Since the completion of this study a group of four female and two male mouse lemurs has been established in Rotterdam Zoo. This grouping seemed much more successful in reproductive terms than those of the study colony and Jersey Zoo. This together with the data of Andriantsiferana et al. (1974) would indicate that the presence of more than one male is required to ensure that a group is successful in reproductive terms. However, the determination of the ideal group structure for the mouse lemur is far from complete and much work is still needed on this topic and its relation to cage structure.

In conclusion, it must be emphasised that the full understanding of the behaviour of a particular species is very important to the successful maintenance and breeding of that species in captivity. With regards to the mouse lemur the following types of information, which have been accrued from the study of its behaviour, can be employed to improve management, conditions and breeding success in captivity. The data on activity patterns can facilitate efficient management of the colony, by indicating those times when the animals are likely to be most active. This means that the time needed for the visual checking of animals, both to confirm that they are in good health and to determine their reproductive condition, is reduced to a minimum. This same basic knowledge of activity patterns also has a diagnostic value, as variations from the expected patterns may herald the onset of disease, the occurrence of oestrus, or possibly indicate the imminence of parturition. Such knowledge ensures that appropriate action can be taken to treat and possibly to isolate a sick animal and to facilitate the introduction of animals for mating in those cases where visual examination of the vulva for signs of oestrus is impossible. It may also be used to ensure that females are not unduly disturbed when they are about to give birth. Further, the knowledge of activity patterns can be employed to ensure that any noisy maintenance procedures are undertaken at a time when they will cause the animals minimal disturbance.

A thorough knowledge of activity patterns and social behaviour is necessary for the successful establishment of mouse lemurs in social groups. Information on both of these parameters is necessary to determine the most propitious time for the establishment of such groups. The data presented in this chapter would suggest that the short daylength period is most suitable for this as the animals are least active and least aggressive at this time. During this part of the year allogrooming is highest and this behaviour may also aid in the formation of amicable groups. Although a knowledge of the social behaviour and grouping of wild mouse lemurs may be indicative of both suitable group size and group structure in captivity, it is only by captive observation and by experimenting with different group sizes and compositions, in combination with various cage designs, that the optimum captive grouping can be established. As can be seen from the preceding discussion, this work is far from complete in the mouse lemur, although some important data, at least on groupings which yield bad results, have been obtained. These observations on inappropriate groupings are important when attempting to establish new groups.

SUMMARY

1) Mouse lemurs are nocturnal. However, their nightly activity pattern is influenced by photoperiod and thus varies over the course of the year. Activity patterns also vary between individuals. This difference is more pronounced between males and females than it is between individual females. This is due to the fact that the difference between male and female activity patterns is structured whereas that between females is random. The propensity towards a bimodal activity pattern is greatest in the female.

2) The pattern of occurrence of specific activities reflects the trends exhibited by the pattern of total activity levels. Exceptions to this are to be found in the social grooming and proximity scores. Both of these activities occur most frequently during the months of lowered activity and during the least active periods of the night. Feeding and marking behaviour occur most frequently during the first hour after "sunset".

3) Data on activity patterns can be of use to captive breeding projects in that deviations from the normal activity patterns may herald disease and indicate oestrus or the imminence of birth. It has been noted that external disturbance, for example that caused by being on display to the public in a zoo, can alter the activity pattern of the mouse lemur.

4) Female dominance seems to be an integral part of mouse lemur social organisation. Female dominance was apparent during introduction experiments in which the female always entered and explored the male's cage while the male adopted a cringing posture on the cage floor. It was also apparent in the group situation. In both groups studied one female was dominant: she was the largest animal in the group and she instigated most of the social interactions.

The hierarchical organisation of animals housed in groups was not a strict one.

5) Several factors were found to modify female dominance. These were: the time of year at which the introduction took place, the degree of familiarity between female and the male participating in the introduction, the age of the female concerned.

6) Two factors of particular interest emerged from the introduction experiments. Firstly, ownership of the cage in which the encounter takes place has no influence on the outcome of the encounter. Secondly, the relative status of each individual participating in an introduction is perceived immediately on first encounter.

7) The bond established between female cage companions appears to be a durable one.

8) Data indicate that environmental conditions can alter the social structure of mouse lemurs housed in groups. Stressful environments such as those encountered in zoos lead to a stricter dominance hierarchy within a group, with a concomitant increase in levels of aggression and decrease in frequency of social grooming.

9) Using the data presented in this chapter together with information on the social behaviour of mouse lemurs in the field, an attempt has been made to list some features which could be used to improve the conditions in captive mouse lemur breeding colonies.

CHAPTER VI

COMMUNICATION

Communication is a facet of mouse lemur behaviour which has been little studied and about which there is very little information available in the literature. It has been included in this thesis not only because it is an integral part of social behaviour and as such its understanding is essential to the interpretation of social interaction, but also because modes of communication, the factors which motivate communication signals and the affect such signals have on their recipient, are important factors to be considered in animal husbandry.

All aspects of communication in the mouse lemur are investigated. Mouse lemurs exhibit a diverse range of visual, olfactory and auditory communication signals. However, as they are solitary, nocturnal animals, visual signals which can probably only be perceived at close quarters are limited. Vocal signals, on the other hand, have the potential to be perceived over distances. However, those used by the mouse lemur are of very high frequency which would result in their rapid attenuation particularly under natural, forest conditions. This obviously imposes some limitations on the use of vocal communication in this species. However, it must be noted in this context, that McGeorge (1978) found that the attenuation of high frequency calls given by nocturnal species in the gallery forest of Madagascar is not as great as that of similar frequency calls given by diurnal species. Olfactory communication would seem to be the most suitable form of communication available to the mouse lemur. As a mode of communication, olfaction would seem to be particularly well adapted to the needs of a semi-solitary nocturnal species as not only can the signals be perceived over distance but, as it also persists in time, the message can be conveyed long after the signaller has departed.

There are three principal ways in which an understanding of communication can benefit captive breeding projects. Firstly, as Petter & Charles-Dominique (1979) have pointed out, a communicative signal reflects the "emotional" state of the signaller. Therefore, by monitoring the animals for the occurrence of various communicative signals it should be possible to determine whether the level of certain undesirable states, such as fear or disturbance, are too high in the specimens under study. (The assessment of when the level of a particular emotional state is too high is subjective but can be based on reproductive success. Thus if suppression of reproduction is observed when the frequency of occurrence of fear or disturbance-related behaviours exceeds a certain level, it is this level which is important in determining the maximum allowable in the colony). Secondly, a knowledge of the probable response of the receiver of a communicative signal is also important. Thus, if particular signals are known to stimulate aggression or to cause a mother to neglect her young, steps should be taken to limit the occurrence or the area of influence of the signal. The third and final way in which an understanding of communication can benefit animal husbandry is by indicating which signals may act as a mediator of social stress. In an investigation which centres on the influence which captivity can have on the reproduction and health of a colony of animals: over-crowding is one factor which must be taken into consideration. In the case of the mouse lemurs in this study, many of the specimens were individually housed. Thus, should they exhibit any of those symptoms which are commonly associated with over-crowding, some factor other than the close physical presence of a conspecific must be acting as mediator. This would seem to implicate one of the modes of communication which can be perceived over distance.

1. Visual Signals

As Hill (1953) has indicated, all prosimians have poor facial musculature which is limited mostly to the labial and nasal areas and accounts for their "blank" expression. Despite this and the limitations that its nocturnal, semi-solitary existence imposes on visual modes of communication, the mouse lemur exhibits a wide range of visual signals. This section focuses mainly on ear positions which are adopted in various situations, in order to establish whether ear position can be used as an "emotional" index in the mouse lemur; to ascertain whether certain ear positions are always adopted in situations of disturbance or nervousness.

1.1. Body Postures and Facial Expressions

Only four signals have been included in this section. They have been included as they were the most frequently recorded visual signals observed in the study colony. These signals are: the swaying body posture, the cringing body posture, and the defensive threat and defensive attack postures.

In defense threat the mouth is open but the lips cover the teeth, while the eyes are wide open and the ears are spread (see section 1.3. below). As the threatening stimulus approaches, the eyes narrow and the ears assume the back and folded position. Defensive attack commonly succeeds defensive threat and both were most frequently observed when removing an animal from its nest-box for condition checking. As the gloved hand approached, the animal would give threat calls and rear up onto its hind legs with its fists clenched. The mouth was open, the teeth were bared and the ears would assume the back and folded position prior to the animals attacking and biting the source of the threat.

During the swaying posture, the body is held low against the substrate and the front half of the body sways from side to side. The movement is sometimes accompanied by head turning in which the head is moved from side to side. This posture is usually observed in situations where an animal is presented with a novel object, although nervous individuals will adopt this posture towards an observer with whom they are not familiar. This swaying is an exaggeration of the head nystagmus frequently seen in young mouse lemurs, in which it is thought to aid in the visual localisation of objects. In the young mouse lemur it is generally seen when the animal encounters a new situation or novel object. Nystagmus gradually attenuates towards adulthood as many situations and objects become familiar.

The cringing body posture was only observed in males during introduction to non-oestrus females. The posture was generally assumed on the floor of the cage, where the male would sit unmoving in a hunched position with his ears held in the back and folded position (see section 1.3. below) and his eyes were partially closed. The posture seemed to function in inhibiting aggression from the female.

1.2. Tail Positions

Using videotape recordings of interaction sequences, an attempt was made to correlate tail position with dominance as in many species the more confident individual in any situation will carry its tail higher than the less confident one (Andrew, 1964). This correlation was not observed in the mouse lemur, in which the tail functions primarily as an organ of balance.

Tail-lashing, that is a vigorous up-and-down movement of the tail, was observed frequently during mating behaviour. As the male approached the female in order to mount, vigorous tail-lashing was always observed. It was also observed when an immature female approached a male. In both of these situations the subject was very cautious in its approach of the other animal. If the animal being approached moved slightly in the subject's direction the subject would retreat.

1.3. Ear Positions

The ear positions assumed during social encounters have been investigated in some detail using videotape recordings.

The pinnae of the grey mouse lemur are large and pale in colour. This enhances their visibility and thus adds to their communication value. However, the rufous mouse lemur, which has small ears, still exhibits similar ear positions. Possibly these movements have been retained in the rufous mouse lemur but are no longer functional.

These ear positions may also be stimulated in a non-social situation and the following analysis aids the interpretation of the motivation of an individual in that situation.

Three basic ear positions have been determined:- "spread", "horned" and "back and folded" (fig. 49).

Spread ears are observed in confident approaches, attacks and chases (table 27).

Horned ears are observed in situations of greater caution or uncertainty (table 27); e.g. in the cautious approaches of female-female introductions, during threat calls and after the chases, attacks and fights of male-female introductions.

The horned ear position is also observed when the male investigates female scent marks. In this situation marked pulling back of the corners of the mouth is also observed.

As an animal becomes more alarmed the angle of the horned ears becomes erect; this upright ear position is an extreme form of the horned position.

The back and folded position occurs in situations of extreme alarm or fear (table 27). The male's ears will assume this position when he is persistently threatened by a female. Folding is frequently observed in association with cringing body posture. A subordinate female will fold her ears when confidently approached or sniffed by a social superior. Ear folding is often seen in association with a marked blinking of the eyes which gives the

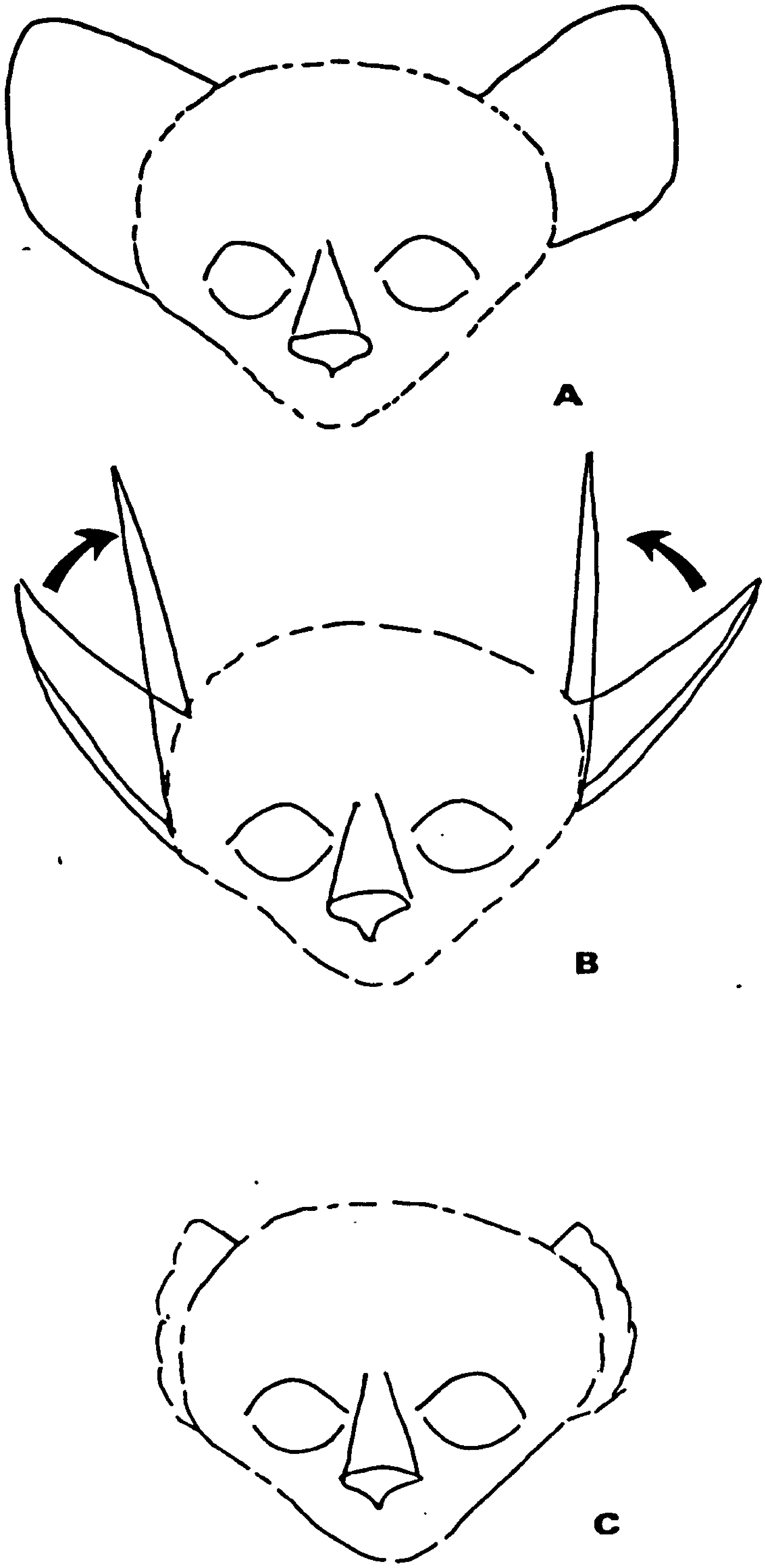


Figure 49

Ear positions

A -alert or spread; B - horned positions; C - back & folded position.

Table 27

Ear Positions, Status and Interactions

	Ear Positions					
	Spread		Horned		Back	
Individual's Status	D	S	D	S	D	S
Interaction						
Chase	++	-	+	+	-	?
Attack	++	-	-	-	-	?
Watch	+	-	+	+	-	+
Threat	-	-	+	+	-	?
Approach	++	-	-	+	-	+
Post Aggression	-	-	++	-	-	++
Amicable Interaction (introduction of non-acquainted individuals only).	-	-	++	++	++	++

- : never observed; + : somtimes observed; ++ : frequently observed;

? : not recorded onto video, but observed or suspected

D : dominant individual

S : subordinate individual

appearance of drowsiness. This is very reminiscent of the sleeping displacement activities of some birds.

2. Olfactory Communication

This section only deals with olfactory communications which are perceived at a distance. There is a second body of olfactory communication involving direct signals. These signals are directly perceived at close quarters and generally involve intrinsic body odours, the perception of which requires actual physical contact.

Olfactory communication which can be perceived over a distance takes two forms: passive scent release and active marking behaviour.

2.1. Passive Release of Scent

Two passively emitted scents are noticeable to the human observer. These are the smell of the oestrous female and a distinct acrid smell associated with fear and the soft faeces produced during fear situations.

2.2. Active Methods of Scent Deposition

Active marking behaviour involves the deposition of urine, glandular secretions and probably faeces. Urine may be deposited in one of three ways: urine washing, rhythmic micturation or urinating directly onto a branch. Urine washing follows the typical pattern first described by Hill (1938). It involves the raising of the hand and foot on the same side of the body. This hand is cupped below the penis or the clitoris and a few drops of urine deposited in it. The foot is then rubbed over the hand several times in rapid succession and both are replaced on the substrate. This procedure may then be repeated several times using alternate hands and feet, while the animal proceeds along a branch. Urine washing is the most frequently observed method of urine deposition. However, rhythmic micturation is also frequently observed. This term was first coined by Ilse (1955) for the slender loris, Loris tardigradus, and during this activity the body is lowered to the substrate and the animal progresses forward with a wriggling motion depositing a thin trail of urine.

In the third method of urine deposition, a copious flow of urine is deliberately discharged onto a branch. "Deliberate" is applicable here as under normal circumstances the animal avoids fouling the branches of its cage with urine or faeces.

The possible methods of depositing glandular secretions are by mouth-wiping or anogenital-rubbing. During mouth-wiping the corner of the mouth, the face and sometimes the head are rubbed along a branch. In this form of marking saliva may also be deposited as a marking substance. It is possible that mouth-wiping may be a method of collecting scents from other sources onto the head region of the body. However, this is felt to be unlikely in view of the observation that mouth-wiping usually occurs in different areas of the cage from those used for other forms of marking; viz. ends of twigs and notches of branches. Nor would it appear to be a method of cleaning the face after eating as its occurrence is in no way related to feeding behaviour.

Anogenital-rubbing involves the animal lowering its hind quarters and dragging its anogenital region along the substrate. This method of marking appears to be responsible for the dark, tacky deposits found on the branches in the mouse lemur cages, which may be the result of faecal matter deposited in the course of rubbing (Petter, 1962). Some prosimians, notably Lemur species, have been observed to back-up to vertical objects and rub their anogenital region against them. This behaviour has not been observed in the mouse lemurs. However, the dark-stained areas on the wooden struts at the corners of the cages seem to indicate that this does occur.

2.3. Histology

Areas of skin from the lip, cheek, scrotum and the epidydimal sac have been sectioned and examined histologically for the presence of specialised scent glands. Although no specialised glandular areas were found, the skin sections from the cheek, scrotum and epidydimal sac were characterised by very large apocrine glands connected to large coiled ducts (figs. 50, 51, 52). In addition large sebaceous glands were found in some parts of the cheek skin (fig. 50). Although no control areas of skin were sectioned to determine the occurrence of large apocrine glands in other areas, two facts would tend to indicate that these glands are only found in certain areas: no enlarged apocrine glands were found in skin taken from the muzzle near the lips; von Fiedler (1959) found no enlarged apocrine glands in the mouse lemur scrotal skin sections which he studied. However, von Fiedler (1959) did report a sub-penile sebaceous area in the mouse lemur which was not observed in the specimens in this study.

2.4. Areas Marked

The areas used for marking were studied in the group-housed animals of the Observation Room and in individually-housed animals. In the group-housed situation the approach taken was to relate the areas used for marking to cage use. This approach gives a further indication of the function of the various marking behaviours and also facilitates the assessment of the role of marking in terrirorial demarcation.

From the work on cage use it became apparent that special areas of the cage were used for feeding, resting, sleeping and grooming. These areas were connected by a series of routeways. The evidence available would support the view that each individual had its own preferred area in the cage and that this preference was more apparent in the case of the females. Both females had

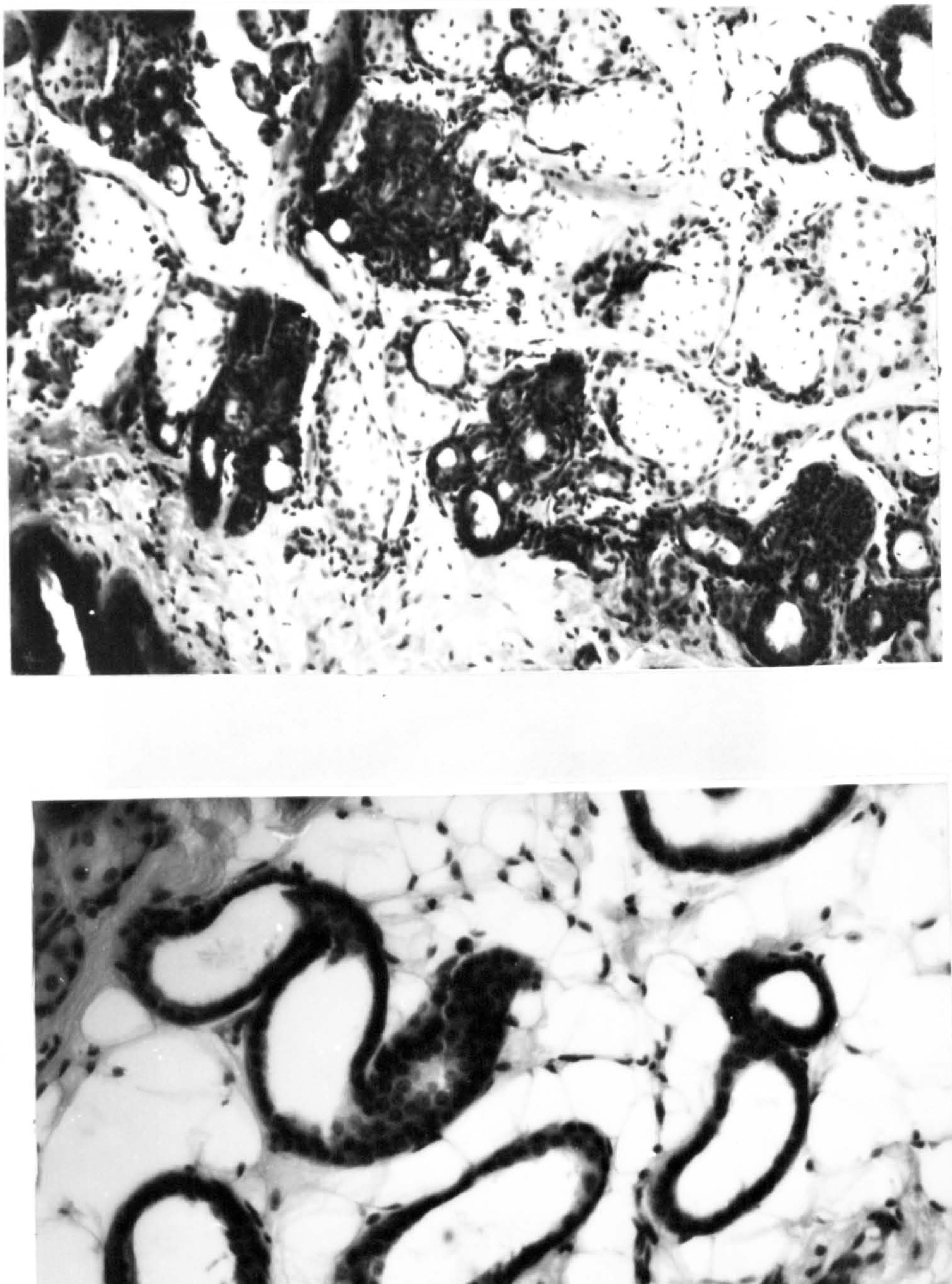
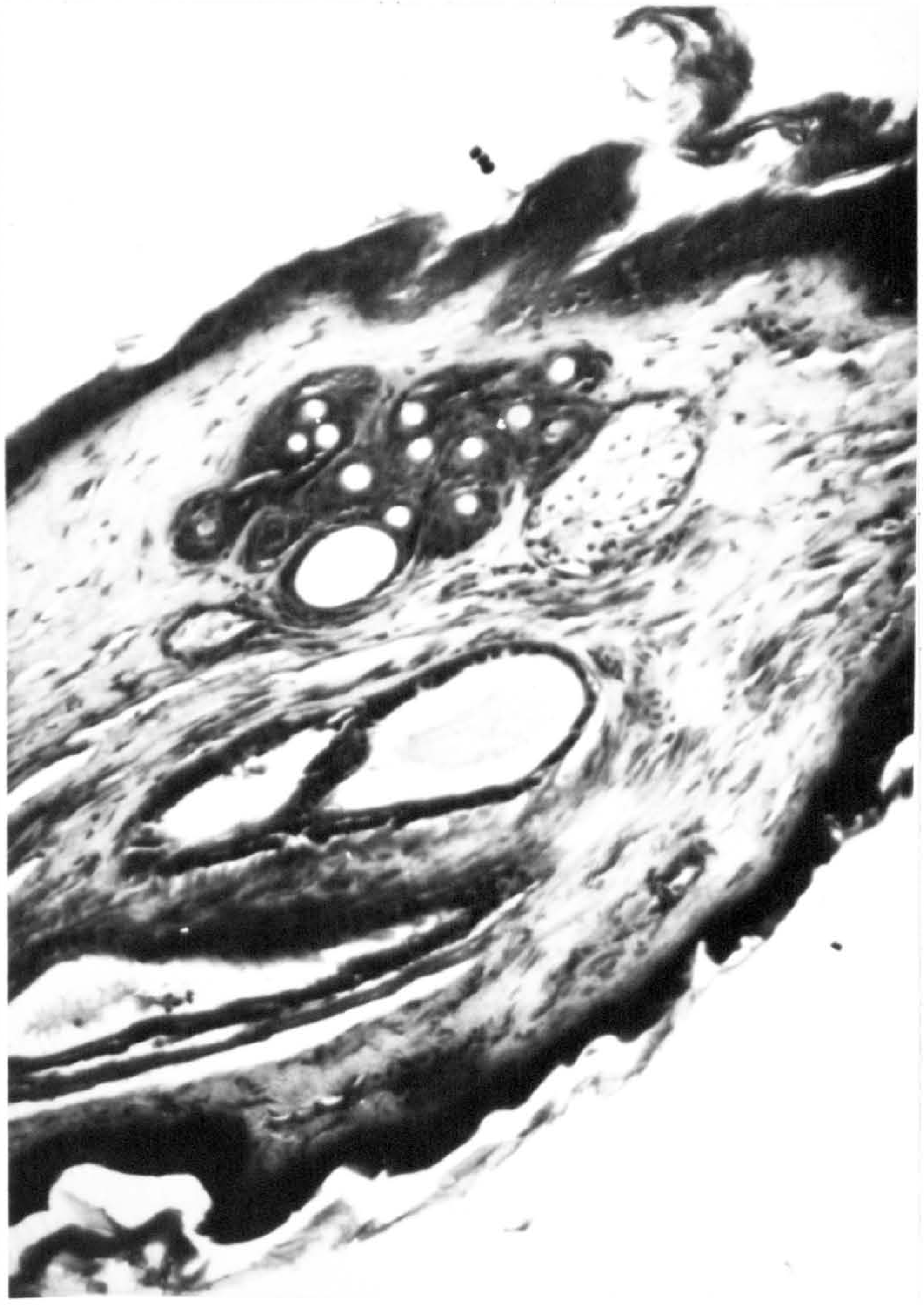


Figure 50

Cheek skin glands

The top figure shows the large sebaceous and apocrine glands characteristic of this region. The lower figure shows the apocrine glands and their ducts at higher magnification.



<u>Figure 51</u>

Scrotal skin glands

The section shows enlarged apocrine glands found in this region.

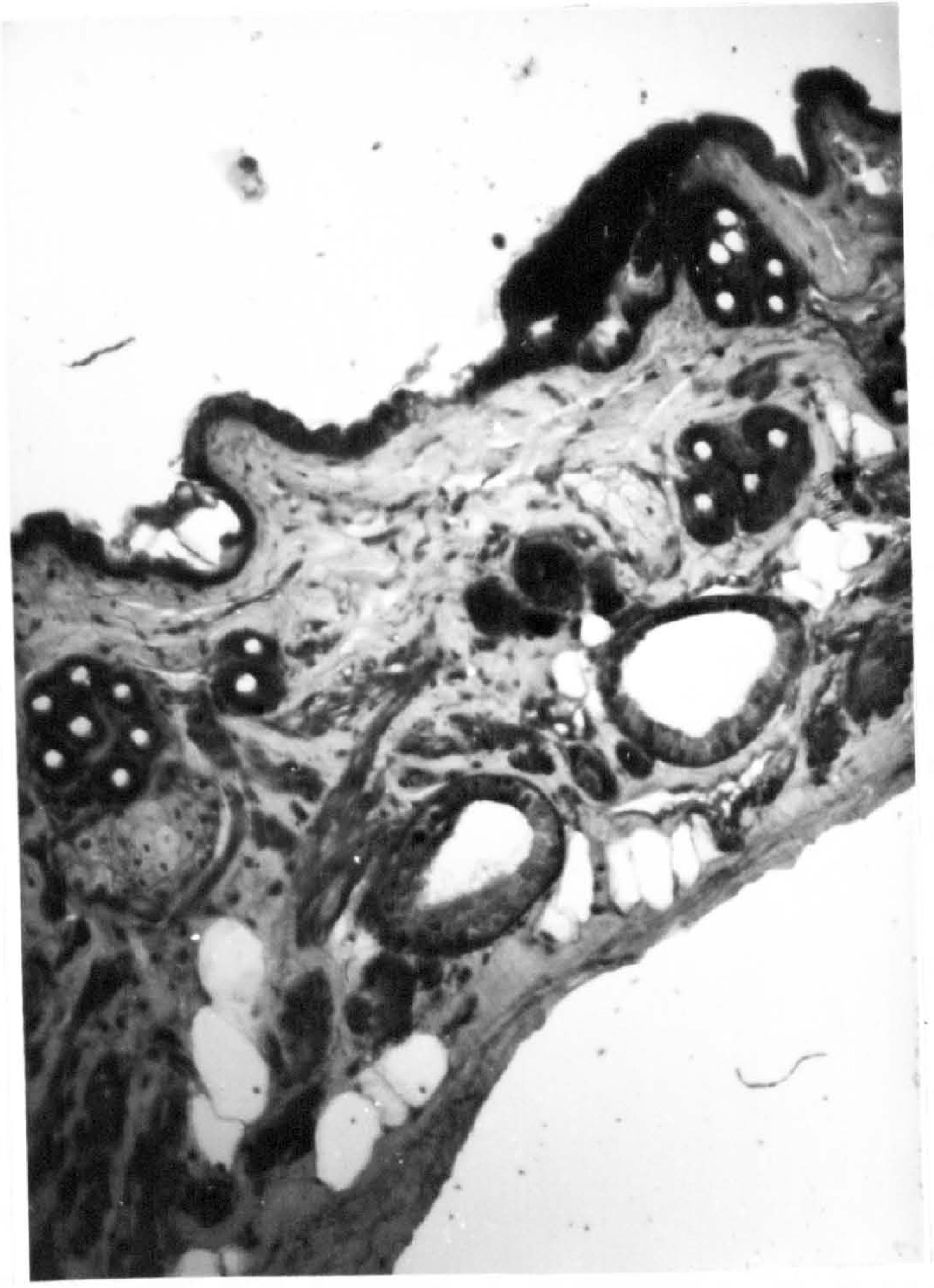


Figure 52

Skin glands from the epididymal sac

Section shows the enlarged apocrine glands found in this region.

their own preferred drainpipe nest-box and food station, whereas the male used all the drainpipes and food stations equally (see tables 28, 29).

Resting areas as shown by the maps (figs. 53, 54, 55) indicate that the females had fewer preferred spots compared with the male. They also indicate an area where the females would commonly associate (outside tube 1) and areas where all the animals might associate (outside tube 2).(nest-tube numbers see fig.5, page 33).

A main routeway linked these areas: each animal had its own individual variation of routeway but there were areas of overlap (see figs. 53, 54, 55).

Marking can be related to cage use as follows:- marking occurs in communal areas, that is on the shared part of the routeway; along personal routes and around preferred resting areas. In addition, the male was observed to mark along part of the females' routes which he rarely used himself (see figs. 53, 54, 55).

Animals housed individually also marked their cages in a preferential manner. Each unit of the "harem" cage contained one wide horizontal branch and this was marked most frequently in all cases. Other branches within each unit were marked with varying degrees of preference. This preference pattern appears to remain constant over time and between individuals, as indicated by the fact that if the branches marked by one individual in two separate years are compared there is no significant difference in preference (Spearman rank test, $p \leq 0.05$, table 30). Similarly, the new occupant of a cage was found to mark the same branches as its predecessor (Spearman rank test, $p \leq 0.05$, table 31).

2.5. Frequency

The frequency of all kinds of marking varies over the day and the year (see Chapter V, section 1.1.1. and 1.2.2.). Variation in frequency within and between individuals is also evident. The

Table 28

a) Chi Squared Test Comparing Nest Site Use

Nest 1

Animal	MF6	MF8	MM3
0	79	38	17
E	44.7	44.7	44.7
0 - E	-34.3	6.7	27.7
$(0-E)^2$	1174.5	44.89	767.3
$(0-E)^2/E$	14.9	1.2	45.1

= 61.2

Nest 3

Animal	MF6	MF8	MM3
0	10	14	20
E	14.7	14.7	14.7
0 - E	4.4	0.7	5.3
$(0-E)^2$	22.1	0.49	28.1
$(0-E)^2/E$	2.1	0.03	1.4

= 3.53

Nest 2

0	9	53	30
E	30.7	30.7	30.7
0 - E	21.7	-22.3	-0.7
$(0-E)^2$	470.9	497.3	0.5
$(0-E)^2/E$	14.9	1.2	45.1

= 61.72

Nest 4

0	51	19	21
E	30.3	30.3	30.3
0 - E	20.7	-11.3	-9.3
$(0-E)^2$	428.5	127.7	86.5
$(0-E)^2/E$	8.4	6.7	4.1

= 19.22

Differential use of each nest-box by the 3 individuals is highly significant in all cases ($p \leqslant 0.001$) except in nest 3.

b) Chi Squared Test Comparing Nest site Preference

MF6

Nest	1	2	3	4
0	79	9	10	51
E	37.25	37.25	37.25	37.25
$(0-E)^2$	1743.3	797.15	741.275	189.79
$(0-E)^2/E$	46.80	21.40	19.90	5.10

= 93.20

MF8

Nest	1	2	3	4
0	38	53	14	19
E	31.0	31.0	31.0	31.0
$(0-E)^2$	48.98	483.6	288.92	143.84
$(0-E)^2/E$	1.58	15.60	9.32	4,64

= 31.41

MM3

Nest	1	2	3	4
0	17	30	20	21
E	22	22	22	22
$(0-E)^2$	25.08	64.02	3.96	1.00
$(0-E)^2/E$	1.14	2.91	0.18	0.045

= 4.275

Nest site preference is marked in both females ($p \leqslant 0.001$), but not in the male.

Table 29

Food Station Use

Food Station	Animal		
	MF6	MF8	MM3
A	13	25	23
B	6	11	20
C	13	12	28

This shows the number of observation periods in which the animal used a particular food station at least once.

Figure 53

MF6, routeways and marking sites

Figure - routeways and resting sites, overlay - marking sites.

Figure 54

MF8, routeways and marking sites

Figure - routeways and resting sites, overlay - marking sites.

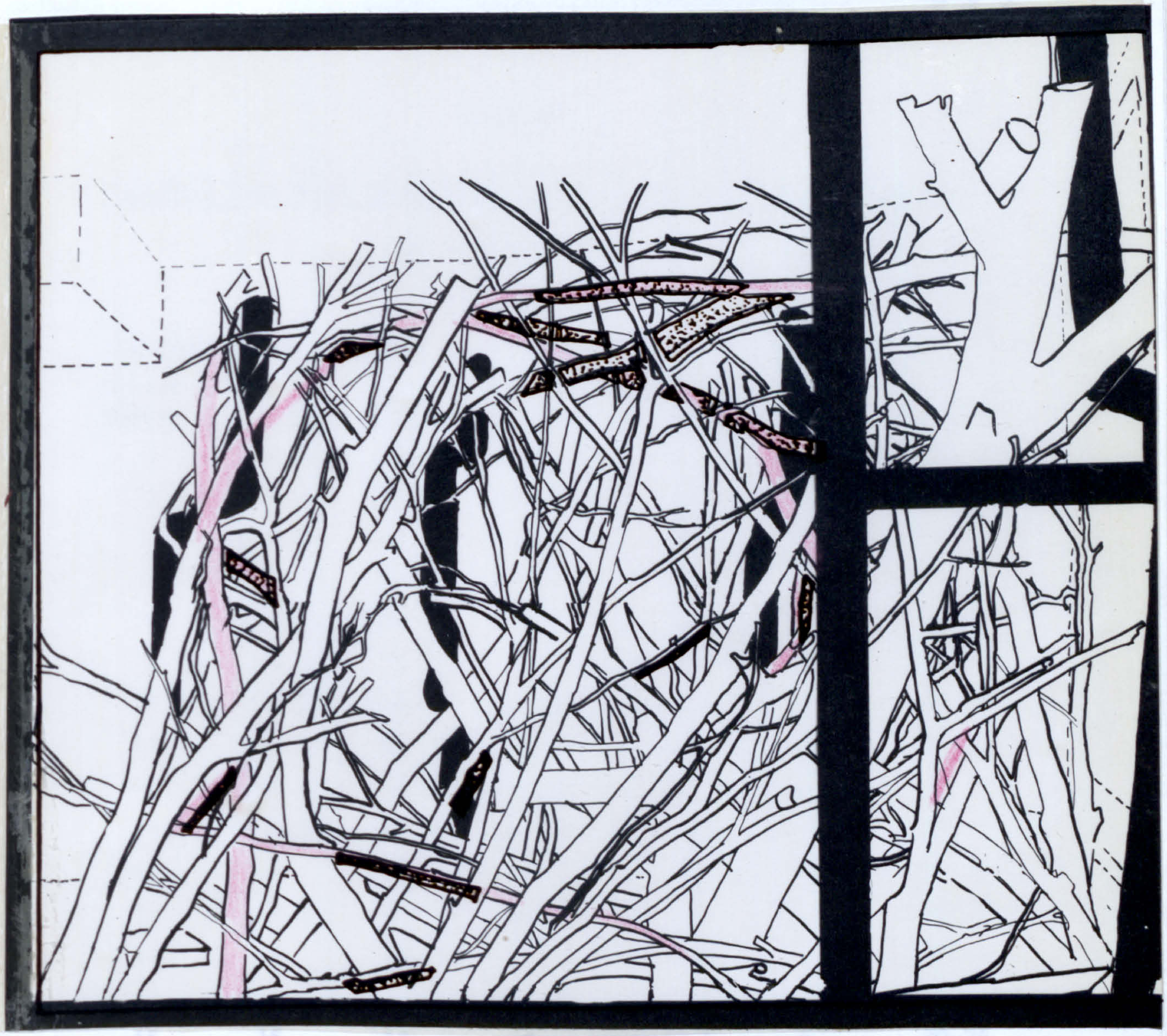


Figure 55

MM3, routeways and marking sites

Figure - routeways and resting sites, overlay - marking sites.

Table 30

Spearman Rank Test Comparing the Branches Marked by MF2 and MF7 in 1975 and 1976

Identification		MF2			MF7	
Branch Number	1975	1976	d^2	1975	1976	d^2
1	1	4.5	12.25	1	1	0
2	6	14	64	13.5	5	72.25
3	9.5	14	20.25	2	3	1
4	16	14	4	13.5	16.5	9
5	16	14	4	13.5	16.5	9
6	4	1	9	13.5	9	20.25
7	9.5	14	20.25	13.5	9	20.25
8	9.5	14	20.25	13.5	9	20.25
9	16	4.5	132.25	13.5	16.5	9
10	2.5	14	132.25	13.5	16.5	9
11	16	14	4	4	16.5	156.25
12	9.5	14	20.25	4	16.5	156.25
13	16	14	4	13.5	2	132.25
14	6	2	16	13.5	16.5	9
15	16	14	4	13.5	9	20.25
16	2.5	14	132.25	13.5	16.5	9
17	16	14	4	13.5	4	90.25
18	16	4.5	132.25	13.5	9	90.25
19	16	14	4	13.5	16.5	9
20	16	14	4	13.5	9	20.25
21	6	4.5	2.25	4	9	28

$$r^s = 1 - \frac{6\Sigma d^2}{n(n^2 - 1)}$$

MF2: $\Sigma d^2 = 745.5$, $r^s = 0.516$

MF7: $\Sigma d^2 = 818$, $r^s = 0.469$

In both cases there is a significant correlation between the branches marked both years; $p \leqslant 0.05$

Table 31

Spearman Rank Test: Correlation of Marking Between MF5 and MF12 in the Same Cage

Branch Number	MF5	MF12	d^2
1	3	1	4
2	1.5	2	0.25
3	18.5	14.5	16
4	5	7.5	6.25
5	15	7.5	56.25
6	1.5	3	2.25
7	9.5	7.5	4
8	15.5	14.5	1
9	9.5	10	0.25
10	5	14.5	90.25
11	15.5	4.5	121
12	4	7.5	12.25
13	15.5	14.5	1
14	11.5	14.5	9
15	15.5	14.5	16
16	11.5	14.5	9
17	15.5	14.5	1
18	15.5	4.5	121

$$d^2 = 470.75$$

$$r^s = \frac{1 - 6\,d^2}{n(n^2-1)} = 0.5142$$

Significant Correlation at 5% level

following table shows the considerable degree of variability in marking frequency between individually-housed females (results for all types of marking have been pooled).

Table 32

Marking Frequency of Individually-housed Females during the Breeding Season

Female	n	Total Marks/ 30 minutes	Urine Marks/ 30 minutes	Approximate % Marks involving urine deposition
MF1	10	0.4 ± 0.2	0.4 ± 0.2	100
MF2	11	2.3 ± 0.5	2.3 ± 0.5	100
MF5	10	4.8 ± 1.0	4.0 ± 1.0	83
MF7	11	1.3 ± 0.6	0.5 ± 0.3	33
MF12	5	7.0 ± 0.8	3.8 ± 4	54

where n is the number of observations.

From the above table (32) it can be seen that the frequency of marking is highest in the females MF5 and MF12. These two females were the most nervous and socially subordinate of all females housed in the "harem" cage. The marking frequency recorded in a sexually active, individually-housed male during the breeding season was found to be of a similar order to that of the females except that no urine marks were observed (1.2 ± 0.6 marks/30 min. period, 0% urine marks, n = 5).

In addition to the variation in marking frequency between individuals there is also considerable variability within individuals as can be seen in table 33 below.

Table 33

Variation of Marking Frequency within one Individual

Date of Obser.	n	Total Marks/ 30 minutes	Urine Marks/ 30 minutes	Approximate % marks involving Urine deposition
Feb-Apr 1975	11	1.3 ± 0.6	0.5 ± 0.3	33
Mar 1976	5	8.0 ± 0.9	2.4 ± 0.6	30
May 1976	6	3.2 ± 0.8	0.8 ± 0.3	25

where n is the number of observations.

In the group situation marking frequency is related to dominance and it is the dominant female which marks most frequently (see table 34). However, after the removal of the subordinate female from the Jersey group, the marking frequency of the remaining dominant female decreased while that of the male increased with the result that marking frequency was approximately the same in male and female (see table 35). Urine marking still remained much higher in the female than in the male although the frequency was lower than when the subordinate female was present.

2.6. Oestrus

As can be seen from the data in table 36, oestrus greatly increases the frequency of marking in both the male and the oestrus female. However, a detailed examination of the table reveals that the increase in total marking frequency does not represent an increase in all of the kinds of marking observed. In the females the increase in total marking frequency is the result of an increase in the frequency of anogenital-rubbing and mouth-wiping while the frequency of urine marks in fact decreases. The frequency of anogenital-rubbing increases still further at the time of peak receptivity in the female. At this time the occurrence of anogenital-

Table 34

Frequency of Marking/45 minute observation period in group housed animals

Subject	Type of Mark				
	Total	Mouth-wiping	Urine-mark	Anogenital-rubbing	n
Dominant female					
London	4.0±1.7	1.8±0.5	2.2±0.9	0.4±0.2	26
Jersey	6.8±2.4	1.2±0.8	4.3±1.8	1.3±0.3	6
Subordinate female					
London	2.3±1.0	0.8±0.3	1.45±0.9	0	26
Jersey	0.3±0.2	0.2±0.2	0.2±0.2	0	6
Male					
London	1.7±0.4	0.1±0.1	1.3±0.3	0.4±0.1	26
Jersey	1.8±0.7	0.3±0.2	0.8±0.3	0.7±0.3	6

n = no. of observations.

Table 35

Effect of removing individuals on marking frequency/45 minute observation period

Subject	Condition	Mouth-wiping	Urine-mark	Anogenital-rubbing	Total	n
Dominant female	subord. ♀ present	1.2±0.8	4.3±1.8	1.3±0.3	6.8±2.4	6
	subord. ♀ absent	1.1±0.5	3.4±1.7	0.25±0.1	4.8±2.2	19
Male	Subord.♀ present	0.3±0.2	0.8±0.3	0.7±0.3	1.8±0.7	6
	Subord.♀ absent	2.5±0.1	1.9±0.9	0.4±0.2	4.7±2.1	19

Table 36

Affect of Oestrus on marking frequency/45 minute observation period

Observations made on Jersey group.

Subject	Oestrus condition	Mouth-wiping	Urine-marking	anogenital-rubbing	Total	n
Dominant ♀	no oestrus	1.2±0.8	4.3±1.8	1.3±0.3	6.8±2.4	6
Subordinate ♀	"	0.2±0.2	0.2±0.2	0	0.3±0.2	6
♂	"	0.3±0.2	0.8±0.3	0.7±0.3	1.8±0.7	6
Dominant ♀	dominant ♀ in oestrus	18	2	18	38	1
Subordinate ♀		0	0	0	0	1
♂		4	0	1	5	1
Dominant ♀	Subord. ♀ in oestrus	0	1	1	2	1
Subordinate ♀		4	0	2	6	1
♂		5	2	1	8	1

n = no. of observations.

rubbing is so apparent that the observation of the frequency of this type of marking can be used to determine oestrus in the absence of any other data.

Male marking frequency also increases when the females are in oestrus. However, in the male it is only mouth-wiping which increases at this time. This, in fact, may not be an increase in actual marking behaviour but rather be indicative of an increased tendency in the male to anoint himself with the scent of the female.

3. Vocal Communication

3.1. Infant Repertoire

Distress Call (fig. 56): This call is elicited when the infant is out of contact with its mother and away from the nest. It has also been observed with sick infants which are in the nest with the mother.

The calls which are high-pitched and piercing, rising in frequency from 12 to 15 Hertz, are given at a rate of 300 per minute.

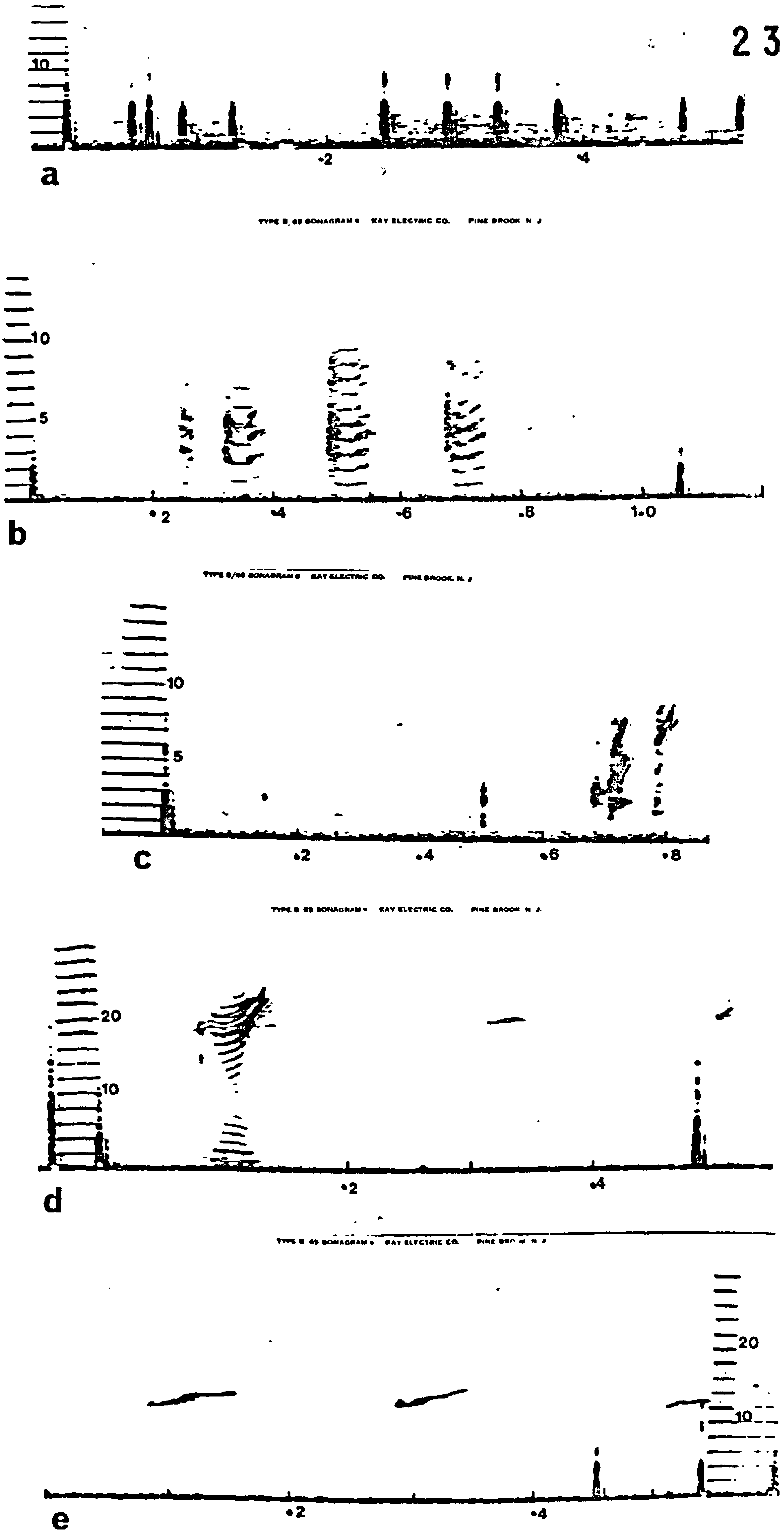
Purr (fig. 56): This sounds very similar to a cat's purr and is given in similar situations. In the very young infant, 1-2 days old, this call is represented by isolated clicks. A click is a burst of noise ranging in frequency from 0 to 16 Hertz but with most of the energy concentrated between 0 and 4 Hertz. As the infant grows older, these clicks are given in irregular bursts at a rate of up to 900 per minute. This call is maintained in adult hand-reared mouse lemurs and is given when they are stroked or are burrowed into the hand-rearer's clothing. This call is probably given by other adult mouse lemurs in situations of allogrooming, associating or nest-box sharing.

Elimination Call (fig. 56): During the hand-rearing of mouse lemurs elimination is elicited by rubbing

Figure 56

Infant vocalisations

a - purr; b - grunts; c - grunts; d - elimination call;
e - distress call
x-axis - time in seconds; y-axis - frequency in KHz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the perineal region with a damp cloth. This simulates the licking of the mother which leads to elimination under natural circumstances. As the infant urinates or defaecates, a soft bird-like call is given. This is a highly harmonically structured call with energy distributed between 0 and 10 Hertz. As the infant develops the call structure changes and most of the energy is centered between 0 and 2 Hertz and between 18 and 24 Hertz. In the grey mouse lemur infants, a trill is also incorporated into this call.

Grunts (fig. 56): A series of grunts in association with clicks are given by the hand-reared infant whilst seeking the feeding pipette. These calls exhibit a rapid change of frequency between 8 and 18 Hertz.

3.2. Adult Calls

Disturbance Call (fig. 57): This is given in situations of visual disturbance, such as the presence of a strange person or object in the animal room. The call consists of a very rapid series of short whistles. Each call lasts about 0.04 seconds, is given at a rate of 400-600 calls per minute, and at a frequency of 14-16 Hertz. A certain degree of individual difference has been noted both in frequency and repetition rate of this call.

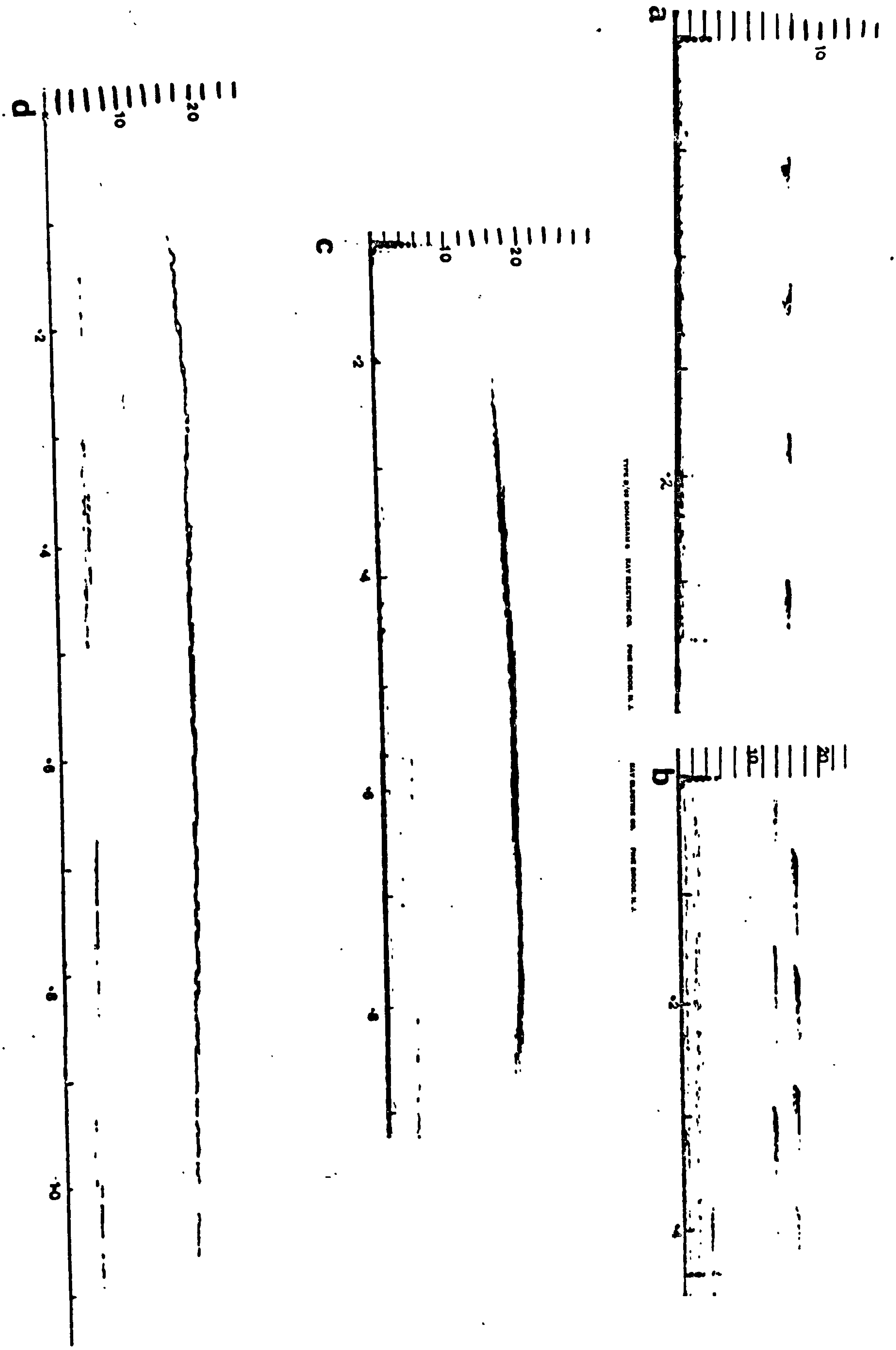
This is a cryptic call, structured so as to render location of the caller difficult. There is no modulation of frequency and this call has a gradual beginning and ending. The cryptic nature of this call is enhanced by the behaviour of the caller, which remains motionless and barely opens its mouth to emit the call. In addition, the call is usually given by more than one individual at a time and chorusing behaviour further camouflages the call.

Scream: This call is also given by the dwarf lemur, *Cheirogaleus medius*, in situations of fear associated with pain. Mouse lemurs give this call in response to sudden loud noises or the proximity of a dominant fellow. The call consists of a series of screams. Screaming in situations of fear or pain

Figure 57

Adult vocalisations

a - unidentified call,a rapid clear whistle;
b - disturbance call, two animals calling; c - long whistle; d - long whistle
x-axis - time in seconds; y-axis - frequency in KHz.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KAY ELECTRIC CO. PINE BROOK, N.J.

is common to most mammals.

Whitter: This is a very soft call given in greeting between two familiar adults and by mothers to their infants.

Whistles: Three categories of whistle call have been identified:- 1) Clear whistle (fig. 58): This call has a fundamental frequency of 7 Hertz. Each whistle lasts about ¼ second and is given at a rate of 60-80 calls per minute.

This call is often heard from a subordinate animal during introduction experiments and may function to inhibit aggression. It also stimulated much interest from other mouse lemurs during play-back experiments.

2) Long whistle (fig. 57): Each of these whistles can last between 0.64 and 0.92 seconds and they are given at a rate of 15 calls per minute. The frequency of the whistle increases slightly during the call and lies between 15-20 Hertz. This call is also heard during introduction experiments though not always from the individuals participating in the experiment. This may indicate a high level of social disturbance.

3) Intermediate whistle (fig. 59): These calls are given at a rate of 38 calls per minute and are between 0.3 and 0.5 seconds in duration. During a series of these calls the structure of the individual call is modified as the intensity of calling increases. At low intensities the call has no frequency modulation and lies between 16-18 Hertz. At higher intensities a frequency modulation develops at the end of the call which increases to 18-19 Hertz and then decreases again. This call is given most frequently within a few hours of "dusk" and may function as a spacing call.

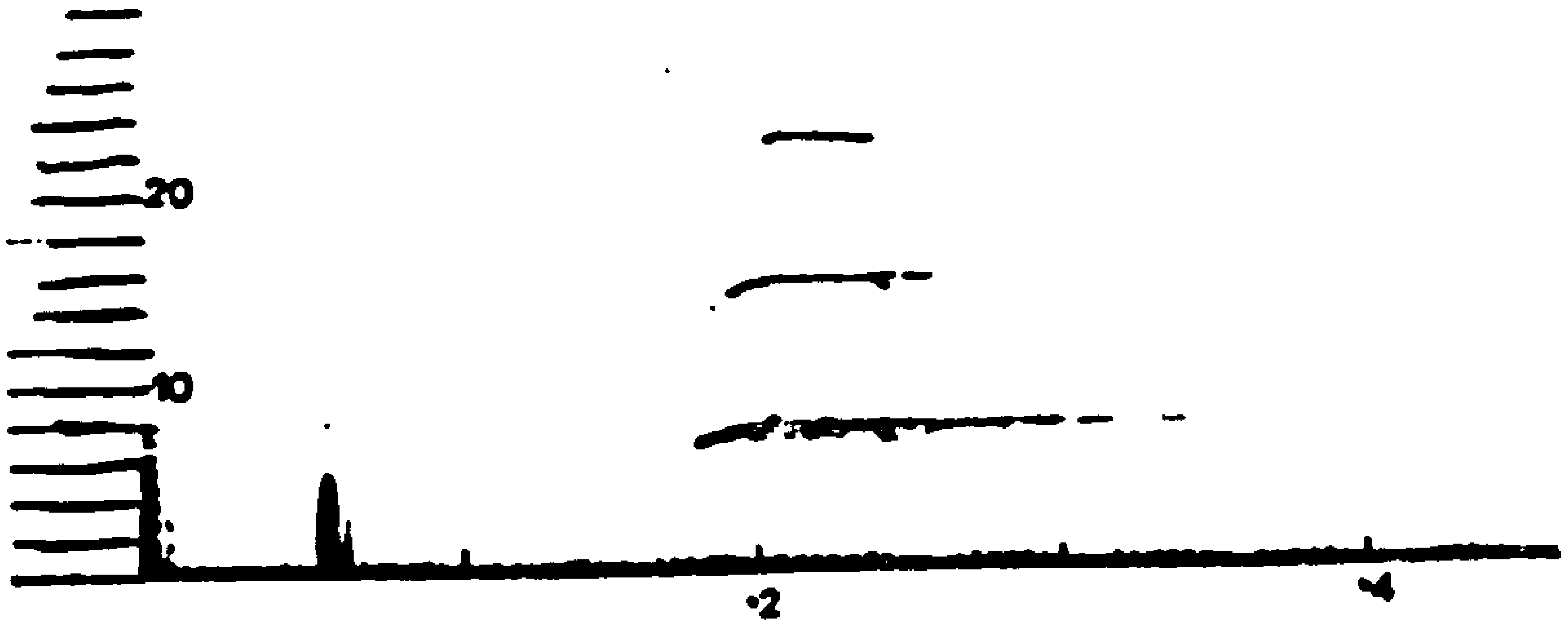
Purr: As mentioned previously, it is considered that this infant call is retained in adult life.

Trill (fig. 60): This call is only given by the grey mouse lemurs. The rufous subspecies gives a very different call in similar situations. The trill is given in two

Figure 58

Clear and hoarse whistles

a - MF1 giving clear whistle; b & c - Spike giving clear whistle; d - MF1 giving hoarse whistle
x-axis - time in seconds; y-axis - frequency in KHz.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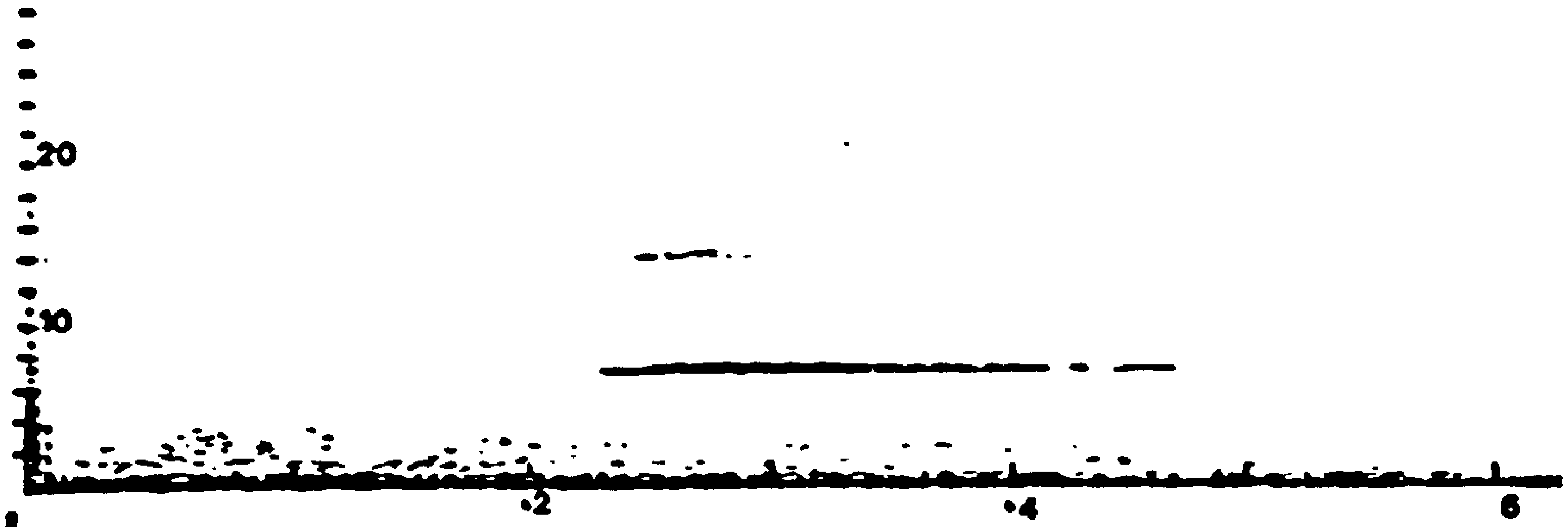
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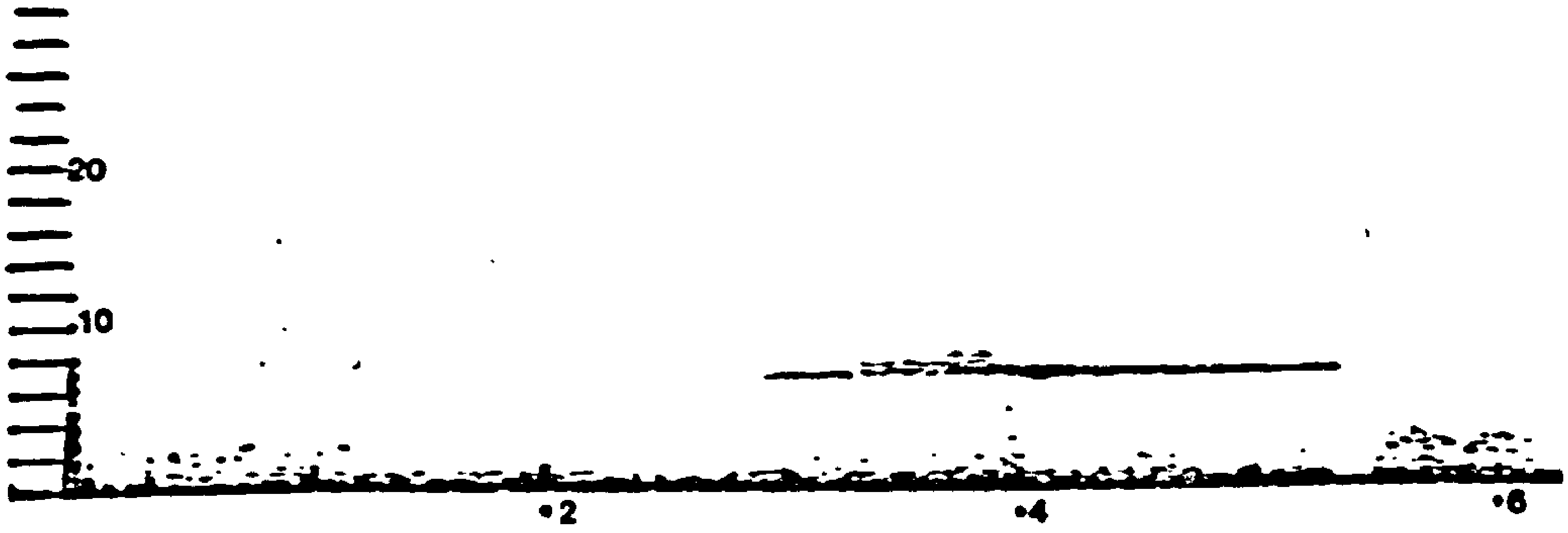
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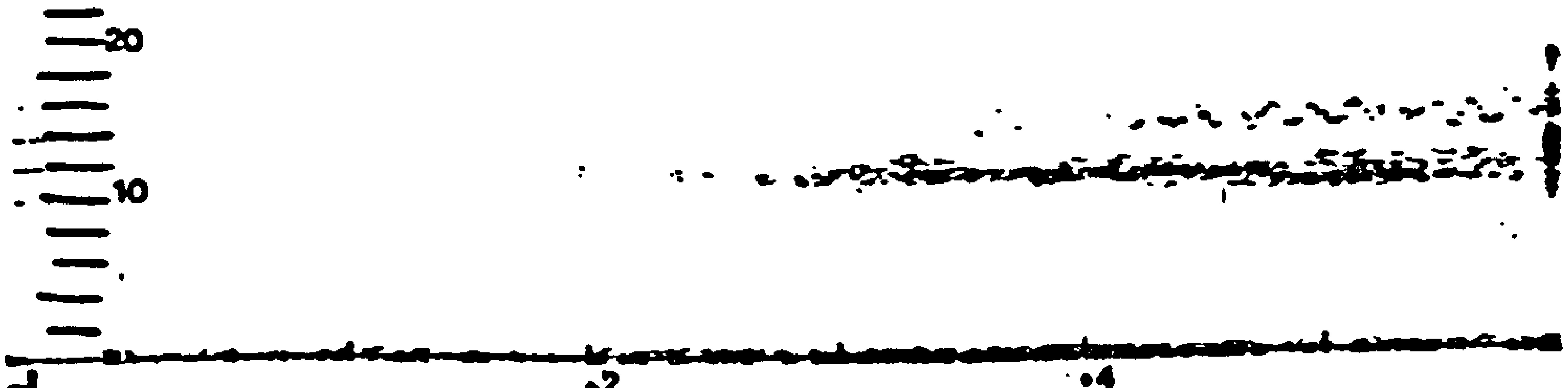
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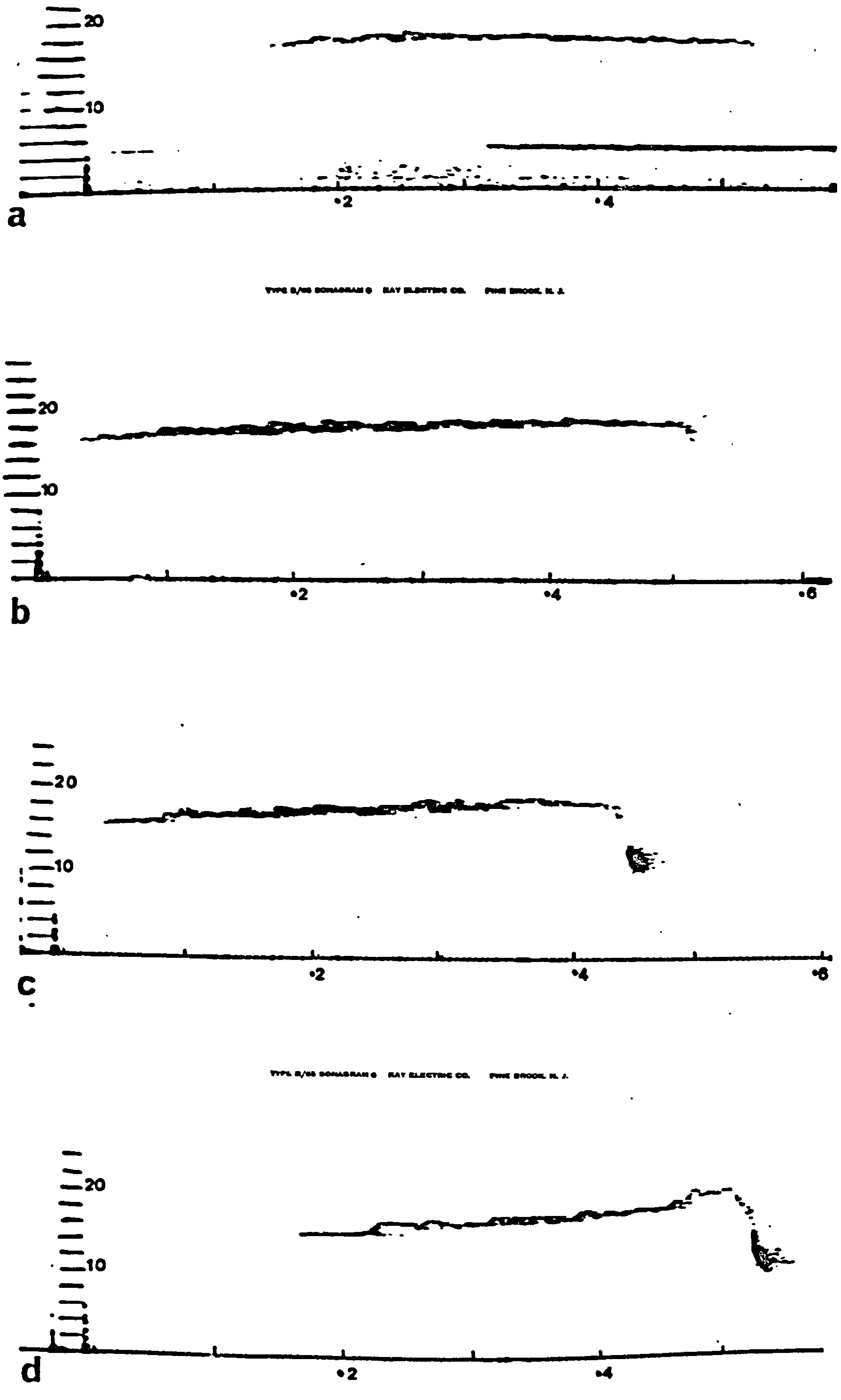
d

Figure 59

Intermediate whistle

All calls given in the same series by the same animal; calls increase in intensity from a - d. x-axis - time in seconds; y-axis - frequency in KHz.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20
10
·2
·4
d

forms, a powerful one and a soft one. The powerful version is a male call given only by the breeding male. It is given periodically throughout the year but is only frequently given during the breeding season. The frequency of calling is increased further if there is an oestrous female in the vicinity and prior to and during actual mating. Subordinate males utter soft trills occasionally, but the soft form is used more frequently by mothers whilst approaching their infants and is also given by hand-reared mouse lemurs whilst trying to reach their foster parent through the bars of the cage.

This call has a trilling structure with frequency modulating between 7 and 18 Hertz and a duration of 0.6 seconds. From the structure and frequency of this call it would appear to be designed to facilitate location of the caller. The call is given over a wide range of frequencies, exhibits great modulation of frequency and has an abrupt beginning and ending.

Gathering Call (fig. 60): This call is given around the time of "dawn" and in a sequence of three or more to form a crescendo. This call ranges in frequency between 4 and 30 Hertz, with harmonics between 16 and 20 Hertz and between 20 and 22 Hertz. The call appears to be in two parts on the sonagraph but is in fact probably one single call which ascends to a very high frequency and which thus could not be detected by the equipment used. The total duration is 0.4 seconds.

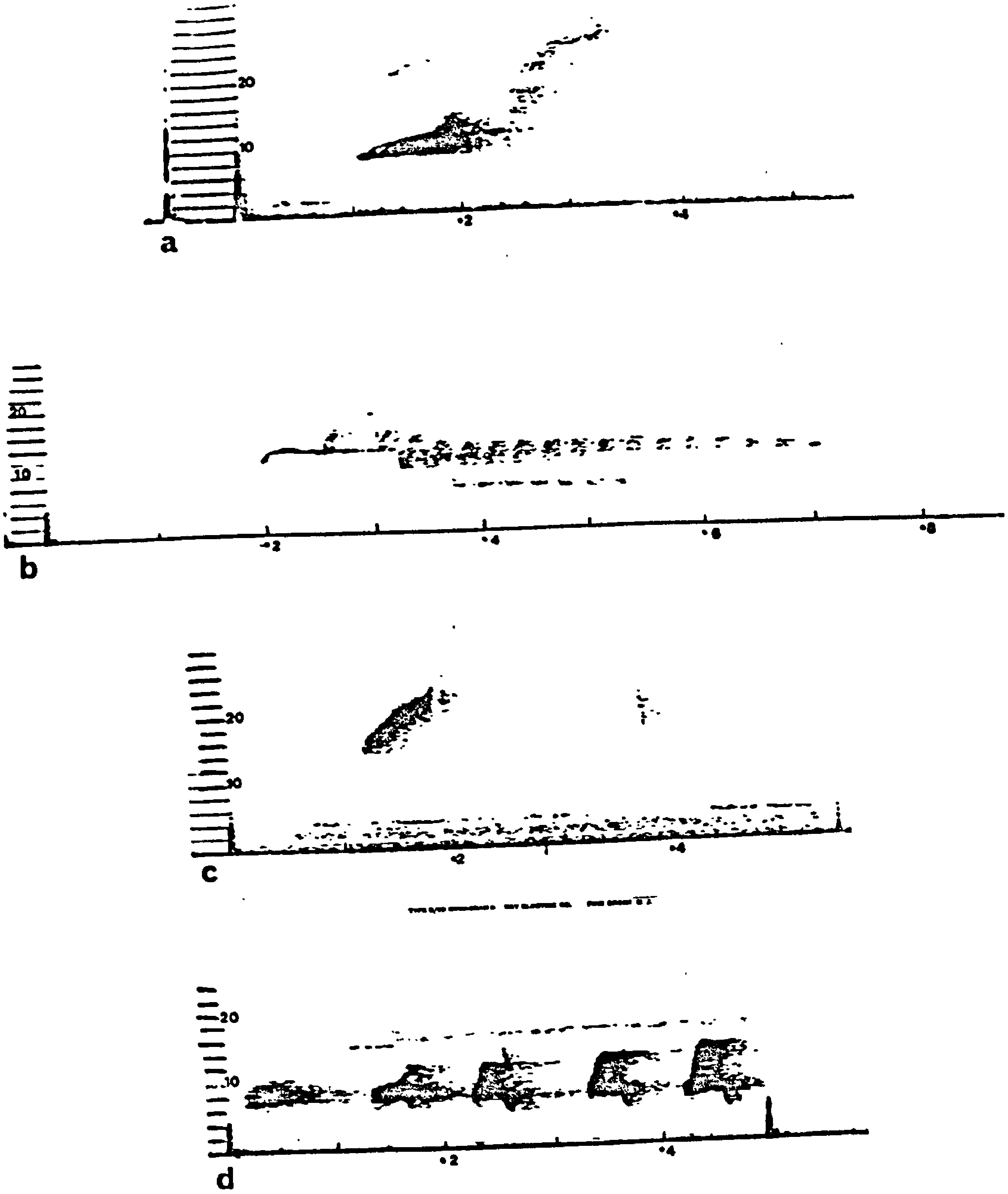
Threat Call (fig. 60): Threat calls generally begin with a hoarse growl. This growl develops into a series of sharp barks, with frequency increasing rapidly from 5 Hertz to a peak of 13 Hertz and rapidly decreasing again. Most of the energy of the call is concentrated between 5 and 10 Hertz.

In addition to the above calls, three calls of unknown function have also been noted, these are:- 1) a call similar to the clear whistle but given at a rate of 150 calls per minute (fig. 57);
2) a hoarse whistle call (fig.58);
3) isolated squeaks.

Figure 60

Calls structured to allow easy location of the caller

a - gathering call; b - trill call; c - mating call of rufous mouse lemur; d - threat call
x-axis - time in seconds; y-axis - frequency in KHz.


20
10
·2
·4
a
20
10
·2
·4
·6
·8
b
20
10
·2
·4
c
20
10
·2
·4
d

3.3. A Comparison of Rufus and Murinus Mouse Lemurs

Differences have been noted in the vocal behaviour of the rufus and murinus mouse lemurs. The most marked of these is the relative lack of vocalisation in the rufus subspecies. Thus, with the exception of the infant vocalisations and threat calls, all the calls described above appertain only to the grey mouse lemurs. This lack of calling behaviour was also noted in a rufous hand-reared infant. While this infant gave purrs and elimination calls, it proved impossible to elicit a distress call from it after the age of 10 days. However, it is possible that this call could be ultrasonic in older rufus infants.

A second major difference between the two subspecies is the absence of the trill call from the rufus lemur's vocal repertoire. In similar situations the rufous males gave a soft squeak (fig. 60), also structured so as to facilitate the location of the caller. This call is of shorter duration than the trill call but appears to be within a similar frequency range, 12-24 Hertz.

4. Discussion

The discussion of this chapter concentrates on two areas: the ways in which an understanding of communication can aid captive breeding projects, and the similarities observed in the modes of communication employed by the mouse lemur and those employed by other prosimians. This latter approach is of interest from the evolutionary point of view. It will become apparent in the course of this discussion that many similarities exist in the modes of communication of the mouse lemur and those reported in the literature for the Galago and Loris groups. These similarities are probably best viewed in the context of primate evolution. One recent opinion holds that the ancestral strepsirhine primate had many characteristics in common with the Galaginae and Cheirogaleinae (Charles-Dominique, 1977a). Thus, many of the modes of communication shared in these groups were probably present in the ancestral condition. Although such comparison, in general terms, lies outside the scope of this thesis it will be included here. The reasoning for this, other than the intrinsic interest of the topic, is that it may be important in allowing one to deduce possible cross-species similarities in the role of communication in reproductive suppression and other problems. From this it may be possible to formulate generalised rules for successful management techniques in captive breeding projects involving other species of prosimians.

The category of visual communication signals, in particular, contains many signal forms in common with other prosimians and with primates and mammals in general. For example the bare-teeth, open mouth, threat face as observed in situations of offensive threat in the mouse lemur, is found throughout the primate line, even in humans (Jolly, 1972a), and tail-lashing in situations of excitement or in a conflict situation when an animal is stimulated both to approach another individual and to flee from it, is common throughout the mammal group (Andrew, 1972). Many of the body postures observed in the mouse lemur are identical to those described for Galago senegalensis in similar situations (Andersson, 1969); these include defensive threat and attack, cringing and swaying. This would seem to indicate that the mouse lemur has no visual signals which are peculiar to itself. Presumably due to its mode of existence there has been no need to evolve a wide range of visual signals. Although the large, light coloured ear pinnae of the grey mouse lemur would appear to be well adapted for visual signalling, the ear positions recorded in this study are essentially the same as those reported in Galago crassicaudatus (Tandy, 1976). Charles-Dominique (1977a) has also reported that the ears of galagos are held in the spread position during aggressive encounters, though they are folded during actual attack to protect them during fighting.

Beyond the lack of development of new visual signals the mouse lemur also lacks some of the visual signals exhibited by the galagos. For example, tail-raising in alarming situations has been reported in Galago demidovii. Charles-Dominique (1976) reported the instance of a male dwarf galago holding its tail vertical in the course of an aggressive encounter with a female. However, within this framework of absence of innovation in visual signals, it is interesting to note the situations in which the mouse lemur withdraws the corners of its mouth. As Andrew (1964) has indicated, many primate facial expressions are derived from protective responses; i.e. from movements used to protect the delicate parts of the face from injury. Eye closure or ear withdrawal are typical examples of this. The ears of the mouse lemur are held in the back and folded position and the eyes are closed when taking insect prey and during fighting (Martin, 1972a and pers. obs.). Withdrawal of the corners of the mouth is also a protective response which is seen in insectivores when sniffing a particularly strong smell or when trying to rid the mouth of a noxious substance (Jolly, 1972a, Andrew, 1963). The fear grin of primates is said to be derived from this response (Jolly, 1972a, Andrew, 1963). The use of this expression in the mouse lemur is of particular interest as it is intermediate between the insectivore and primate conditions. The male mouse lemur exhibits this expression when sniffing the scent marks of the more dominant female or when approaching her in order to mate.

Olfactory communication, as Charles-Dominique (1977a) has noted, is of particular importance to nocturnal animals. Clark (1978) has suggested that the well-developed olfactory structures of the prosimian group are a specialisation rather than a retention of a primitive characteristic and that they represent part of the solution to a communications problem posed by the ecological adaptations of these animals; i.e. nocturnal activity and spatial separation of individuals. However, this view is contrary to the accepted view that olfactory communication is a primitive retention in lemurs. The latter view is supported by the data of Martin (1979). He has indicated that the olfactory bulbs of the nocturnal prosimians are the same size relative to body weight as those of the insectivores and of representative Mesozoic mammals.

As with visual signals, many of the methods of scent production are common to other species. The discharge of odour, usually from scent glands, in fright is common to a wide range of species (Mykytowycz, 1970) and may elicit avoidance of the area by conspecifics; e.g. as in the dog (Donovan, 1967, cited in Mykytowycz, 1970). The active modes of scent deposition recorded in the mouse lemur are shared with other prosimians and, in some cases, with some of the old world monkeys. Urine-washing has been recorded in many prosimians; for example Galago senegalensis (Bearder & Doyle, 1969, Andersson, 1969), Galago demidovii (Charles-Dominique, 1977a), Loris tardigradus (Ilse, 1955) and in Galago crassicaudatus (Clark, 1978), and in some of the cebids; e.g. Aotus trivirgatus and Saimiri sciureus (Andrew & Klopman, 1974). Rhythmic micturation was observed by Ilse (1955) in Loris tardigradus and mouth-wiping has been reported in Galago crassicaudatus (Clark, 1978), Galago demidovii (Charles-Dominique, 1977a) and Galago senegalensis (Andersson, 1969). Anogenital-rubbing has also been reported in Loris tardigradus (Ilse, 1955) and Galago senegalensis (Andersson, 1969).

The methods of scent deposition in the mouse lemur are essentially primitive. No specialised scent glands, similar to those found in the chest patch of Galago senegalensis (Andersson, 1969), occur in the mouse lemur. However, those areas of skin which are rubbed onto the substrate, namely the cheek and scrotum, are characterised by large apocrine glands. These large apocrine glands are similar to those found in the anogenital region of Perodicticus potto (Montagna & Ellis, 1959) and in Galago crassicaudatus (Montagna & Yun, 1962). These authors have suggested that the apocrine glands they described had a scent-producing function.

It is suggested that the forms of marking behaviour observed in the mouse lemur, urine marking, mouth-wiping and anogenital-rubbing, are probably representative of those occurring in the ancestral primate condition. They are all relatively simple forms of marking and are all associated with common body products: saliva, faeces, urine and vaginal secretions. It is possible that scent glands were developed later in the course of evolution to enhance the marking potential of these substances.

Before examining vocal communication, it is necessary to indicate the possible functions of the olfactory signals observed in the mouse lemur. As Charles-Dominique (1977a) has noted there is always a problem in the interpretation of olfactory marking behaviour as it is so alien to us. Schilling (1979) has listed many factors which complicate the interpretation of olfactory signals. These include: the spatio-temporal nature of the signal, which makes it difficult to determine whether the signal is followed by a response; the need of a receptive conspecific to respond to the signal; the difficulty in specifying through observation alone the scent which serves as a signal; the possible visual significance of the marking procedure.

The approach adopted to interpret olfactory communication in this study was to look at the areas marked and the frequency of marking in different situations. However, in order to understand which areas were marked it was first necessary to understand cage use, particularly in the group situation. Cage use in this situation coincided with the use of territory postulated by Hediger (1955), with different activities occurring in different parts of the cage. It is also reminiscent of the situation found in the field (Martin, 1972a) with separate female home ranges being overlapped by the male home range.

From the data collected it would seem that marking does not have a territorial function as few of the scent marks are deposited around the perimeters of the cage. This is contrary to Charles-Dominique's (1977b) findings for Galago alleni in the field. However, as Schilling (1979) has emphasised, caution must be used in interpreting the territotial significance of marking behaviour based solely on data collected in captivity. The size of the cages in which the animals were housed bore no relation to the size of their natural territories.

Marking behaviour does not appear to have a trail-laying function in mouse lemurs as not all of the main routes used by the animals are marked. However, in both the group-housed and the individually-housed situations marking does seem to coincide with the most frequently used parts of the cage and thus within those areas where the individual concerned is most likely to be encountered. This relationship between marking behaviour and cage-use was more apparent in this study than that reported by Doyle et al. (1975) between urine-washing and cage-use.

From the results obtained there is clearly a relationship between marking frequency and dominance in the group situation. This relationship is reflected most accurately in the frequency of occurrence of urine-based scent marks. This relationship between dominance and urine-washing in mouse lemurs was also noted by Doyle et al. (unpublished paper). Further, as can be seen from the lowered frequency of marking exhibited by the dominant Jersey female after removal of her subordinate, marking frequency is in fact related to dominance over individuals of the same sex.

Similar elevated levels of marking have also been reported to occur in dominant female common marmosets, *Callithrix jacchus*, in situations where there are more than one female present in the group (Epple, 1970).

The increase in marking in the nervous females is not necessarily at odds with the relationship between dominance and marking. The marking in the nervous females may have been induced by fear of the observer and may have occurred less frequently at other times. Presumably fear can induce a different scent in the urine and thus these scent marks could have an advantage in warning other individuals of danger in the vicinity. In addition, urination in response to fear is a common phenomenon throughout the animal kingdom.

Oestrus also apparently increases marking frequency in the mouse lemur. This situation would seem at first sight to be the converse of that found in *Galago senegalensis* (Doyle *et al.*, 1967) and *Galago crassicaudatus* (Clark , 1978) in which urine-marking decreases at oestrus. However, close examination will reveal that this is not the case. The results presented in this chapter indicate that it is only the frequencies of anogenital-rubbing and mouth-wiping which increase at oestrus. Urine-washing decreases in frequency at this time. It might be anticipated that urine would be a good medium through which a female mouse lemur could advertise her reproductive condition (it is in the urine that the various hormones are excreted from the body). Should this be the case it could be argued that urine-marking decreases at oestrus because the female needs to limit the advertising of her condition to certain males. However, this is felt to be unlikely in view of the fact that the subordinate female in the Jersey group did not urine-mark at all at this time and secondly because at peak oestrus no urine-washing is observed. In addition, as female receptivity is of short duration (see Chapter IV, section 2.1.), if she limited the distribution of signals indicating her oestrous condition the male might not detect the signals until it was too late. It is therefore suggested that the decrease in urine-marking may reflect the need in the female not to express her dominance at this time and thus to facilitate the approach of the less dominant male. Information of reproductive condition is provided by anogenital-rubbing and possibly by mouth-wiping. The fact that anogenital-rubbing occurs only infrequently in the female except when she is in oestrus would tend to support the contention that this method of scent-marking provides information on reproductive condition. In addition, should vaginal secretions form a constituent part of the olfactory signal produced by anogenital-rubbing in the female, the fact that the vulva is sealed except during oestrus and parturition would limit the deposition of the vaginal constituent to those periods when the female was reproductively active. The identification of receptivity in the female based on an odoriforous vaginal discharge at oestrus has been suggested in other prosimians, including for example: Galago senegalensis (Lowther, 1940), Galago crassicaudatus (Jolly, 1966), Nycticebus cougang and Loris tardigradus (Seitz, 1969) and, Lemur catta (Evans & Goy, 1968) (all cited by Schilling, 1979).

The relationship between mouth-wiping and the indication of oestrus remains obscure unless mouth-wiping during oestrus has a scent collecting rather than a scent depositing function. Thus the female may be collecting her own scent, indicating oestrus. Should the female then encounter a male both facial sniffing and genital sniffing of the female by the male would convey information as to her condition. However, the possibility that a chemosignal (under the control of sex hormones) is present in the saliva or in the secretions of the facial glands should not be ignored. A chemosignal under androgen control in the saliva of male mice is postulated by Lee & Ingersoll, (1979).

To summarise, marking in the mouse lemur tends to occur in those areas where the individual concerned is likely to be encountered or in those areas in which a suitable recipient is likely to encounter the scent. Urine-based marks provide information, particularly in the group situation, on the dominance status of the individual depositing the mark. This is most evident in the presence of other individuals of the same sex. In the individually-housed situation such marks, even if they do not carry information on status, probably do indicate the propensity of the marker to behave in a dominant fashion. Conversely, urine marks can also carry information on the presence of alarming stimuli in the vicinity. Urine-based marks decrease at oestrus presumably to facilitate the approach of the less dominant male while information on reproductive status is provided by anogenital-rubbing and possibly mouth-wiping.

The calls described in this chapter have, in many instances, also been reported in the field by Petter (1962) and Martin (1972a). In addition, Petter & Charles-Dominique (1979) have also published descriptions of some mouse lemur calls together with some sonograms which agree with the findings in this chapter. The following table (37) equates the calls in the different studies.

The only differences which arise between the data presented in this chapter and those available in the literature are in the infant distress call and in the interpretation of the high intensity version of the intermediate whistle.

Only one type of infant distress call was noted in the current study. Thus, there is no evidence to support Martin's (1972a) report that there are two types of distress call produced by infant mouse lemurs which differ from each other in pitch and in the situation of elicitation.

Petter & Charles-Dominique (1979) have reported a call which would seem to be identical to the high intensity version of the intermediate call discussed in this chapter. However, they have attributed an alarm function to this call, an interpretation of function which would not agree with that presented in this chapter.

Table 37

Mouse Lemur Vocalisations

Author	Petter	Martin	Petter & Charles-Dominique
infant distress call	call 6	infant distress call	summoning call of infant
-			
purr			purr
-			
disturbance call			alarm call, short whistle of elevated frequency
-			
clear whistle		high-pitched squeal	
-			
intermediate whistle	call 1	high-pitched squeak	distant communication calls
-			
intense version of intermediate whistle			short reinforced whistle
-			
trill	call 4		mating call
-			
soft trill			adult contact call "fee-tsi"
-			
gathering call	call 3	crescendo call	
-			
threat	call 5	explosive snort	contact rejection call
-			

This call was always heard in series with the intermediate whistle (the distance communication call of Petter & Charles-Dominique, 1979). This form of the call apparently developed when the call was being given at maximum intensity. As such this call is not feld to have an alarm function but rather is given to facilitate the spacing function of the basic call. (See below for discussion of call structure relative to its function).

As with visual signals, some categories in all mammals; for example, the secream in association with fear. Purring in situations of physical well-being is also widely found amongst mammals. Other signals are shared with the galago and loris groups; for example, a hoarse growl given in threat (Charles-Dominique, 1972a). In addition to the similarity in the actual call there is also a similarity in the organisation of the calls. This is indicated by the fact that mouse lemurs and the lesser bushbaby, Galago senegalensis (Andrew, 1963), use one call both as a mating call and when trying to reach a desired object which is denied to them; e.g. a human foster parent who is outside the cage. In both species the vocalisation is accompanied by withdrawal of the corners of the mouth. Generally, however, the basic call types vary between the different groups. Both mouse lemurs and galagos give click calls. In the galago these clicks are given by the infant when it is away from its mother or at the sight of an unfamiliar object (Andrew, 1964), whereas in the mouse lemur they are given in situations of physical well-being. However, in Galago demidovii clicks are also given by the infant in the nest.

The structure of a vocalisation must be taken into consideration when deciding on the function of a particular call. According to Marler (1955) lower pitched calls, with modulating frequency and with a sudden beginning and ending, are those calls by which the caller is most easily located. The converse is true of those calls by which it is extremely difficult to locate the caller. Thus, from the sonograms of the mouse lemur vocalisations it can be said that the infant distress call, the trill, the gathering call and the threat call and also the mating call of the rufous mouse lemur are all structured to facilitate the location of the caller. This as also true of the intermediate whistle call when given at high intensities. All other mouse lemur calls are structured so as to camouflage the position of the caller. This camouflage is most apparent in the disturbance call in which chorusing acts to enhance the cryptic effect.

The frequency at which a particular call is given affects the attenuation of that call. McGeorge (1978) has provided data on the attenuation of various frequency sounds in the gallery forests of Madagascar. Unfortunately her work deals only with sounds of between 0-6 Hertz. well below the calling frequencies of most mouse lemur vocalisations. However, McGeorge (pers. com.) has indicated that sounds of the frequency range of most mouse lemur vocalisations do attenuate rapidly. However, the lower notes of all these calls, which are structured to facilitate the location of the caller, lie within the frequency range of low attenuation in nocturnal calls. The exception to this is the infant distress call which, despite being structured so as to facilitate the location of the caller, is given at high frequencies, presumably so as not to attract the unwanted attention of predators.

Generally the calls of the mouse lemur are higher pitched than those of Galago senegalensis (Andersson, 1969) and less harmonically structured than those of Galago demidovii (Charles-Dominique, 1977a). As can be seen, many of the mouse lemur calls are pure tonal whistles which are very similar to the calls of birds and of marmosets (Pook, pers.com.). The frequency and structure of these calls is probably a function of the environment in which the mouse lemur lives and the necessity for the location of a small animal to remain undetected by predators.

The differences noted in vocalisation between rufous and grey mouse lemurs are of particular interest. The absence of many of these vocalisations in the rufous mouse lemurs must not be taken to indicate that the rufous mouse lemur does not produce the same range of vocalisations as the grey mouse lemur, but only that it does not do so in captivity. This absence of vocalisation in the rufous animals cannot be accounted for by their lower numbers as the three grey mouse lemurs maintained in the Observation Room were more vocal than the seven rufous mouse lemurs housed in one room in University College. Whether this relative lack of vocalisation has any adaptive significance is difficult to ascertain. However, the difference in mating calls between the two sorts of mouse lemur is significant and may act as a separation mechanism between these two sorts of mouse lemur.

This difference, together with other evidence for reclassifying the rufous and grey mouse lemurs into separate species, will be discussed in more detail later (Chapter X). However, the functional reasons for these differences in vocalisation patterns must be considered. It may well be that the absence of vocalisation in the rufous form may be a reflection of the rain forest habits in which high-pitched calls would attenuate too rapidly to be of use. It is however, impossible to tell whether the difference in mating calls is also related to this habitat difference.

From the data presented in this chapter it can be seen that in general the modes of communication used by the mouse lemur are not very specialised. Most of the modes of visual and olfactory communication are found throughout the prosimian group. It is suggested that these were also present in the ancestral prosimians. Vocalisation shares less affinity with the other prosimians and is probably adapted to the mouse lemur's environment.

As was indicated in the introduction to this chapter there are three principal ways in which an understanding of the communicative signals of a particular species can aid in the captive breeding of that particular species. The first is of a diagnostic nature while the other two could aid more specifically in the prevention of problems.

One of the four aspects of the act of communication as listed by Marler (1955) is motivation or the "feelings" of the animal sending the signal. In other words a communicative signal reflects the "emotional" condition of the signaller. Thus the monitoring of signals can be used to determine the state of fear or disturbance of the animals in question. Should it then be determined that these types of signals occupied an undesirably large proportion of the animals' time or of their repertoire of captive signals, steps could be taken to alter the environment so as to reduce fear or disturbance-producing elements. In the case of the mouse lemur the frequency of occurrence of fear or disturbance calls could be monitored to this end. In addition, the continuous adoption of the cringing posture in the male was taken to indicate that it was time to separate him from those females to which he had been introduced for mating purposes. The observations on ear positions could also be important in this context: in particular the horned ear position. The horned ear position was generally adopted in situations of uncertainty, such as when a subordinate individual was being approached by a dominant one or when two unacquainted individuals attempted an amicable interaction. This same ear position is also seen in non-social situations such as when giving the disturbance call or when confronted by an alarming stimulus. Thus, its occurrence may be said to be associated with a low level of stressful arousal. It is therefore suggested that the frequency with which this ear position is adopted may be used to quantify stress in rather the same way as von Holst (1974) used the occurrence of piloerection along the tail of the tree shrew, Tupaia belangeri. It may also be possible to use the technique postulated above for mouse lemurs on other prosimians: for example, Tandy (1976) indicated that an ear position, similar to the horned position of the mouse lemur, is adopted by Galago crassicaudatus in situations of uncertainty.

An understanding of communication can also aid in the elimination of certain undesirable characteristics. If it is known that certain communicatory signals elicit an undesirable response in the recipient of that signal, steps can be taken to ensure that the signal is not elicited or to limit the sphere of influence of that signal; i.e. to house the animals in isolated groups. In the case of the mouse lemur, the disturbance call has been observed to disrupt maternal care and it has been postulated that the trill call may be instrumental in testis suppression in male mouse lemurs (See Chapter VIII, sections 3.4. and 5.). As it has also been observed that the disturbance call is elicited by sudden noise or other startling factors in the environment, limiting the occurrence of these should also limit the occurrence of the disturbance call. It has also been postulated that the affect of the disturbance call is magnified by chorusing; therefore, if the animals are kept in small groups this affect should be minimised. In addition, small group size or small numbers of animals housed in a single room would minimise the sphere of influence of both the disturbance and trill calls by limiting the numbers of recipient animals.

Finally an understanding of communication can indicate ways in which stress can be mediated. This is particularly important in captive situations, such as those of the study colony mouse lemurs, in which the animals are individually-housed. This point is very much linked with the preceding point in which it is suggested that an understanding of communication can be used to eliminate undesirable responses in the recipient which are elicited directly by a communicative signal. In the case of the stress responses under discussion here, the undesirable responses arise indirectly as a result of the stimulation of the pituitary-adrenal axis. This division into direct and indirect undesirable responses is sometimes difficult to determine; for example, the testis-suppressing effect of the trill call discussed above. In this case it is difficult to determine whether the trill call is itself stressful,and that the testis suppression arises as a result of this stress,or whether the animals which suffer testis suppression are also suffering from stress due to the action of other agents, and that the trill call acts directly to inhibit testis development in these animals.

In the case of the mouse lemurs in this study it was observed that some specimens showed indications of social stress (see Chapters VIII and IX), despite the fact that they were mostly housed alone and often out of visual contact with conspecifics. The role of the trill call in reproductive suppression has already been discussed. However, other vocalisations are probably also important in indicating to an individual animal the presence of many conspecifics in the vicinity. In this context olfactory communication is of particilar importance. It has been postulated in this chapter that scent marks convey information regarding social status and fear. As the effects of stress are found to be more pronounced in subordinate animals (Chapter VIII) some factor must indicate relative status in individually-housed animals. As in this case it cannot be the immediate physical presence of the dominant animal which is responsible for the subordinate status of the individual concerned, it is highly probable that scent, which is clearly related to dominance in the group condition, plays an important role in the determination of relative status in individually-housed animals. The magnitude of scent production is also related to the numbers of animals housed together and could thus give an indication of crowding.

To conclude, it must be emphasised that an understanding of communication mechanisms can aid captive breeding projects. Firstly because it indicates a mechanism whereby animals which are housed individually can suffer from overcrowding. Being human we tend to consider visual signals important. Thus, if an animal cannot see any conspecifics, we tend to think of it as isolated. In the case of other species in which vocal and olfactory mechanisms of communication predominate, this is patently untrue. Having a knowledge of the actual signals employed by a particular species may aid in the construction of methods of preventing the dissemination of such signals. The frequency of vocal signals comes to mind in this context. A knowledge of the frequencies most used by a species can aid in sound insulation as well as indicating frequencies of extraneous noise which might affect the animals.

Thus the data presented here should be of use to those who will work with mouse lemurs in the future by indicating possible improvements which could be made to captive conditions. It is felt that if due consideration were given to the reduction of communication between individually-housed specimens at the planning stage of an animal colony, so that efficient isolation of the animals into small groups in terms of communication were achieved, "psychological overcrowding" and its deleterious effects could be substantially reduced.

SUMMARY

1) The study of visual communication signals centred mainly on ear positions. Three primary ear positions which are adopted during social encounters were identified: spread, horned and back and folded. The occurrence of these ear positions was correlated with the social status of the signaller and the type of behavioural interaction.

2) Olfactory communication can take two forms: the passive release of scent and active scent deposition. This study concentrated on the latter. Three prime mechanisms of scent deposition were recognised: urine-washing, mouth-wiping and anogenital-rubbing. Other mechanisms of urine deposition were noted but urine-washing was the most usual.

3) Scent marking in the mouse lemur is postulated as being primitive, as the three principal mechanisms of scent deposition involve the deposition of common bodily products: urine, saliva, faeces and vaginal secretions. The observation of enlarged apocrine glands in those sections of skin which are rubbed along the substrate during marking behaviour may indicate that these glands have been developed to enhance the marking potential of the body products listed above.

4) Study of the areas of the home cage which are frequently marked has revealed that mouse lemurs mark those areas where they are likely to be encountered by a conspecific or those areas where a conspecific would be most likely to come across the scent.

5) Various factors influence the frequency of marking behaviour. From these, certain inferences can be made as to the functions of marking behaviour. In the mouse lemur urine marks would seem to be important in expressing social status and fear, while anogenital

marks and possibly mouth-wiping marks are important in indicating reproductive condition.

6) Three infant and thirteen adult vocalisations were recognised in this study. Most of these vocalisations, with the exception of the infant distress call, the gathering call, the threat call, the trill call of the grey mouse lemur and the mating call of the rufous mouse lemur are all structured so as to camouflage the location of the caller.

7) Similarities are noted between the modes of communication adopted by the mouse lemur and those reported in other prosimians, notably the galagos. This similarity is pronounced in the modes of visual and olfactory communication. Vocal communication signals tend to differ from those used by the galagos in both pitch and harmonic structure. However, the threat call and the organisation of vocalisations are the same in the mouse lemur and the galagos.

8) Differences were noted in vocal communication between the rufous and grey mouse lemurs. The rufous mouse lemurs were observed to vocalise less frequently than their grey counterparts. In addition, the rufous mouse lemurs never produce the trill call. In similar situations the rufous mouse lemur produces a soft squeak. This difference in the mating calls of the two subspecies of mouse lemurs is taken as an indication of the need to reconsider the classification of the two forms of mouse lemur.

9) An understanding of communication signals of a species is important to the successful maintenance and breeding of that species in captivity. It is suggested that some of the stressful facets of the captive environment could be eliminated if due consideration were given to communicative signals, both as a means of diagnosing stress and as a mechanism through which the stressful influences of the environment can be transmitted.

SECTION C

EFFECTS OF CAPTIVITY

This section deals with reproductive abnormalities arising in captivity and post-mortem findings.

CHAPTER VII

THE STIMULATION OF REPRODUCTION AND RELATED PHENOMENA

This chapter sets out to investigate the possibility that environmental changes might stimulate reproduction in certain circumstances and to determine how far such changes could be considered stressful. In other words can stress stimulate reproduction? Darwin (1905) touched on this question when he observed that lions maintained in travelling menageries bred better than those in zoos. He wrote, "... probably the constant excitement and irritation produced by moving from place to place or change of air may have considerable influence on the matter" and concluded that "... a slight change in treatment sometimes makes a great difference to fertility". However, if indeed the environmental changes which stimulate reproduction can be considered stressful in the accepted sense, that is to say, that they lead to the stimulation of the pituitary-adrenal axis, then the problem arises in reconciling this observation with the classical view of stress expounded by Selye (1950) in which adrenal stimulation arising as a result of stress leads to the inhibition of reproduction.

The data obtained during the course of the study on the relationship between environmental change and reproductive stimulation must needs be very limited due both the limited number of specimens and also to the limited possibilities for producing environmental change. However, in consideration of the potential impact that the exploitation of this phenomenon could have on captive breeding projects, an extensive literature search was deemed necessary both to establish the occurrence of this phenomenon in other species and to determine whether any evidence existed linking adrenal activity to reproductive stimulation. It is hoped that the picture that has emerged, sketchy though it is, will stimulate further research into this topic in the future.

1. Changes in the reproductive cycle.

In 1974, after the mouse lemurs had been transferred from University College to the Wellcome Institute, many of them were found to be in breeding condition despite being subject to decreasing daylength (fig.61). This observation was made both in the males and the females and was accompanied by loss of body weight and increase of behavioural activity.

1.1. Males

1.1.1. Grey Males

Within four weeks of the move, 3 months before the main breeding season, all the adult males exhibited enlarged testes (see fig.61); this reproductive condition was accompanied by the characteristic weight loss observed in the normal breeding season. Maximal testis development was only maintained for 2-3 weeks, after which the testes regressed and weight was regained. The onset of testis development during the following season was delayed; maximal testicular development of the breeding grey male occurred thirteen weeks after the shortest day, five weeks after expected maximal development. Testicular development of the non-breeding grey males began even later; sixteen weeks after shortest daylength These observations would tend to support the hypothesis of a refractory period postulated in chapter III. Unfortunately, no further data on this could be obtained as all other grey males in the colony at the time were either not fully mature (see section 1.3.) or were in the Observation Room where observation on testis size was not possible.

As a result of these observations, during the following non-breeding season, in the winter of 1975, a grey male, Sam, was

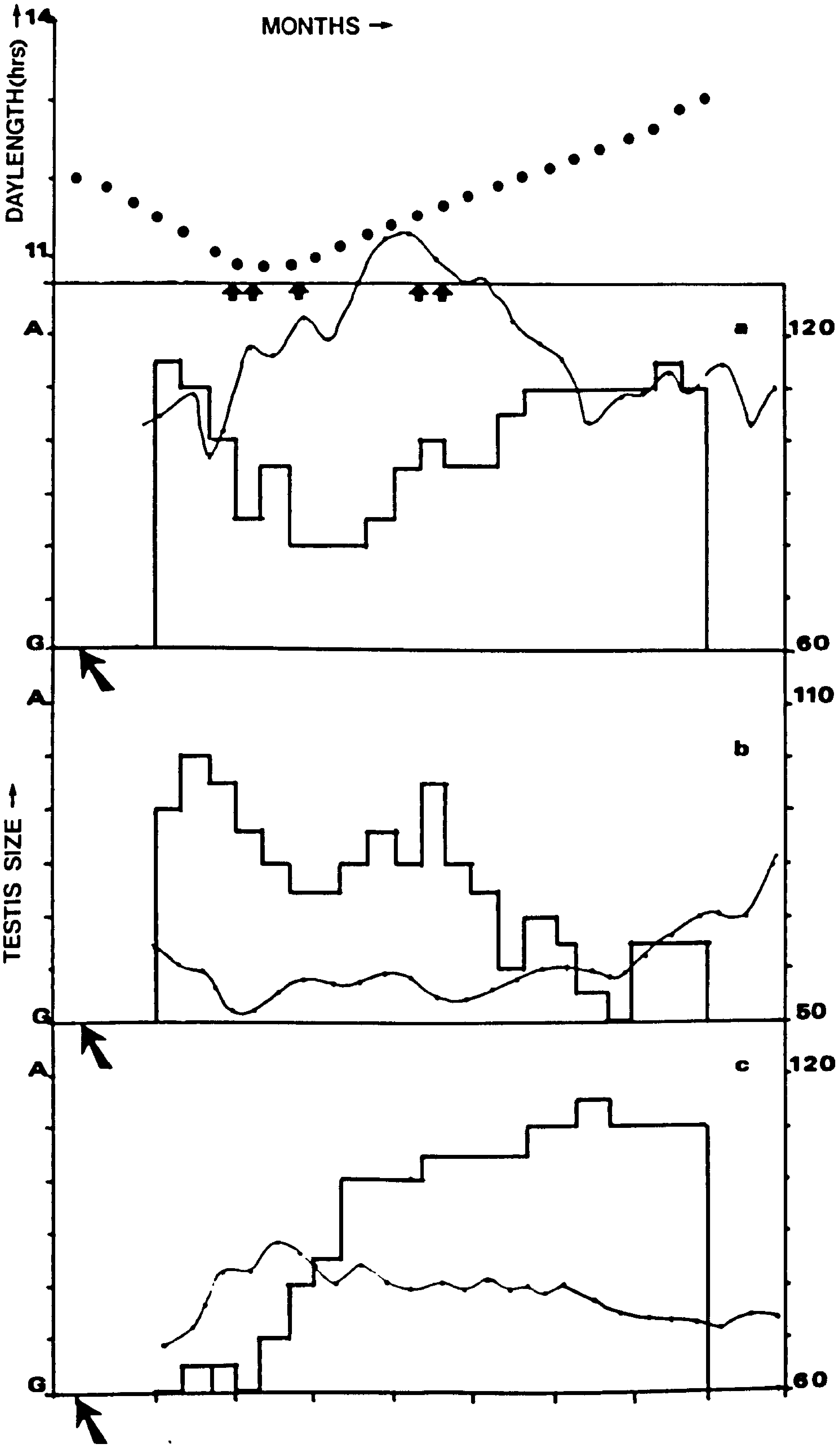


Figure 61

The effect of transfer to the Wellcome Institute on reproductive condition

a- shows weight (graph) and testis size of an adult grey male
b- shows weight and testis size of an adult rufous male
c- shows weight and testis size of a maturing male.
Oblique arrows indicate the timing of the move, and straight arrows indicate the occurrence of oestrus.

transferred from one cage room to another and placed in a cage identical to that to which he had been accustomed. Sam was observed to come into breeding condition within four weeks of this move, immediately after the period of shortest daylength and 1-2 months before the expected time of maximal testicular development (see fig.62). Again reproductive stimulation was accompanied by weight loss (see fig.62). Testicular development was maintained for 12 weeks in this case and development in the following breeding season was again retarded: maximal testis growth occurred thirty-one weeks after the shortest day, twenty-three weeks after expected maximal development. The prolonged period of reproductive stimulation observed here can probably be attributed to photoperiodic stimulation. The cage transfer of Sam was effected later in the non-breeding season than the original move to the Wellcome Institute; thus Sam's testes were still maximal when the photoperiod started to increase and it is presumed that this photoperiodic stimulation led to the prolongation of the reproductive activity which was triggered in the first instance by cage transfer.

1.1.2. Rufous males

The rufous male mouse lemurs also exhibited stimulation of testicular growth and loss of body weight in association with the transfer to the Wellcome Institute (see fig.61). However, unlike their grey counterparts, the rufous males failed to come into normal breeding condition in response to photoperiodic change later in the year. A minor burst of testicular growth occurred two months after the shortest day, but this lasted only five weeks, and otherwise there was no normal breeding season.

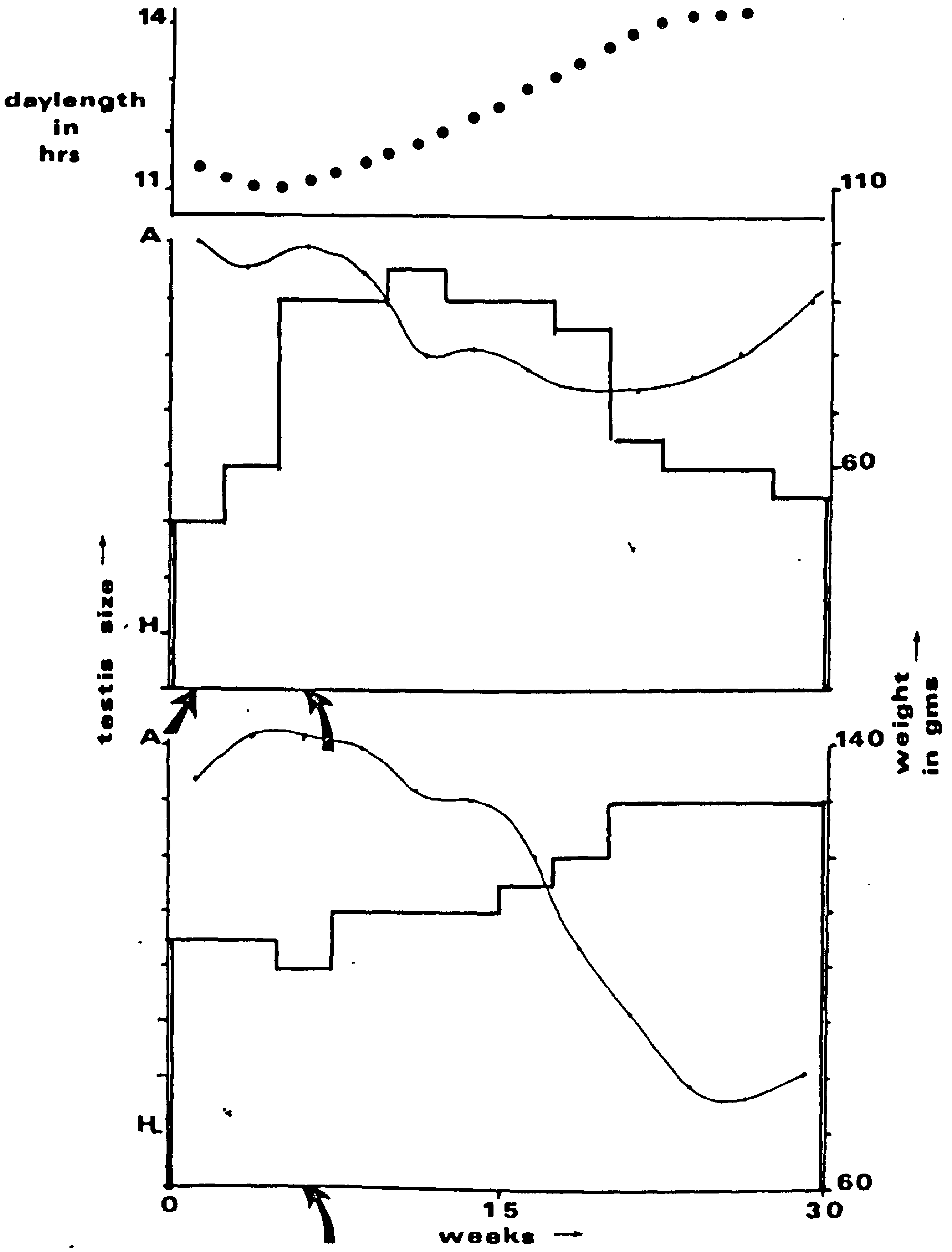


Figure 62

The affect of transferring Sam to a new cage on his reproductive state

Top figure shows weight (graph) and testis size (histogram)of Sam, lower figure those of a non-transferred male. Straight arrow indicates the transfer, the curved arrows indicate the expected onset of testicular growth.

1.2. Females

1.2.1. Grey Females

Data on stimulation of reproduction in the female grey mouse lemur are rather more limited than for the rufous females, as all except one adult female had been introduced into non-handling cages and had not yet been trained to jump onto the cage bars for condition checking (Chapter II, section 3.1.). The adult female which was checked exhibited an abnormally short period of oestrus (vaginal opening lasted only one day) within four months of the move. This oestrus was only 2-3 weeks earlier than the expected first oestrus periods of the breeding season. However, the stimulation of feeding behaviour (see section 2.), the delayed onset of reproduction during the sollowing breeding season and the fact that the first periods of oestrus occurred five weeks later than would normally be expected, indicated that the grey females were affected by the move in a similar fashion to the grey males.

1.2.2. Rufous Females

All three of the rufous females came into oestrus within 2½ and 3½ months of the move, 1½-2 months before the breeding season. One of these females cycled to come into oestrus again 62 days later. On this second oestrus, she became pregnant and successfully gave birth to an infant 62 days later. One of the other females failed to come into oestrus again with increasing daylength, but remained in the non-breeding condition for 6 months before coming into oestrus just prior to the longest day, a further indication of the refractory period. The third rufous female continued to cycle in phase with the grey mouse lemurs. It should be noted, however, that this female always exhibits abnormal oestrous cycles(Chapter VIII, section 2.4.) and has never had any breeding success.

Following the above observations, the pre-arranged transfer of a rufous female from Jersey Zoo to the Wellcome Institute was organised so that the light conditions prevailing, both in Jersey and in London, at the time of transfer were such that oestrus would not be expected. In Jersey the daylength was just increasing from the minimum and in London it was decreasing. This decrease should have inhibited breeding condition (Petter-Rousseaux, 1972). However, within four weeks of arriving in London the female came into breeding condition exhibiting a normal period of oestrus (fig.63). It was also observed that the onset of reproductive activity was slightly delayed during the following season.

1.3. Juvenile Animals

Data collected have indicated that the young mouse lemurs born in the previous breeding season (i.e., animals which were approximately 3-6 months old at the time of the move to the Wellcome Institute) were not affected by the move in the same way as the adults (see section 1.1. and 1.2. above).

1.4. Sick individuals

Unseasonal reproductive activity similar to that discussed above has also been observed in connection with illness. Two female mouse lemurs, one of each sub-species, were observed to come into oestrus during the short daylength period. In both instances this observation was made in the month prior to their deaths; 3-4 months before the expected onset of reproductive activity. One of these females was diagnosed as suffering from leukaemia and the other from a variety of problems. Both exhibited the respiratory distress commonly observed in sick mouse lemurs (see Chapter IX, section 2.1.).

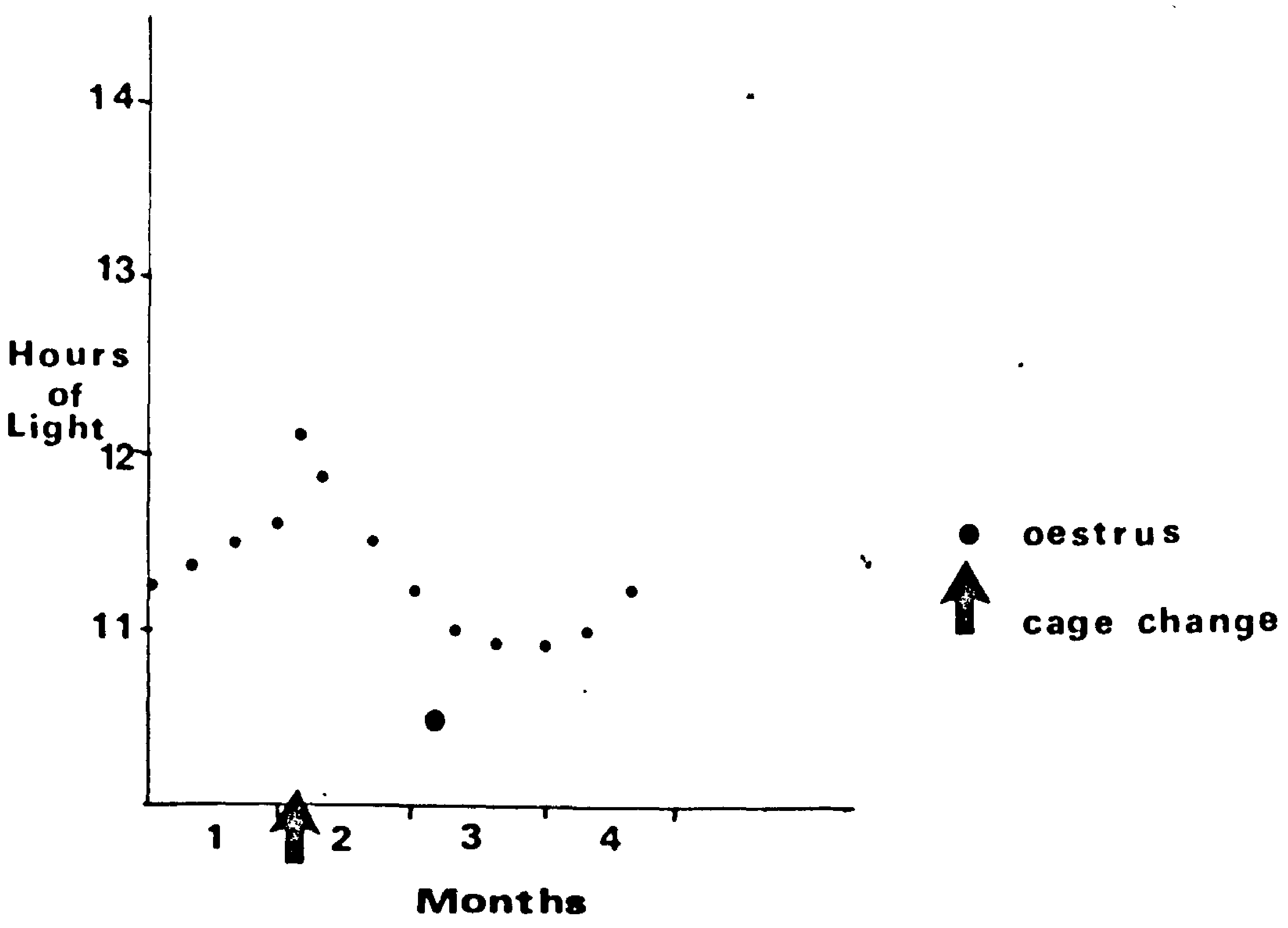


Figure 63

Stimulation of oestrus following transfer of a rufous female mouse lemur from Jersey Zoo to the study colony

Dotted line indicates the daylength change to which the female was subjected.

2. Changes in activity patterns

The transfer of the animals to the Wellcome Institute resulted not only in the changes in reproductive activity described above but also in some variations in behavioural activity levels. Routine observations of activity levels conducted on the three animals housed in the observation room indicated that two of them, the dominant female and the male, were exhibiting feeding frequencies in excess of those predicted from the photoperiodic conditions to which they were being subjected (see fig.64 and table 38).

2.1. The effect of loss of cage mates on activity patterns

Similar changes in activity patterns to those mentioned above were also recorded on occasions when individuals were removed from their cage mates. On three occasions (1 in the Jersey Zoo group and 2 in the Observation Room group) the removal of one member of a group of mouse lemurs was deemed necessary. In all cases the group was composed of one male and two females and the individual removed was a female. On these occasions the activity patterns of the two remaining individuals in each group were followed for 2 or 3 days and the results compared with those obtained before the disruption of the group. In all cases the loss of a female cage mate had no effect on the remaining females but in two of the three cases the remaining male was affected by the loss of a female and exhibited increased levels of feeding (table 39). In the third case, however, a decrease in feeding behaviour was observed.

3. Experimental Data

Two experiments were set up to investigate the phenomenon of out-of-season reproductive stimulation in more detail. These involved cage transfer and ACTH injections.

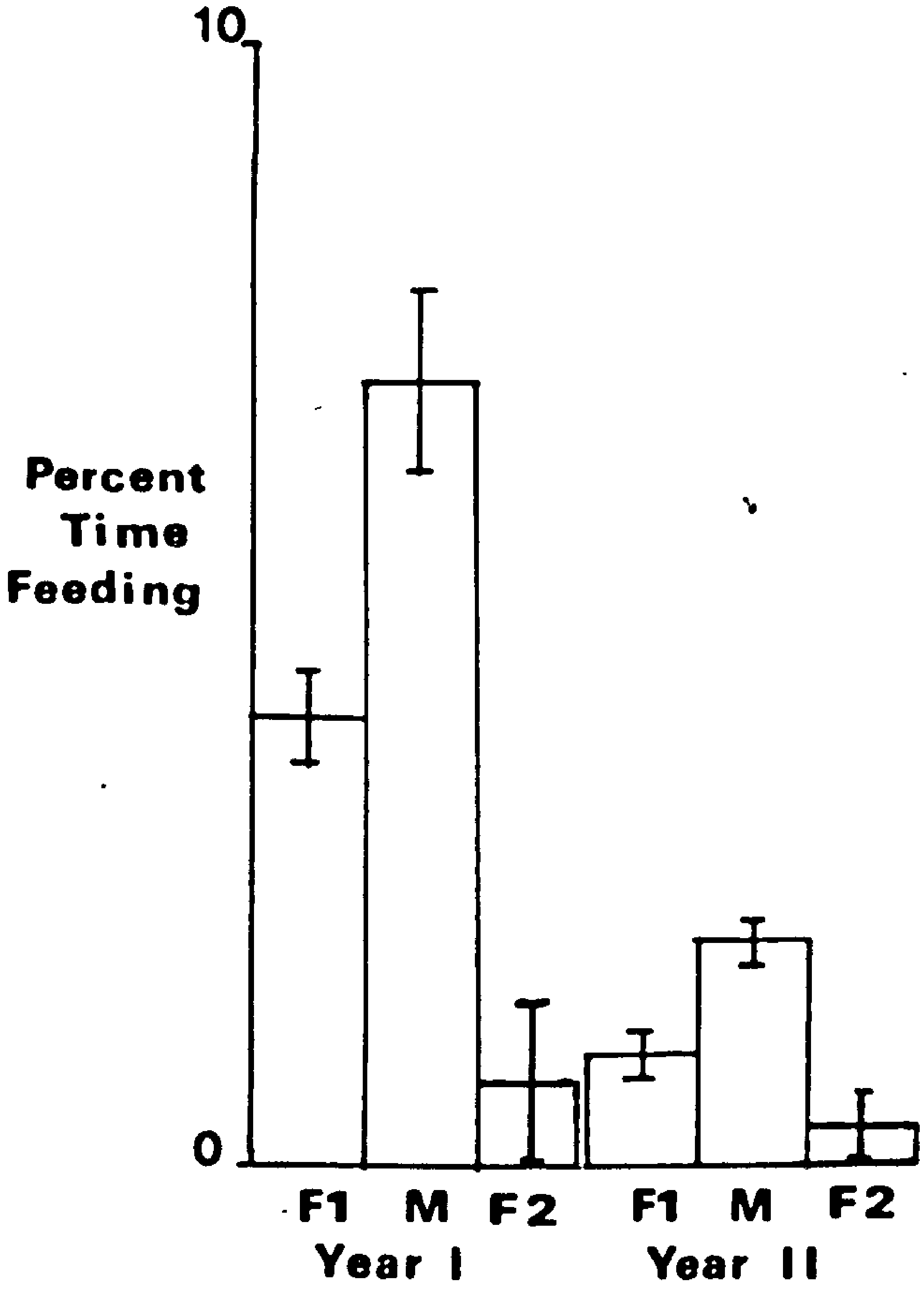


Figure 64

The effect of cage transfer on feeding frequency

Year I was the year the animals were transferred to the Wellcome Institute. Year II shows the feeding frequency during an equivalent period of a non-transfer year.

Table 38

The effect of transfer to the Wellcome Institute on percentage of time spent feeding

Date	MF6	MF8	MM3
Oct.-Nov. 1974 (after transfer)	0.83±0.83	3.67±1.47	7.33±2.80
Oct.-Nov. 1975 (normal feeding)	0.33±0.30	1.00±0.52	2.00±0.92

n, the number of observations = 12 in all cases

t-test comparing feeding in 1974 and 1975

Subject	6	t	Significance
MF6	0.88	0.57	No
MF8	1.56	1.71	Yes at $p \leqq 0.10$
MM3	2.95	1.81	Yes at $p \leqq 0.10$

Table 39

Effect of removing one member of the group on the feeding levels of the other individuals

London Group

	Dominant female	Subordinate female	male
Normal per cent of time feeding	3.5±1.0 n=27	1.3±0.5 n=27	7.85±2.2 n=27
Removal of subordinate female	5.3(o-12) n=3	-	2.7(0-8) n=3
Removal of dominant female	-	1.3(0-4) n=3	13.3(0-24)

Jersey Group

	Dominant female	Subordinate female	male
Normal per cent of time feeding	8.8(0-20) n=5	-	0.8(0-4) n=5
Removal of subordinate female	8.5(0-28) n=8	-	6.5(0-28) n=8

Figures in brackets represent the ranges.

n is the number of observations

3.1. Methods

Both experiments were implemented during the period of decreasing daylength; four months after testicular regression and three months before the onset of the next breeding season.

Cage transfer: Five males, including one juvenile, were transferred from their normal quarters to much smaller cages, 99 x 30.5 x 30.5 cms, in a new room. They were left in these cages for a period of five weeks during which time they were maintained on the same light schedule as the rest of the colony. The effects of this treatment on testis size and behavioural activity were recorded.

ACTH treatment: This treatment involved three males. Two males were injected with a synthetic ACTH preparation, Synacthen depot 1M, whilst the third male was given a placebo; this was a preparation identical in all respects to the Synacthen but lacking the ACTH component. The schedule of injections was as follows:-

Day	Test Male 1	Test Male 2	Control
1	0.03 ml ACTH prep.	0.03 ml ACTH prep.	0.03 ml placebo
3	0.06 ml " "	0.06 ml " "	0.06 "
6	0.03 ml " "	0.03 ml " "	0.03 "

The effect of this treatment on testis size and behavioural activity was also recorded.

Behavioural activity: Behavioural activity was monitored for four of the cage-transfer males and one of the ACTH-treated males. The reason behind this selection was that not all of the males were housed in suitable cages for observation, and the main aim of the experiment was to duplicate the cage-transfer situation. The behaviour of these animals was monitored for one week prior to treatment and for the three weeks following treatment. The procedure was as follows:- the occurrence of feeding, locomotion and inactivity was recorded for each individual male at 15 second intervals over a period of five minutes. Three of these five minute periods were observed in one day: the first was within the first half hour after lights out, the second was two hours later and the third two hours after that, and there were three such days of observation each week. The reason why such a short period of observation was chosen was to ensure that each batch of observations could be completed in less than 45 minutes and thus the influence of time difference between each observation in the batch was minimal. The sequence in which the different males were observed was randomised using a Latin square technique.

3.2. Results

The results of these two experiments were rather equivocal in that neither of the two groups showed any signs of testis development. However, in the case of ACTH treatment the activity pattern results confirmed the link between ACTH and feeding stimulation (fig.65 and table 40) by demonstrating an increase in feeding immediately after treatment.

4. Discussion

There can be little doubt that transfer of the mouse lemurs to the Wellcome Institute led to unforeseen changes in their reproductive condition and metabolism. These were confirmed by the observations made on Sam and the Jersey female. Before going on to consider how environmental change might be implicated in this phenomenon it is first necessary to consider whether light, the most important mechanism of physiological control in the mouse lemur, might be involved. The intensity of red light used in the "night" phase of the daily light cycle was much greater in the Wellcome Institute (where 100 Watt red bulbs were used) than in University College (where 40 Watt bulbs were used). Light intensity has been reported

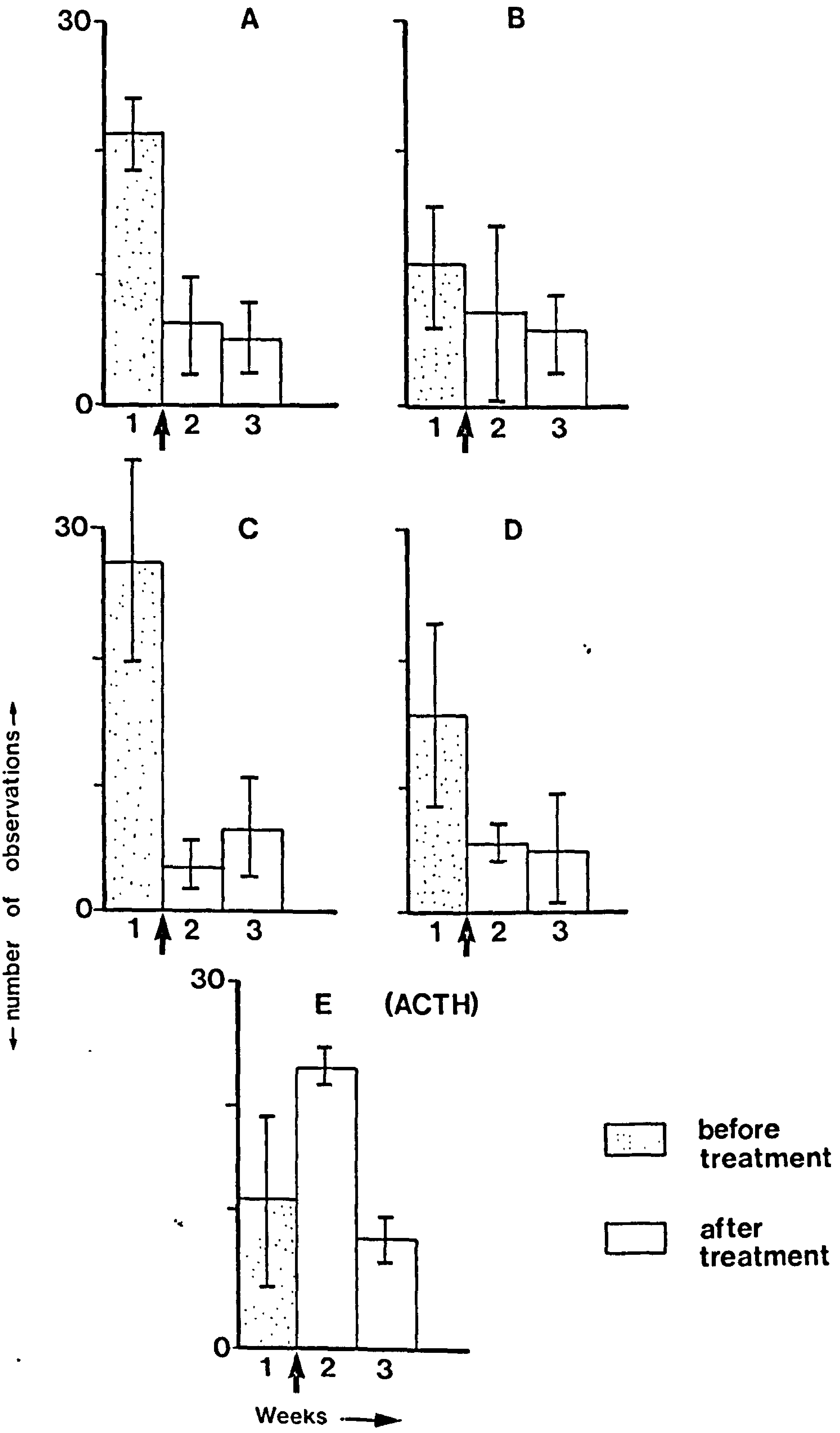


Figure 65

The effect of cage transfer and ACTH treatment on feeding in males

Arrows indicate the onset of treatment.

Table 40

The effects of ACTH treatment on the amount of feeding observed

	Period 1	Period 2	Period 3	Total
Week prior to treatment	3.0(0-9) n=3	9.7(3-13) n=3	0.3(0-1) n=3	4.3±1.09 n=9
Treatment week	8.0(5-11) n=2	17.5(15-20) n=2	0 n=2	8.5±2.9 n=6
Week following treatment	3.3(0-6) n=3	2.7(0-8) n=3	3(0-9) n=3	3.0±1.3 n=9
Two succeeding weeks	1.5(0-6) n=4	6.7(0-20) n=3	1.5(0-3) n=4	2.9±1.8 n=11

Figures in brackets represent the ranges.

n is number of observations

as important in the stimulation of reproduction in some birds (Murton, 1977). However, two observations would tend to refute this hypothesis in the case of the mouse lemur. Firstly, the behaviour of the mouse lemurs indicated that the red lighting was significantly darker than the white lighting. They did not leave their nest boxes until after the red lights came on and the timing of their behaviour was controlled by the red light. Secondly, if light intensity were responsible for the reproductive stimulation it would be anticipated that a normal breeding season would have ensued rather than the short period of reproductive stimulation observed.

Therefore, it is considered that it was the move itself which led to the observed reproductive stimulation. Mouse lemurs are not alone in the exhibition of this phenomenon, several reports of similar observations on other species exist in the literature. Petrusewicz (1957, 1963, cited by Sheppe, 1965) found that, by moving an established colony of laboratory mice to a new cage, by introducing new mice to established populations or by removing mice from a colony, population growth was stimulated. This growth was due both to a higher birth rate and to better survival of the young. Sheppe (1965), has reported that unseasonal reproduction was induced in deermice, Peromyscus leucopus, which had just been moved to a new environment. Observations of a similar nature have also been made on the prairie vole, Microtus ochrogaster. Richmond and Conaway (1969), whilst investigating the Witten and Bruce effects in this species, found that manipulation of housing conditions could stimulate reproductive activity in females. The response of the females varied from opening of the vagina to persistent vaginal cornification and receptivity. Perry (1971), reviewing the relationship between stress and reproduction, cited the case of an American team investigating infertility in sows. Animals which had failed to become pregnant over a series of matings were transferred to a university farm for closer study. The sows then became pregnant at first service, a fact which was attributed to the "natural cussedness of things".

Zoo management has also used the strategy of husbandry change to stimulate reproduction. Davis (1962) has reported the success of what he terms the "stimulus of separation" in stimulating reproduction. The technique involved separating animals which were not reproducing and then reintroducing them two or three months later. Success of this technique was reported for mandrills, *Mandrillus sphinx*, Geoffroy's spider monkeys, *Ateles geoffroyi*, and Grivet guenons, *Cercopithecus aethiops*. Davis (1962) also reported a case where the introduction of a strange female to an established, non-breeding pair of lion-tailed macaques, *Macaca silenus*, led to successful reproduction; both the original female and the new female gave birth to infants. This latter case could be attributed to social stimulation. However, it is possible that this "social stimulation" effect, is in fact a "stress" effect in response to stimuli transmitted from the pituitary to the adrenal. Perry (1971) made this suggestion to explain the oestrus synchronizing effect of introducing a ram to a flock of ewes.

From the above it can be seen that environmental changes of various sorts can lead to the stimulation of reproduction. According to Perry (1971) such changes can be considered as stressors. Certain of the observations made in the course of the mouse lemur study would tend to indicate that adrenal stimulation is associated with such changes. The stimulation of feeding behaviour which occurred in association with the transfer to the Wellcome Institute and with the loss of cage mates is indicative of this stimulation. The relationship between feeding and ACTH or adrenal activity was postulated earlier (Chapter III, section 3.) and the experimental data reported in this chapter (see later in this discussion) confirm this link. The individual variation in the levels of feeding behaviour observed probably reflect individual variation in tolerance to stressors. In the case mentioned of absence of feeding stimulation as a result of cagemate loss, this may have been due to the fact that the remaining female, at the loss of her subordinate which she chased regularly, turned her attentions to the male and began to chase him, thus allowing him less time to feed. The decrease in feeding behaviour recorded in this case may therefore not be an accurate reflection of adrenal activity. It must be noted that in the mouse lemur adrenal stimulation apparently occurred at the loss of the dominant female of the group. This is contrary to the findings of Sassenrath (1970) in rhesus monkeys, Macaca mulatta, where removal of the dominant animal from a group resulted in decreased social stress while removal of a subordinate led to an increase in ACTH in the remaining subordinates. This may be a reflection of the strictly hierarchical structure of rhesus monkey groups as compared to the loose social structure of the mouse lemur groups.

Before going on to discuss how stress might stimulate reproductive activity and how such stimulation can be reconciled with the classical picture of stress as expounded by Selye (1950), there are two further points raised in connection with the mouse lemur observations which need discussion. These are the absence of any effect observed in the young mouse lemurs, and the absence of positive results from the cage transfer experiments. In the case of the young mouse lemurs the lack of reproductive stimulation was probably due to the fact that they were too young to come into breeding condition (see Chapter IV, section 1.1.). However, it is possible that under natural conditions animals of this age leave their mothers to establish home ranges of their own and, thus, they are more adaptable to environmental change than are the adults.

The fact that the experiments were unable to confirm the original observations on the effects of cage transfer may not conflict with the hypothesis that acute stress can lead to reproductive stimulation but rather may be a reflection of problems in the experimental method. In the case of the ACTH treatment the dosage of ACTH duration of treatment chosen were arbitrary. The choice of dosage was based on the dose known to cause adrenal hypertrophy in experimental rats. Therefore, this experimental situation bears little resemblance to the hormonal changes resulting from natural causes. In addition, the dose of ACTH chosen may have been too low to stimulate reproduction. Reproductive stimulation might have a higher threshold than the stimilation of feeding. Secondly, it is possible that the stress caused by cage transfer is reflected in the stimulation of other endocrine functions. One possible site of such stimulation is the thyroid. Jenkins & Kruger (1973) have suggested that thyroid activity might be stimulated be stress and Perret (1975) has postulated that, whilst chronic stress leads to hypothyroidism, acute stress leads to thyroid stimulation. In the mouse lemur and other seasonal species, activation of both the thyroid and adrenal glands are associated with seasonality (Chapter III, section 3.). Thus, reproductive activation in the mouse lemur may involve both adrenal and thyroid activity. The role of the thyroid in reproduction is evident from the literature in that hypothyroidism is known to be correlated with infertility: e.g., goiterous cretins are sterile and the maturation of their genitals is also delayed and thyroidectomy in infancy leads to reproductive failure (Underwood, 1971). In addition, thyroxine and the catecholamines are intimately related in that thyroxine potentiates the activity of the catecholamines and vice versa. Removal of the thyroid leads to a reduction of adrenal size in rats and thyroxine treatment leads to adrenal hypertrophy (Underwood, 1971).

The lack of testicular development and the decrease in feeding observed as a result of the cage transfer experiment are more difficult to explain. The absence of females in the transfer room may have been responsible. Vandenbergh (1971) reported that the presence of adult females increases the rate of testicular development in young male mice. In addition, the cages used in the transfer were very much smaller than the mouse lemurs' home cages, thus possibly stressing the animals further and leading to a chronic stress situation and reproductive suppression. A third possibility could be that the experiment was conducted earlier with respect to the light clock than the original move to the Wellcome Institute. However, a full four months had elapsed between the onset of testicular regression and the experiment. This period was identical to that in the original transfer.

From the preceding sections one can say that both the adrenal and reproductive stimulation occurring in association with environmental change are related, but the question arises as to whether this relationship is a caused or a coincidental one. The observation of reproductive stimulation occurring in sick mouse lemurs and similar observations of reproductive stimulation in wounded prairie voles, *Microtus ochrogaster*, as reported by Richmond and Conaway (1969),would tend to indicate that there is a causal relationship between adrenal and reproductive activity. However, these observations alone do not provide sufficient data from which to draw any firm conclusions. Thus, in order to demonstrate the relationship between the adrenal stimulation and reproduction, recourse had to be made to the literature. In fact, the relationship is well documented; Parkes & Deansley (1966) wrote an extensive article reviewing this topic and to this end cited as evidence the numerous seasonally reproducing species in which corresponding adrenal activity such as that described in the mouse lemur (Chapter III, section 3.) is also observed. These species include the vole, *Microtus agrestis*, the ground squirrel, *Cittelus tridecemlineatus*, the hedgehog, *Erinaceus europaeus*, and the dormouse, *Eliomys quercinus*. In addition to these seasonal breeders, Parkes & Deansley (1966) also reported that there is an increase in adrenal size at oestrus in the dog, *Canis familiaris*.

The annual cycle of adrenal activation and its relationship to the reproductive cycle in the mouse lemur has already been discussed (see Chapter III, section 3.). In addition to this relationship, Perret (1972, 1974) has noted that female mouse lemurs in oestrus, particularly those near the time of ovulation, show a clear increase in all morphological parameters of adrenocortical function. She has also demonstrated that ACTH secreting cells in the pituitary are most active at the time of ovulation. She has postulated that these cells have luteotrophic activity. This was deduced from the timing of maximum activity and from various staining properties of the cells (Perret et al.,1971). Luteotrophic activity of ACTH cells is also found in other species (Perret et al.,1971).

Experimental work further demonstrates the relationship between the adrenals and reproduction. Testicular degeneration resulting from adrenalectomy has been observed in rats and mice (Parkes & Deansley, 1966) and adrenalectomy also alters the response of prepubertal rats to exogenous gonadotrophins (Ramaley, 1974) and suppresses ovulation in the cat (Parkes & Deansley, 1966). Results of corticosteroid treatment have been rather more equivocal; for example, Friedman et al.,(1950) found that it led to suppression of testicular function, whilst Leroy et al.,(1952) found that it led to stimulation (both cases cited by Parkes & Deansley, 1966).

Ramaley (1974) published a paper reviewing the relationship between the adrenals and gonads at puberty. As she has stated that the events leading to the first ovulation are similar to those in any oestrous cycle, although more easy to recognise, her observations are relevant to this chapter. She has postulated that adrenal activity is intimately involved with the onset of puberty and in particular that the adrenal cortex is the timer of the oestrous cycle and she has cited the results of many studies which have led her to this conclusion. Evidence for the involvement of the adrenal in the onset of puberty can be deduced from various studies on the effects of adrenalectomy or treatment with adrenal extracts. For example, Gorski & Lawton (1973) found that removal of the adrenals led to a delay in ovulation and the onset of vaginal opening in rats and Cory & Britton (1931) induced precocious puberty in mice by means of treatment with adrenal extracts. The way in which stress-induced adrenal activation can lead to reproductive stimulation can be seen from the observations of Paris & Ramaley (1973) in which they found that in some instances heat stress could induce early vaginal opening in prepubertal mice,and from the work of Lawton (1971) and Nequin & Schwartz (1971) who found that in the adult rat surgical stress at pro-oestrus advanced the ovulatory surge and LH and the onset of mating behaviour whereas adrenalectomy prevented these advances. Further, the daily cycle of adrenal activity is intimately associated with fertility; in the rat the daily corticosteroid secretion rate is at its highest on the afternoon of pro-oestrus. In addition, induced changes in this daily cycle are associated with changes in oestrous cycle (Ramaley, 1974). Other experiments on the effect of stress on rats have indicated that stress before puberty delayed the onset of puberty and reduced fertility thereafter; whereas stress after puberty increased the litter size in cycling females and induced mating and ovulation in old persistent-oestrous females (Paris & Ramaley, 1974). In all these cases there was alteration in the daily adrenocortical rythm. In the old persistent-oestrous females no daily cycle of adrenocortical activity was present. However, stress was observed to stimulate this daily adrenal cycle prior to the return to fertility.

There would appear to be sufficient evidence to indicate that not only is adrenal activity of fundamental importance to fertility but also that it can in fact stimulate reproduction. Therefore, as the environmental changes discussed in this chapter led to adrenal activation and this in turn probably led to the observed reproductive stimulation it seems that stress can be said to stimulate reproduction in this instance. It must be noted that the stress of cage transfer during the breeding season in the mouse lemur does not inhibit reproduction although there is no clear evidence that reproductive stimulation arose as a result. This hypothesis of the stimulatory effect of stress on reproduction runs contrary to the accepted theory of stress as expounded by Selye (1950) in which stress is held to inhibit reproduction (See Chapter X for a more detailed discussion of this theory). One way to explain this anomaly is to suggest that while chronic stress, such as prolonged exposure to dominant conspecifics, leads to reproductive suppression, acute stress, such as cage transfer, leads to reproductive stimulation. This hypothesis is similar to Perret's (1975) theory on the relationship between stress and thyroid activity. In addition, her theory of stimulation of thyroid activity resulting from acute stress is compatible with this explanation of reproductive stimulation. However, this explanation may be too simplistic, as various other factors have to be taken into consideration, notably the timing of the adrenal stimulation. Ramaley (1974) has noted that the timing of adrenal output of steroids can enhance or depress fertility in the female, depending upon whether the peak output of corticosteroids coincides with a critical period in the oestrous cycle. Thus, one may envisage a situation where adrenal stimulation, if well timed, may stimulate reproduction, but when prolonged or not well timed can result in the converse happening which may lead to the type of results obtained by Paris & Ramaley (1973). They reported that the same heat stress inflicted on groups of pre-pubertal mice of the same age in some instances advances the onset of puberty and in other cases delays it (this point will be taken up again in the final discussion, Chapter X).

The observation that stressful agents could stimulate reproduction was also made by Zuckerman (1952) more than 20 years ago, when he reported that many non-specific stressing agents could stimulate reproduction through ovarian hypertrophy and precocious puberty. Since that time little work has been undertaken to investigate this phenomenon despite its potential advantages to the breeding of laboratory animals. It would appear that we are dealing with a phenomenon which has been reported in diverse species and,although the evidence is not overwhelming, it does apparently exist and as such needs to be reported and discussed. It is not felt that the hypothesis given in this chapter will explain the whole story. The actions of the phenomenon do not appear to be predictable;for example when some of the mouse lemurs were transferred to Rotterdam Zoo during the non-breeding season of 1978 no reproductive stimulation occurred as a result of the move. This may well indicate the involvement of other factors the existence of which has not been taken into consideration here. However, a hypothesis which proposes that it is acute stress which can stimulate reproduction reconciles the findings with Selye's theory of stress. In conclusion it is felt that much work is needed to investigate this phenomenon which,when fully understood,could have positive repercussions on captive breeding projects in the future. In the mouse lemur such work could easily be initiated with a series of cage transfer experiments under rigorously controlled conditions.

SUMMARY

1) In some situations changing the environment can stimulate reproduction in the mouse lemur. Cage transfer was observed to lead to unseasonal reproductive activity, as demonstrated by an increase in testicular size in the males and by oestrus in the females. In some instances this unseasonal reproductive stimulation can lead to fertile mating, resulting in the birth of young out of season.

2) This reproductive stimulation is accompanied by the changes in body-weight and behavioural activity which are normally observed in the breeding season. The stimulation of feeding behaviour which accompanies reproductive stimulation may be taken to indicate that this is a stress response. The observation that sick animals also exhibit unseasonal reproduction in some instances supports the idea that stress is involved in reproductive stimulation.

3) The onset of reproductive activity in the breeding season succeeding the unseasonal reproductive activity is delayed in the grey mouse lemurs and non-existent in the reds indicating the existence of a refractory period during which reproductive activity cannot be stimulated.

4) An experiment which was undertaken to examine the effects of cage transfer under controlled conditions failed. Various possibilities are put forward to explain this. An attempt was also made to mimic the stress of cage transfer by ACTH treatment. This experiment also failed to induce reproductive stimulation. However, the stimulatory effect of ACTH on feeding behaviour was demonstrated.

5) The hypothesis is put forward that in some circumstances acute stress can stimulate reproduction. This does not contradict Selye's

hypothesis that stress inhibits reproduction, as his hypothesis is dealing with the effects of chronic stress.

6) Due to the paucity of data available from the study itself, recourse has been made to the literature to find support for the proposed hypothesis. Much evidence is available which implicates the adrenal in reproductive activity. In addition, similar reproductive stimulation has been reported in other species in situations of trauma or environmental alteration. It is therefore believed that the hypothesis, that acute stress stimulates reproduction, is supported by the data available in the literature. However, the understanding of this phenomenon is far from complete and a call is made for further research to be undertaken on this topic in the future as it could have far-reaching effects on captive breeding projects.

CHAPTER VIII

THE SUPPRESSION OF REPRODUCTION

One of the most common problems which can beset the establishment of a viable, self-sustaining colony of an exotic species in captivity is the suppression of the reproductive potential of its members. This suppression may be the result of inhibition of fertility in the male or the female, of inadequate parental care by the female, and possibly in some species also by the male, or of the production of non-viable offspring. The mouse lemur colony investigated in this study suffered from such suppression due to all the causes listed above. Thus the mouse lemur lends itself to the study of the suppression of reproduction in captivity and in this chapter this phenomenon is examined in some detail. In particular an effort is made to determine the extent to which a single underlying cause might be responsible for the various mechanisms of reproductive suppression which were observed. To this end the General Adaptation Syndrome of Selye (1950) is invoked. The details of Selye's hypothesis of a specific syndrome arising in response to stress is discussed elsewhere (Chapter X). However, his prediction that the reproductive potential of an individual would be impaired as a result of chronic stress is of obvious relevance to this chapter. Should it be the case in the mouse lemur that stress is the cause of reproductive suppression then this stress can be visualised as originating from two separate sources: the conditions of captivity *per se* and the presence of too many conspecifics in a confined area. Mouse lemurs are by nature solitary or semi-solitary; under natural conditions their main social contact is that of the sleeping group (Martin, 1972a, 1973). It is not surprising therefore that this species should be susceptible to social stress in the conditions prevailing in the colony. Even though the individuals were housed separately in most instances, they were subjected to the sight, sound and scent of conspecifics constantly and at a population density far exceeding that encountered in the wild.

This investigation centres mainly on stress, looking to the literature for supporting data from studies on other species with the hope that in elucidating the underlying stressors, it may be possible to eliminate them to some degree in the future. In this way, by manipulating captive conditions, it should prove possible to improve reproductive turnover.

1. Male

1.1. Suppression of testicular development

The annual cycle of testicular hypertrophy and atrophy undergone by the male mouse lemurs was discussed earlier (Chapter III, section 1.1.). It was also noted (see section referred to above) that not all the males attain the maximal testis size of A/B - B, as measured by the testis size chart (fig. 9, page 39, table 41). During the study it was found that only one male of each subspecies in each cage room attained this full breeding condition (table 41), and that it was only those males which developed maximum sized testes that appeared to be fertile. The other males (in the case of the greys, 1 in 1973-4, 4 in 1974-5 and 9 in 1975-6; in the case of the reds, 2 in 1973-4, 0, no normal development due to move to Wellcome Institute in 1974-5 and 4 in 1975-6) exhibited maximal testicular sizes of C or less as measured by the testis size chart (fig. 9, table 41). In addition, testicular growth both commences earlier and lasts longer in those males which develop the largest testes (fig.66). Thus, non-breeding males exhibit testes smaller than those given in table 41 during part of the breeding season.

From the above observations it would appear that the presence of a breeding male inhibits the testicular development of the

Table 41

Body weight, date of onset of testicular development and maximum testicular size

Year	Cage Room	Animal	Body weight	Duration of testis development	Maximum testis size
1973-74	University College murinus	MM4	126	19 Oct. - 31 May	A-B
		MM1	128	29 Nov. - 21 Feb.	C
	rufus	RM3	51	29 Oct. - 11 Apr.	B
		RM4	50	29 Nov. - 7 Apr.	B-C
1974-75	Room I murinus	MM4	131	2 Feb. - 23 June	A-B
		MM1	119	17 Feb. - 26 May	C-D
	no rufus				
	Room II murinus	Sam	82	16 Dec. - 29 July	A-B
		Clyde	78	13 Jan. - 29 July	C
		Plato	62	3 Mar. - 26 May	D
	no rufus				
1975-76	Room I murinus	MM4	143 133 74	5 Jan. - 14 July	A-B
		Sam	100 82 74	3 Nov.-2 Feb.& 10 Jan.-27 Sept.	A-B
		Josh	71 68 60	22 Dec. - 19 Apr.	B
		Jon	62 68 60	22 Dec. - 29 Mar.	B
		Bran	74 74 63	22 Dec. - 19 Apr.	B-C
	rufus	RM3	84	22 Dec. - 10 June	B
		RM4	76	15 Jan. - 19 Apr.	B-C
	Room II murinus	Clyde	100	26 Jan. - 5 Apr.	D
		Plato	70	26 Jan. - 29 Mar.	D
		Big Ears	73	15 Jan. - 17 May	B-C
	rufus	RM7	67	26 Jan. - 23 Feb.	D
		Henry	63	5 Jan. - 22 Mar.	C

Table 41 cont'd (explanation)

The body weights given are those obtained at the onset of testicular growth of the earliest developing male of that particular subspecies in a particular cage room. Thus, in 1974-75, the weights given for MM4 and MM1 were obtained on 2 Feb. whereas those for Sam, Clyde and Plato were obtained on 16 Dec.

In 1975-76, the male, Sam, was transferred to a new cage room. This move resulted in stimulation of his reproductive condition and suppression of that of the resident male (see Chapter VII, section 1.1.1., and this chapter section 1.1.). Thus, for 1975-76 three body weights are given for the grey males in room I, the first are those obtained on 3 Nov. (Sam coming into reproductive condition), 5 Jan. (MM4 coming into reproductive condition) and 10 June (Sam coming into reproductive condition for a second time).

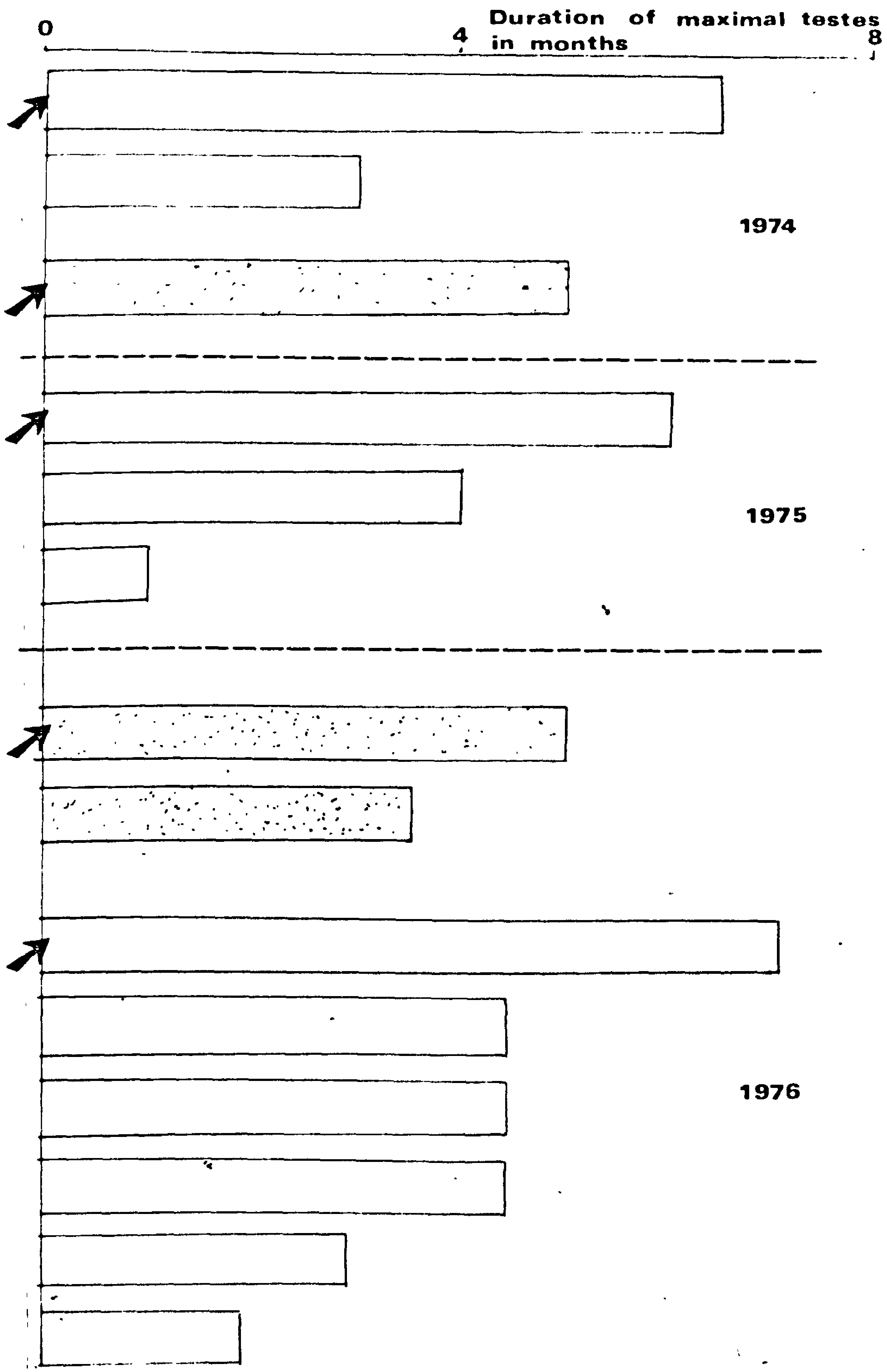


Figure 66

Duration of testis development

Arrows indicate the breeding males, stippled columns represent _rufus_ males.

other males. This conclusion is confirmed by observations made during the course of a cage transfer experiment. When the male mouse lemur, Sam, was transferred to new quarters in Room 1 in the winter of 1975, his testes were stimulated into unseasonal development (Chapter VII, section 1.). As a result of this the testes of the breeding male normally housed in that room failed to develop in phase with increasing daylength. The development of the testes of this male was retarded until after the onset of testicular atrophy in Sam. In turn, Sam, also normally a breeding male, did not come into full breeding condition again until the end of the succeeding breeding season after the testes of the original male had begun to regress (fig. 67).

Suppression of testicular development is not always caused by the presence of a breeding male. In some cases it appears that the presence of a dominant female has a similar effect. One particular instance of this situation was recorded in the Observation Room. The male housed together with two females in the Observation Room failed to show any signs of testicular development in response to photoperiodic stimulation during the second year he was housed in these conditions. This year also saw a change in the dominance structure of the group (see Chapter V, section 2.2.3.) and the development of pseudomale behaviour in the females. This observation is discussed further in section 2.5.1.1. of this chapter.

1.1.1. The relationship between testis suppression, social status and body weight

It was noted in the preceding section that testicular development commenced earliest in those males which develop the largest testes. The onset of testicular development in the other males does not occur simultaneously but rather is staggered over time and related to some extent to the final testicular size attained.(table 41).

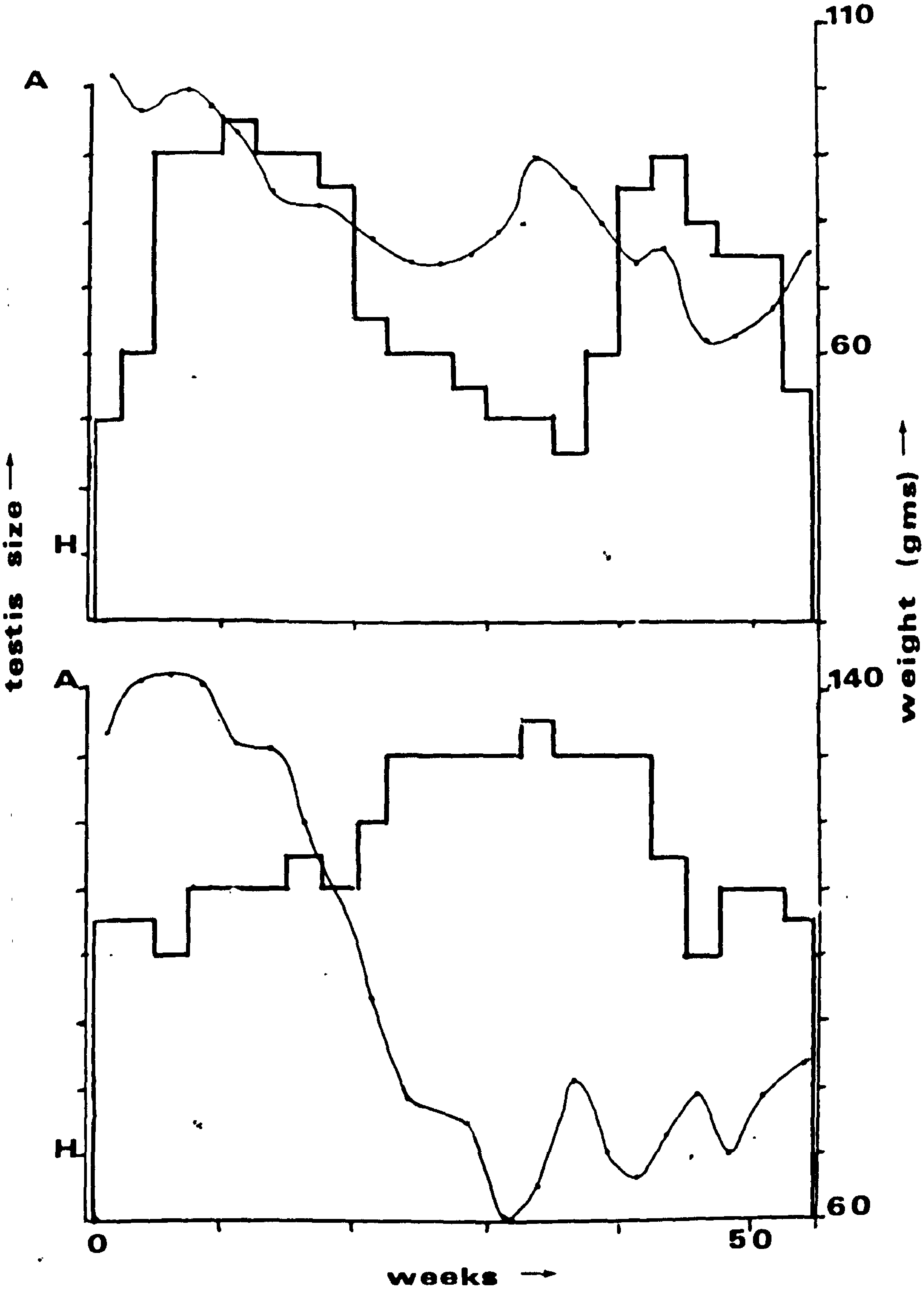


Figure 67

Sequential testis development in two males

The two males are represented in separate graphs and histograms. Testis development is represented by the histograms and body weight by the graphs. The time base is the same for both males.

As a result of this observation an attempt was made to correlate the onset of testicular development with body weight. If one assumes that testicular development is related to dominance, then one might expect a relationship between these two parameters assuming that body weight is a fair reflection of dominance status. From the results presented in table 41 it can be seen that while this relationship appears to occur in some instances; for example in Sam, Clyde and Plato and in RM3 and RM4, it was not the norm. For example in some instances males of quite different body weights commenced testicular development simultaneously. Further complications which arose were due to the presence of exceptionally heavy males in the colony. These males, which were chronically fat, were also socially subordinate and never developed large testes despite the fact that they were the heaviest males. This simple model was also not adhered to in the case of unseasonal reproduction as a result of cage transfer. In this situation the transferred male exhibits loss of body weight in association with the reproductive stimulation induced by the move. Thus he exhibits a lower body weight than that of the other males in the same room which are in their winter short daylength condition (Chapter III, section 2.). In the case of Sam discussed above and in the previous chapter (section 1.), at the time of testicular development as a result of cage transfer he had a body weight of 100 grams while that of the other adult male housed in the same room, was 143 grams. (The other three males housed in the room at this time were not yet mature). Later in that season when the male normally housed in that room, MM4, came into breeding condition, he had a weight of 79 grams compared to Sam's 69 grams, and when Sam came into breeding condition a second time at the end of the breeding season (see above) his weight was 74 grams while the other males housed in the same room ranged in weight from 63-74 grams.

1.1.2. Observations of testis suppression in wild mouse lemurs

Data collected in the field by Martin (unpublished) indicate that the suppression of testicular development is a naturally occurring phenomenon. The data show that a variety of testis sizes occur in wild mouse lemurs during the breeding season. Table 42 indicates the testis sizes found in wild mouse lemurs. In general these can be seen to be very similar to those of captive males, although the degree of suppression found in some captive males (for example, Plato) is much greater than that found in the wild.

The observation made in captivity that only the males which develop maximal sized testes breed is supported by observations in the field. Martin (unpublished) found that only those males with the largest testes were found sharing nests with females in oestrus, while the males with smaller testes were found sleeping with non-oestrus females.

However, unlike the situation found in the study colony of mouse lemurs, Martin (unpublished) found that there was a positive correlation between body weight and testis size (fig. 68). In the wild the relationship between testis size and body weight was not complicated by the presence of exceptionally heavy males which never developed large testes (see section 1.1.1. above).

In the field the heaviest body weight encountered was 85gms. and these males exhibited maximal sized testes.

2. Female

The causes of reproductive suppression in the female mouse lemur can basically be divided into five categories: uterine abnormalities, oestrus abnormalities, apparent failure to become pregnant, failure of a pregnant individual to produce full-term young and behavioural abnormalities. These categories are by no means independent: for example, oestrus abnormalities and uterine abnormalities are

Table 42

Testis Sizes found in Wild Mouse Lemurs

Male identification no.	Testis dimensions in mm.			Approx. Chart size
	Height	width	product	
1	26	24	624	B
2	24	20	500	C
3	27	23	621	B
4	28	23	644	B
5	27	21	567	B-C
6	26	23	598	B
7	27	20	540	B-C
8	28	21	588	C
9	24	20	480	C
10	26	19	494	C-D
11	24	19	456	A-B
12	29	23	667	B-C
13	27	27	567	B
14	29	22	638	B-C
15	25	22	550	B-C
16	29	19	550	B-C
17	28	19	532	C-D
18	24	18	432	C-D
19	26	18	468	B-C
20	26	21	576	C
21	25	15	375	A-B
22	25	20	500	C
23	30	23	690	B
24	25	20	500	B
25	28	23	644	B-C
26	27	24	648	B
27	25	21	525	B-C
28	27	23	621	B-C
29	26	21	546	B-C
30	27	21	567	C
31	27	21	567	B-C
32	25	27	525	C
33	28	21	588	B-C
34	28	22	616	B
35	28	24	644	B
36	27	21	567	B-C
37	27	21	567	B-C
38	28	21	588	B-C

The approximate testis chart size equivalent was obtained by comparing the scrotal area of the wild mouse lemurs and the area of the various scrotal sizes represented on the chart. (fig. 9, page 39).

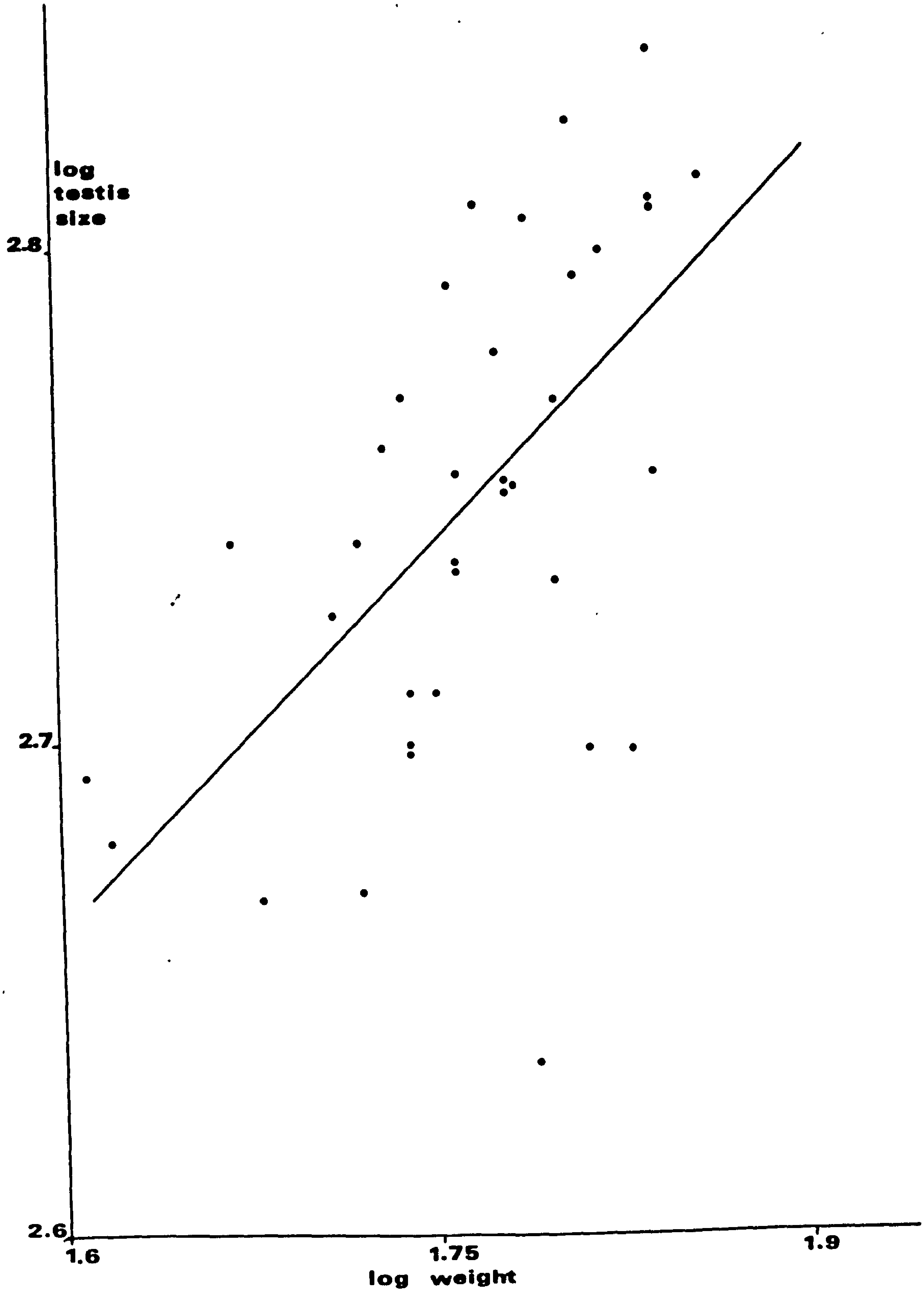


Figure 68

The relationship between testis size and body weight in wild mouse lemurs

Slope = 0.478; correlation coefficient = 0.5; n = 36; p = 0.01.

obviously related to an animal's failure to become pregnant, while behavioural problems may also account for an animal's failure to become pregnant or for its inability to maintain full-term young immediately after parturition. However, these categories will be used here for ease of presentation of the results.

2.1. Histological abnormalities of the uterus

The reproductive tracts of eight captive female mouse lemurs were examined histologically during the course of this study. Of these females five had failed to reproduce in captivity and in the case of three of these, infertility was associated with endometrial cystic hyperplasia (diagnosed by P.F. McMullin).

This is a condition in which the endometrium is much thickened and the uterine glands are distended and in many cases lined with non-secretory columnar epithelial cells (figure 69). In the case of the specimen in the figure, the overy exhibited areas of an abnormal fibrous appearance (figure 69).

Unfortunately, this condition proved difficult to diagnose in the living animal. In two cases a white vaginal discharge was noted at oestrus in these females and careful abdomal palpation can be used to detect the swollen uterus but otherwise external indications of this condition in the mouse lemut are not very marked. To date, no vaginal smears have been obtained from females which were subsequently found to be suffering from this condition. These might have given some indication of the problem.

2.2. Abnormalities associated with oestrus

The abnormalities associated with oestrus are often inter-related and include: abnormal oestrus, abnormal vaginal smears and abnormal inter-oestrus periods.

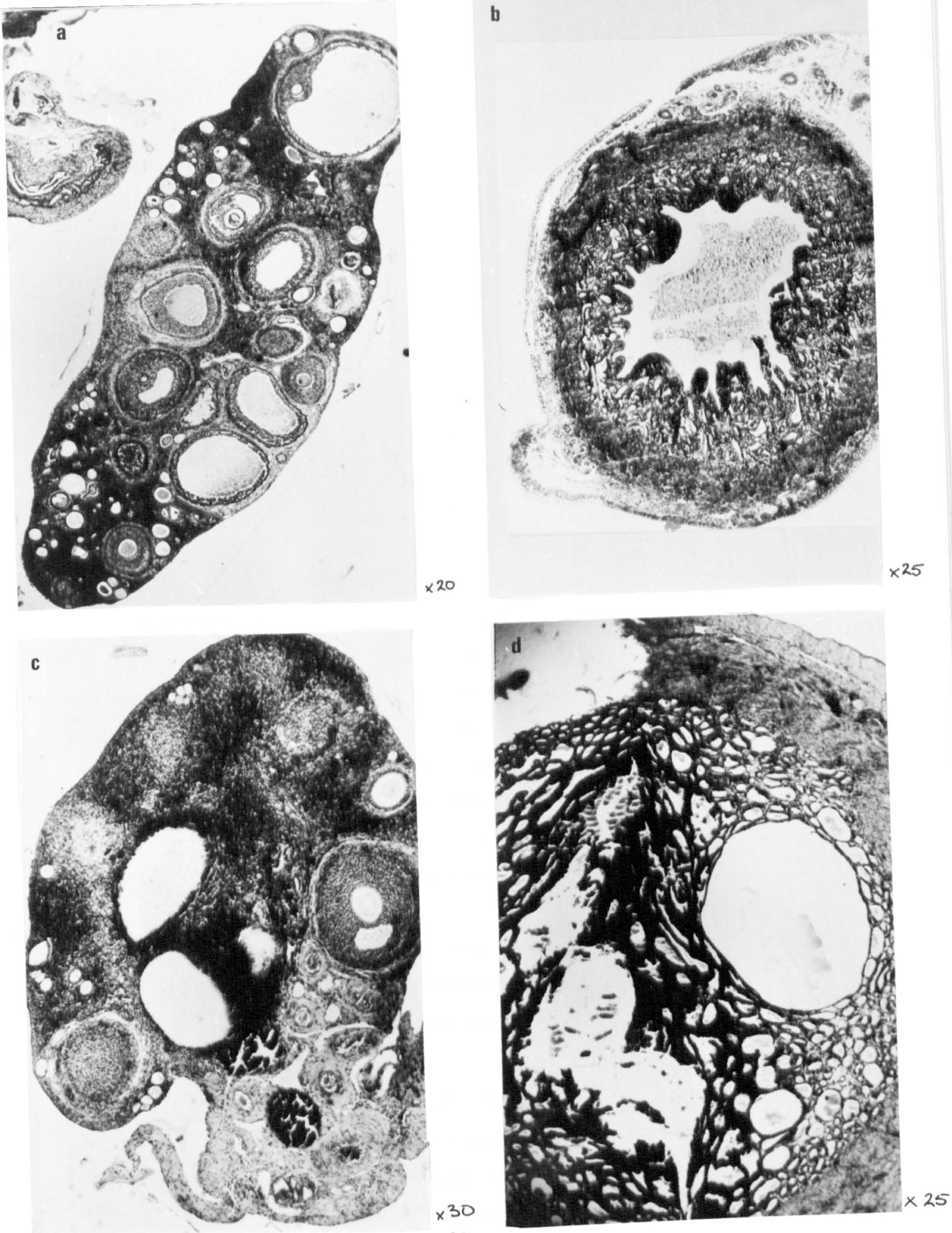


Figure 69

Uterine cystic hyperplasia

a & b - ovary and uterus of a normal female; c & d - ovary and uterus of a female suffering from cystic hyperplasia. Note fibrous appearance of ovary c.

2.2.1. Abnormal oestrus

As discussed previously (Chapter IV, section 2.2.), the normal duration of vaginal opening is 3 - 7 days. However, of the total of 48 periods of oestrus recorded during the study, seven cases were recorded in which vaginal opening only persisted for one day. Six of these cases were observed in one particular female, RF3 (a female which never produced any young during the eight years she was maintained in the colony). These abnormally short oestruses have always occurred in association with an abnormal swelling pattern. Instead of the normal pattern of vulval colouration and swelling, this female exhibited a transparent blister-like structure over the vagina prior to opening. Another case of abnormal oestrus was recorded after the transfer of the colony to the Wellcome Institute (this was mentioned earlier, Chapter VII, section 1.2.1.). In addition to these very short oestrous periods, one case was recorded in another female where vaginal opening occurred without swelling. In this case a normal oestrus occurred 20 days later during which the female conceived.

All these abnormal oestruses have proved to be infertile. No data are available on the occurrence of abnormal oestrus during the time that the mouse lemurs were studied by Martin (1971-73).

2.2.2. Abnormal vaginal smears

The female, RF3, discussed in the previous section had vaginal smears taken from her on 3 occasions: once during one of the abnormal one-day oestrus periods and twice during oestrus periods of more normal length. From table 43 it can be seen that the smear obtained during one-day oestrus had the typical appearance of the post-ovulation smear of a normal female. Although the other two smears were obtained from oestrous periods of more normal duration, in both cases the abnormal swelling pattern described above was noted. In neither of these cases were the

Table 43

Abnormal Vaginal Smears

Day	Epithelial Cells	Cornified Cells	Leucocytes	Small Epithelials
1	++++			
2	+++	+++		
3	+	+	+	++
4		+	+	+
5		debris		
1	+++			
2	++++			
3	++			
1	++	++	++	

smears normal, a paucity of leucocytes was noted in the five-day oestrus and no change in the cells recovered in the smears of the three-day oestrus were recorded: only epithelial cells were found, this is typical of an ovulatory smear.

2.2.3. Inter-oestrus periods

The normal inter-oestrus period recorded in the mouse lemur is between 48-56 days (see Chapter IV, section 2.4.). During the course of the study, of the 36 inter-oestrus periods recorded, 12 cases were noted in which the inter-oestrus period was less than 48 days: 1 of 20 days, 1 of 33 days, 1 of 36 days, 1 of 37 days, 1 of 38 days, 3 of 39 days, 3 of 42 days and 1 of 46 days (fig.20,p.94). The oestrus periods succeeding these short inter-oestrus periods were infertile (of the eleven inter-oestrus periods recorded ranging between 33-46 days, none were succeeded by pregnancy). The exception to this was the inter-oestrus period of 20 days succeeding the oestrus in which vaginal opening was unaccompanied by vulval swelling (see section 2.2., above). In this case the oestrus following the short inter-oestrus period was fertile. Usually (on seven of twelve occasions) these short inter-oestrus periods were associated with an abnormal swelling pattern, (see 2.2. above).

During the period the mouse lemurs were studied by Martin; 38 inter-oestrus periods were recorded of which nine were less than 48 days: 1 of 38 days, 1 of 39 days, 2 of 40 days, 2 of 41 days, 1 of 44 days and 2 of 45 days. Unfortunately no data are available as to the fertility of the oestrus periods succeeding these short inter-oestrus periods.

2.3. Apparent failure to become pregnant

As can be seen from table 44, which summarises the reproductive histories of all of the females in the colony, the most prevalent breeding problem encountered in the colony was apparent failure to become pregnant; i.e., the females were never determined to be

Table 44

Reproductive Histories of the Female Mouse Lemurs in the Study Colony

Female	Breeding Season					
	1971	1972(1)	1972(2)	1973/4	1974/5	1975/6
MF1	-	+(0.67)	A	-	-	d
MF2	-	+(1.0)	+(0)	+(h.r.)	-	-
MF3	-	-	d			
MF4	-	-	-	d		
MF5	-	+/S(0)	+/S(0)	++(0,h.r.)	+/S(0)	+/S(0)
MF6	+(0)	S	+(1.0)	-	-	-
MF7	-	-	-	-	+(1.0)	+(1.0)
MF8			+(0)	++(0,0)	-	-
MF9			-	+(h.r.)	+(1.0)	+(1.0)
MF10			-			
MF11					+(1.0)	+(0.3)
MF12						-
MF13						-
RF1	+(h.r.)	+(0.5)	+(0.67)	A	+(h.r.)	d
RF2	-	-	-	-	d	
RF3	-	-	-	-	-	-
RF4	-	-	-	d		
RF5	-	-	d			
RF6	-	d				
RF7	+(h.r.)	+(h.r.)	+(0)	d		
RF8			+(0.5)	died resorbing foetus		
RF9			-	d		
RF10				-	-	-

+ is a birth, - is no birth, A is abortion, S is still birth, d indicates that the female in question is dead. The figures in brackets indicate the proportion of infants surviving to the weaning stage and h.r. indicates the infants were hand-reared. ++ indicates two births in one season.

pregnant by palpation or by the visual method of determination (see Chapter II, section 3.1.). Of the 48 oestruses recorded in the course of the study, during which the female was with a fertile male, 33 were not followed by pregnancy. In 11 of these cases the female in question was suffering from one of the oestrus problems discussed above (sections 2.2.1., 2.2.2. and 2.2.3.). During the period in which the colony was studied by Martin, of the 65 oestruses observed 48 failed to result in pregnancy.

Due to the absence of a reliable, hormonally based method of detecting pregnancy in its early stages, it was impossible to determine whether this absence of detectable pregnancy was due to failure of conception or to intra-uterine loss occurring early in pregnancy. Evidence of both of these phenomena has been found in the mouse lemur colony.

2.3.1. Failure of conception

Actual failure to conceive may be due to one of the following causes: non-receptivity of the female, failure of the female to ovulate or failure of the fertilised ovum to implant. Observations made during the course of this study indicate that female non-receptivity is the cause of conception failure in some cases. Oestrus abnormalities such as those described above (see sections 2.2.1. and 2.2.2.) may be indicative of problems associated with ovulation. The absence of any hard evidence indicating a failure of implantation does not mean that this does not occur in the mouse lemurs but rather that evidence indicating the occurrence of this phenomenon cannot be obtained from simple observation.

2.3.1.1. Non-receptivity

Observations have confirmed that non-receptivity is the cause of the failure to conceive in some cases. During the course of the study, observations were made on eight oestrous female/male introductions. In three of these introductions the female remained unreceptive and aggressive towards the male despite the fact that the vulval condition of these females indicated peak oestrus. Non-receptivity does not appear to be a permanent condition, the females mentioned above have bred normally on occasions previous to or succeeding that of the observation.

2.3.2. Resorption of the embryo

As discussed previously (see section 2.3.) the low birth-rate of the mouse lemur colony could in most cases be attributed to failure of conception or intra-uterine loss. As was also discussed it is difficult to differentiate between these problems without adequate hormonal diagnosis of pregnancy. Pregnancy determination has depended on visual assessment and abdomenal palpation. These methods of pregnancy determination are unreliable in the early stages of pregnancy.

As the diagnosis of pregnancy is unreliable it is impossible to tell whether and with what frequency resorption of the embryo does occur. Evidence of its occurrence must therefore depend on those cases where it can be verified: for example, through histological evidence or through observation of resorption in the later stages of pregnancy (after pregnancy has been reliably determined).

The apparent resorption of the young during later pregnancy has been recorded on two occasions. Prior to the current study, two females which were determined to be pregnant by abdomenal palpation, subsequently failed to give birth or to exhibit any indication of abortion (i.e., a non-oestrus vaginal opening), (Martin, unpublished data). These females were assumed to have resorbed their young. These females were housed in overcrowded conditions: together with two other females in a relatively small cage.

A further case of resorption has been verified histologically. One female, RF8, which died of pneumonia during early pregnancy, was found, on histological examination of the uterus, to be in the process of resorbing three embryos.

These observations indicate that resorption of embryos does occur in the mouse lemur. It apparently occurs in early pregnancy (RF8) as well as in later stages when pregnancy can be determined by palpation. It is impossible to estimate the frequency of its occurrence in early pregnancy at this stage. However, it would seem safe to say that resorption of the young is responsible in some instances for the apparent failure of some females to conceive.

2.4. Failure of the pregnant animal to produce healthy full-term young

Reproductive failure during late pregnancy can result from four causes: resorption of the young (see the preceding section), abortion, premature birth accompanied by foetal death, full-term parturition followed by immediate death and/or cannibalism of the young (see section 2.5. below). Of the 34 pregnancies recorded in the colony (17 in the current study and 17 by Martin), 12 failed to produce live infants. On 2 occasions the foetuses were resorbed. On five occasions, the foetuses were expelled too early: at 44, 49, 50, 59, and 59 days respectively. On the remaining five occasions full-term young were presumably produced (parturition occurred after a gestation of 60 or more days) but the resulting infants were either born dead or cannibalised immediately after birth.

On seven occasions these phenomena have occurred in association with various stressors: firebells (1 case), handling (1 case), x-rays (1 case) and overcrowding (4 cases; these cases occurred in the group of four females housed together in a relatively small cage).

Most cases of foetal loss have occurred in one particular female, MF5, (5 cases). This female was a particularly nervous individual and one of the most subordinate females. Her nervousness may be related to the fact that she had been used for blood collection by heart puncture (using ether anaesthesia) on eight occasions prior to the study.

2.5. Behavioural abnormalities

This section includes abnormalities in reproductive behaviour and in maternal behaviour.

2.5.1. Abnormal reproductive behaviour

Two types of abnormal reproductive behaviour have been recorded in the female mouse lemurs: non-receptivity (see section 2.3.1.1. above) and pseudomale behaviour.

2.5.1.1. Pseudomale behaviour

Pseudomale behaviour is included in this section as it seems to be effective in suppressing reproduction in some instances. It is unknown whether this behaviour causes non-receptivity in the females but it does probably lead to the suppression of testicular development in the male. During the course of the study it has only been observed in one group of animals, those housed in the Observation Room.

During their second year together, the females in the Observation Room developed a peculiar pseudomale behaviour which became most apparent when the females were in oestrus. The females would follow behind each other in turn, anogenital-rubbing and sniffing the genital region of the female in front. The first female would facilitate this sniffing by carrying her tail higher than normal. This behaviour would continue for a variable amount of time and was either followed by a period of nest-box sharing or the females became more excited and began to grapple with each other. They appeared to hold each other with their hands and hang from a branch by their feet and wrestle. Often they would let go of the branch and fall to the floor but, remaining oblivious to the fall, would continue wrestling and rolling on the cage floor. The "trill" call which is normally only given by the grey breeding males (Chapter VI, section 3.2.) was produced throughout the interaction but was most frequent during the wrestling phase.

As mentioned above, this behaviour emerged during the second year in which the animals were housed together. Over the same period a change in the social organisation of this group was also observed. The structure of the group became much more strictly hierarchical and many more antagonistic interactions were observed (see Chapter V, section 2.2.3.). In all cases, the pseudomale behaviour described above was initiated by the dominant female.

2.5.2. Maternal behaviour abnormalities

Of the 14 gestations which have come to term during the course of the study and of the 13 gestations which came to term during the observations of R.D. Martin, 23 infants survived to the weaning stage. In some cases the infants were cannibalised at or shortly after birth (see section 2.5.1.1. and 2.5.2.2.2.), whereas in other cases the litters were abandoned (2 cases), the young were cannibalised at a later stage (2 cases) or the infants died of undetermined causes. In many of these cases it is impossible to tell whether the infants were deserted or killed because they were weak and sickly or whether they died as a result of insufficient maternal care and attention.

In the following sections, therefore, in addition to discussing actual instances of infant death, factors in the mother's behaviour which indicate that there may be problems with the infant and factors in the environment which may disrupt maternal care are also discussed. Abnormalities in maternal behaviour can be divided into those associated with birth, and those associated with care of the infants.

2.5.2.1. Birth

It was hoped that with the aid of infra-red video equipment it might prove possible to observe an habitually bad mother,(i.e., a mother which usually lost her young) MF5, giving birth. Unfortunately, this proved impossible. However, it became apparent that this female gave birth at an inappropriate time of day. Females normally give birth during the period of daylight (see Chapter IV,section 3.2.). However, this female gave birth during the dark phase of the daily light cycle. Martin (unpubl. data) has also noted that this female may have given birth during the night period on other occasions.

Despite the inability to study birth and post-partum behaviour in MF5 it is felt that some of the observations made on the birth of the last of triplets to a normally competent mother may provide some indication of the problems which may cause infants to fail to survive. In this case the mother delivered her first two infants normally (see Chapter IV, section 3.2.). The delivery of the third infant was preceded by only one obvious labour effort and followed that of the second infant by an interval of only ten minutes. The female failed to deliver the third infant with her hands and groom him as observed in the normal births. She continued to direct her attention to her first two infants and failed even to sever the umbilical cord of the third infant. The infant remained struggling on the floor of the nest-box with his mother sitting on him.

This infant was of low birth-weight, which may have contributed to the problem. However, he would not have survived had it not been for the presence of the two other siblings. These stimulated the mother's grooming behaviour which occasionally was directed to the third infant and also stimulated her to adopt suckling postures from which the third infant also benefitted.

This infant eventually died at 17 days old. His eventual demise did not apparently result from lack of maternal care but from an infection which also killed one of his larger litter mates.

2.5.2.2. Problems associated with infant care

A multitude of problems in infant care are mirrored by the mother spending too little time in the nest-box with her young. This topic will be considered in the following sections together with the specific problem of cannibalism of the infants and unusual behaviours observed in the nest-box.

2.5.2.2.1. Time spent with the infants in the nest-box

As discussed earlier (Chapter IV, section 3.3.) due to the altrical condition of the young all early maternal care in the mouse lemur must needs occur in the nest-box. This being the case, it can be assumed that if the time apent by the mother in the nest with her infants is substantially reduced, infant development will suffer. Thus, the proportion of time that a mother spends with her infants is an important indication of the well-being of the infants. In the case of one particular mother it was noted that when the percentage of time spent in the nest-box dropped to 64% by day 8 and to 6% by day 9, the infant died.

A comparison was then made between the amount of time that successful mothers spent in the nest with their infants (this represents the results from two different mothers over two separate years) and that spent by a mother which lost two of her three infants. The data indicate that there is no significant difference between the amount of time these mothers spend with their infants or between the rate at which this time declined as the infants developed. In other words the regression lines obtained by plotting the amount of time a mother spends in the nest-box against time do not differ significantly in the different mother (table 15, fig.26, pages 111, 112).

It must be noted that the mother discussed above which lost two of her three infants had previously been a good mother and the two infants which died did so as a result of an overwhelming infection (as indicated by the discovery of lymphocytes in all the major organs at postmortem) and not from maternal neglect. As such the mother should not necessarily be expected to show reduced maternal care. However, observations made of this mother in the nest-box indicate that she did ignore her infants when they were ill.

2.5.2.2.2. Cannibalism

The disappearance of one or both infants from the nest-box during their first few days of life has been verified on ten occasions of the twenty-seven full-term births recorded. This phenomenon was particularly associated (5 observations) with MF5, a female which probably gave birth at the wrong time of day (see section 2.5.2.1. above). Also, it was observed in the case of four females housed together. Three of these females gave birth but all the infants were eaten.

The infants which are eaten may well have been still-born or have died shortly after birth in many cases. Observations on the behaviour of females at the birth of their infants indicate that the activity of the infants is a potent releasing factor for maternal behaviour. For example, where a female was observed to give birth to a still-born infant its lack of activity soon caused her to lose interest and return her attention to the first-born infant.

2.5.2.2.3. Unusual behaviour in the nest-box

With the aid of the infra-red equipment it was possible to determine those environmental disturbances which appear to disrupt maternal care. The effect of any sudden noise (e.g. doors slamming, dishes breaking or aircraft engines) which occurred during the course of an observation was noted. These types of sudden noise appeared to have little effect on maternal care, apart from attracting the female's attention momentarily. The vocalisations of other mouse lemurs, particularly disturbance calls, do have a disruptive influence. The effect of disturbance calls on maternal behaviour has been observed on three occasions. On one of these the calling was due to an external noise, a dish smashing, but in the other cases the cause of calling could not be determined. On hearing these calls, the mother would leave the nest-box for a variable amount of time. When she returned again she did not settle but continued to run in and out of the nest-box for some time, even after the calling had finished.

Another problem arising in relation to maternal care was demonstrated by the behaviour of the mother of triplets towards her third-born, low birth-weight infant (see section 3.1. of this chapter). This infant tended to be neglected and when the mother transferred her infants to a new nesting site this infant was left behind. When it was reintroduced to the mother, she accepted it only to leave it again when she changed her nesting site for a second time. It would appear that this infant could not stimulate proper maternal care, probably as a result of its small size.

3. Infants

3.1. Low Birth-weight

The normal birth-weight range for healthy infant mouse lemurs is between 5.5 - 7.0 grams (Chapter IV, section 4.1.). However, the birth of full-term infants which body-weight lies below this range, 3.0 - 4.5 grams, appears to be a common occurrence in this species. Four of the nine infants born at University College exhibited this problem. However, after the animals were transferred to the Wellcome Institute no further instances of low birth-weight infants were recorded with the exception of the one triplet mentioned in the preceding sections.

All low birth-weight infants seem to fail to survive. This may, in part, be due to the fact that they fail to elicit proper maternal care (see section 3.4. above). However, it must be noted that all attempts (3 in total) to hand-rear these infants have failed.

4. Discussion

From the results presented in this chapter it is apparent that the reproductive output of the animals in the mouse lemur colony was well below their potential maximum. The study colony of mouse lemurs was not alone in suffering from these problems. Similar observations of the various aspects of reproductive suppression recorded here have been reported by other authors in other mouse lemur colonies. Petter-Rousseaux (1964) and Martin (1972a) have both reported the births of infant mouse lemurs weighing below 4.5 grams. Andriantsiferana et al. (1974) have reported the occurrence of short inter-oestrus periods which occur in association with incomplete oestrous swelling and colouration patterns in their mouse lemur colony, and Perret (1974) reported a low rate of reproduction in her female mouse lemurs. In addition, Perret, (1977) has reported the suppression of testicular development in some of her male mouse lemurs due to the presence of other dominant males or dominant females. In this same paper she describes the histological appearance of the suppressed testes which would appear to be similar to that of the normal testis during the non-breeding season (fig. 18, page 89); the seminiferous tubules are regressed, the Leydig cells are small and degenerate and no spermatozoa can be found in the epididymis. This observation on testis histology would confirm the observation that the males with suppressed testes are infertile.

However, there are two principal points of difference between

Perret's work on testis suppression and that reported in this chapter. Firstly, Perret (1977) reported that there was a relationship between testis size and body weight. In her colony of mouse lemurs, she found that the heaviest males in groups of mouse lemurs commenced testicular growth earlier than their cage mates and also developed the largest testes. The onset of testicular growth in the remaining males occurred in order of decreasing body weight. The final testicular size attained by these males was also related to body weight with the lightest males having the smallest testes. This relationship between testicular size and body weight is similar to the situation observed in the wild. Results in the study colony indicate that this relationship between testis size and body weight is not always clearcut.

The confusion arises from the fact that body weight does not necessarily reflect dominance in mouse lemurs. This results from two factors: firstly the annual cycle of body weight changes and secondly the presence of chronically obese animals in the colony. The role of the annual body weight cycle is most apparent in the case of a male artificially induced to come into breeding condition in the non-breeding season. As a result of the stimulation he will exhibit lowered body weight when the other males have maximal body weight and thus will have the lowest body weight and largest testes of any adult male in the colony. On the other hand, chronically obese males which are the heaviest males in the colony also exhibit small testes and their presence in the colony disrupts the simple model of a constant relationship between body weight and reproductive potential. Chronic obesity may well be due to hypothyroidism which in turn is probably related to social stress (Chapter IX, section 2.5.1.).

The second point of difference between the current study on testis suppression and that of Perret (1977) is that Perret's observations were made on male mouse lemurs housed together in the same cage, whereas those made in the study colony were made on animals housed individually. Thus, the data collected in this study demonstrate that testis suppression can act between males which have no physical contact with each other and in some instances between males which also had no visual contact with each other.

From the data presented in this chapter and from that available in the literature mentioned above there can be little doubt that there are some factors in captivity which act to suppress reproduction in the mouse lemur. The observed suppression of reproduction may result directly from unsuitable techniques of husbandry or management or it may arise as a result of stress. It has already been indicated in the introduction to this chapter that, as the mouse lemur is by nature a semi-solitary species, it is to be expected that it is likely to be susceptible to social stress in conditions of captivity, where individuals are maintained in close proximity with a number of conspecifics.

One of the basic tenets of the stress concept is that, in order to maintain homeostasis in a stressful situation, the individual will sacrifice certain physiological functions. Reproduction is postulated as one of those functions. Selye (1950) suggested that there was a reciprocal relationship between the formation and secretion of ACTH and of the gonadotrophins. Thus stress, by the stimulation of ACTH secretion, inhibits gonadotrophin secretion and thereby interferes with reproduction. Barnett (1964) has indicated that stressors are known to influence reproductive organs. Selye (1950) reported various occurrences of this: for example, rats forced to take exercise exhibit, in addition to adrenal enlargement, ovarian atrophy and anoestrus. In addition, the influence of social stress on reproductive success has been demonstrated by experimental work on various species, notably rodents: for example, those conducted by Christian (1950, 1955a, 1955b, 1956) on the mouse, *Mus musculus*.

In this discussion an attempt will be made to rationalise the contribution of stress to the observed suppression of reproduction. Currently few data are available in the literature on the effect of stress on mouse lemurs although some mention of the phenomenon is made by Perret (1974, 1975, 1977). Thus, in order to evaluate the role of stress the only approach available is to compare the observations made in the study colony with those available in the literature for other species in situations of stress, and to attempt to evaluate whether suppression of reproduction occurs more frequently in subordinate individuals or in situations of increased crowding.

In the case of reproductive suppression in the male, Selye (1950) reported that testicular involution could arise in the human male as a result of stress. Christian (1955b) on the other hand, found during the course of his work on mice, that no affect of increasing population density could be discerned in the testes of mature male mice and, although the accessory glands declined in weight, there was no proof that this change in any way altered the fertility of the mice. However, he did find that high population density suppressed the development of the testes of prepubertal mice.

The relationship between male reproductive suppression and dominance has already been discussed above. It would appear that the suppression of testicular development is a phenomenon associated with lower social status.

A similar relationship between stress and reproductive suppression as observed in the male is also apparent in the female. Selye (1950) reported that stress leads to ovarian atrophy, anoestrus, anovular cycles and delayed puberty. These changes he found to be associated with the alarm phase of the General Adaptation Syndrome. Christian (1956, 1971) and Christian & Davis (1964) report that in studies on experimental populations of mice at high density, females exhibit prolonged oestrus cycles, a decline in ovulation, and implantation, an increase in intra-uterine mortality and inadequate lactation. All these phenomena they found to be most marked in the subordinate females.

Disruptions of maternal behaviour have also been reported in association with social stress. Southwick (1955) reported poor litter survival in colonies of mice at high population densities. This was due to inadequate maternal care, which took the form of cannibalism of the young, desertion of the young in the nest or construction of inadequate nests. He also noted that this effect was most marked in the subordinate females.

These results from the literature would tend to indicate that many of the findings on the study colony of mouse lemurs are paralleled by findings for other species in situations of social stress. However, observations made on inter-oestrus period length in the mouse lemur do not accord with the literature. As discussed earlier (Chapter IV, section 5) the inter-oestrus period of the mouse lemurs seems to be inordinately long. According to the observation of Christian & Davis (1964) cited above, it is possible that this long inter-oestrus period could be an artifact of captivity and a result of stress. However, this is not felt to be the case as it was the short inter-oestrus periods which seemed to be infertile and there was no indication that the inter-oestrus periods were more prolonged in the subordinate females. Rather the converse was true, the short inter-oestrus periods were mostly observed in RF3, one of the more subordinate rufous females.

A note must also be added here on uterine cystic hyperplasia, one of the causes of failure to conceive amongst the colony's females. This is a condition which in domestic species and women is known to be produced by prolonged, unremitting oestrogen secretion due to the failure of progesterone production. It can result from ovarian tumours, excess adrenocortical function and, in women, from hormonal imbalance at menopause (Robbins, 1967). The link with adrenocortical function is of obvious relevance here, but also of interest to those who deal with captive mouse lemur is the fact that this condition can be treated with progestin therapy.

The evidence from the literature, which would tend to support the hypothesis that stress is involved in the observed suppression of reproduction in the study colony mouse lemurs, is supported by the observation that in many instances the animals which exhibited the more recurrent forms of reproductive suppression were socially subordinate. For example, all the suppressed males were subordinate, and the females MF5 and RF3, which were habitually suppressed in some form, were low-ranking and/or highly nervous individuals. In addition, other reproductive problems such as abortion, resorption and cannibalism, were observed in association with highly stressful situations: for example, abortion was observed in association with firebell ringing or severe overcrowding, cannibalism with overcrowding and resorption with overcrowding and terminal illness.

However, the possible direct influences of captivity on reproductive suppression must not be neglected or example, the possible relationship between dietary deficiency on cannibalism. Manocha (1976) found that a low protein diet was responsible for the cannibalism of the young in the squirrel monkey, <u>Saimiri sciureus</u>. It would seem unlikely that dietary deficiency is related to the observed cannibalism in the study colony of mouse lemurs. MF5, a female which habitually cannibalised her young, has been given a variety of diet without any obvious improvement in her maternal behaviour. A further possibility which must not be neglected is that cannibalism may in fact be the result of infant death or the production of non-viable infants. It was noted earlier in this chapter (section 3.2.) that, as in other primates: for example, the common marmoset, <u>Callithrix jacchus</u> (Stevenson, 1976) and the rhesus monkey, <u>Macaca mulatta</u> (Hinde <u>et al</u>., 1964), it is the presence of strong and vital infants which is an important releasing mechanism of maternal care. The presence of still-born or low-birth weight infants does not have this effect. However, as the production of low-birth weight infants may not be totally related to stress (see below) one could argue that cannibalism is not a stress related phenomenon. This does not appear to be the case; observations from the study colony of mouse lemurs indicate that cannibalism occurs most frequently in subordinate or nervous females or those housed in overcrowded conditions.

To date no details are available on the effects of stress on infant birth-weight. However, it is well known that stress is associated with poor litter survival (Christian, 1971). Therefore, it is possible that the low birth-weights, with their concomitant effect on infant survival, which were recorded in the study colony of mouse lemurs were the result of stress. However, there is no evidence which links the occurrence of these births to particularly subordinate females or to those housed in particularly crowded conditions, except if one included the case of cannibalism cited above. It is therefore more probable that the production of low birth-weight infants is an artifact related directly to techniques of management. As no low birth-weight infants (with the exception of one triplet) were born at the Wellcome Institute, an investigation into the differences in mouse lemur husbandry between University College and the Wellcome Institute may indicate a cause of this phenomenon. There were two important differences between the management of the mouse lemurs in University College and in the Wellcome Institute. Either of these differences might be associated with the reduction in the number of low birth-weight infants. These were diet and housing density.

At University College large amounts of Vionate vitamin powder and Abidec and Adexolin vitamin additives were included in the diet of the mouse lemurs.

If vitamin poisoning were the cause of low birth-weight then one of the fat soluble vitamins (A,D,E or K) must be involved as these are the only vitamins which can be stored in the body and so are the only ones which can build up to toxic levels. Toxic effects are normally only associated with A and D. Hypervitaminosis A was suspected in the mouse lemur colony when two animals fell ill showing signs of Vitamin A poisoning. It was subsequently calculated that the mouse lemurs were receiving 175 IU per animal per day. The minimum requirement dose of Vitamin A is between 20-50 IU/kg body weight (Brody, 1945). According to this estimate the minimum requirement dose for a mouse lemur would be 1.5-3.8 IU per day. A mouse lemur receiving 175 IU/day would be susceptible to Vitamin A poisoning. In addition, very high concentrations of vitamin A have been found in the livers of two mouse lemurs (one adult female suffering from leuçaemia, and one infant, 14 days old, suffering from an acute infection of undetermined origin) which died during the course of the study. The results indicated a level of Vitamin A greater than 1000 μg/gram liver. This is a level of Vitamin A storage which has proved toxic in tree shrews, Tupaia belangeri (Rivers et al., 1977). However the birth defects normally associated with hypervitaminosis A (e.g., cleft palate, Moore, 1957) were not found in the low birth-weight infants.

If stress were the cause of the low birth-weight infants this may have been mediated by the thyroid. The birth of weak young is often associated with iodine deficiency (Underwood, 1971). The observations that adrenal hyperfunction leads to decreased thyroid activity (Underwood, 1971) and that hypothyroidism arises in the mouse lemur in captivity (Perret, 1975) may indicate the link between these infants and stress. There is some indication that hypothyroidism occurred in the study colony (Chapter IX, section 2.5.1.).

A final possible cause of low birth-weight is the combined affect of overcrowding stress and Vitamin A. Calhoun (1962) reported that stress increased both the storage of and the tolerance to Vitamin A. Therefore, stress could potentiate the effect of Vitamin A on the developing foetus of a mother housed in stressful conditions.

Another direct effect of captive management techniques on reproductive suppression which should not be confused with the effects of stress is that due to poor photoperiodic alteration (see Chapter III, section 4.2.). It has been noted that failure to alter daylength at both ends of the day results in poor synchrony of reproductive condition. This would in turn act to reduce reproductive output. If the importance of proper photoperiodic alteration were unknown the resultant lowering of reproductive output might be attributed to stress.

If it is to be assumed that the reproductive suppression observed in the study colony arose as a result of stress some attempt must be made to identify the stressor or stressors. Stressors of environmental origin can be identified as: noise, such as firebells or in the case of the Observation Room the noise of a working laboratory adjacent to the animals; the direct effects of enforced confinement; the proximity of humans; dietary inadequacies; absence of adequate temperature variation between day and night; other problems associated with captivity and an artificial environment. Stressors of social origin are easy to identify where animals are housed together. However, in the mouse lemur colony, stressors of social origin apparently acted on animals which were individually housed. This leads to a problem in identifying what signals act to indicate to an animal that it is both overcrowded and socially subordinate to animals it has had no contact with. The role of olfactory signals is not to be ignored in this context. However, it is very difficult for an observer to detect these signals or their action (see Chapter VI, section 4.). In the course of the study, two signals, vocal in origin, which had a suppressing effect on reproduction, have come to light. These are the trill call of the male mouse lemurs and the disturbance call (see also Chapter VI, section 3.2. and 4.).

Data collected during the course of this research indicate very strongly that the male "trill" vocalisation is involved in testis suppression (see Chapter VI, section 4.). As discussed previously, this call is specific to the *murinus* subspecies and this is relevant as no inter-subspecific testis suppression has been observed; each cage room could contain one rufous and one grey breeding male. Also, as discussed earlier, this call is sonically structured in such a way as to allow both easy location of the caller and slow attenuation over distance. These characteristics may function in the mating season to attract females. However, this call is still given although less frequently when there are no oestrous females in the vicinity and it is also given sporadically during the non-breeding season. Therefore, it would appear to have a function other than that of a mating call and presumably this is to inform other males of the presence of a breeding or potentially breeding male in the vicinity.

Non-breeding males do not appear to give this call and neither do females with one exception. This involved the two females kept in the Observation Room with one male. During their first year together they behaved normally, with the male exhibiting a full breeding condition. However, during their second year the females starting exhibiting pseudomale behaviour (see section 2.6.) and giving the trill call in association with this behaviour. During this season it was noted that the male's testes failed to develop.

Unfortunately, similar data have not been collected for the *rufus* subspecies. This is partially due to the fact that fewer of the *rufus* specimens were maintained in the colony and also to the fact that they rarely gave any vocalisations. The soft squeak of the rufous males (Chapter VI, section 3.3.) was only heard during mating encounters and, thus, may not have had a testis suppression function. However, as this call was very difficult for the human ear to detect it may have been given more frequently than was noted. Alternatively, some other factor, such as scent, could mediate in testis suppression in these animals.

Observations undertaken on maternal care in the nest-box indicate that the factor which has the most disruptive effect on maternal care is the disturbance call. This is a vocalisation which would be anticipated to be most pronounced in overcrowded conditions. This is due to the fact that one might expect the probability of an animal being sufficiently aroused to give the call would increase with increasing population density. In addition, under crowded conditions the chorusing effect would be greater due to the greater number of individuals giving the call. In this way a density-dependent effect due to the response to the disturbance call can be envisioned.

To summarise, it can be said that several factors were acting to reduce birth rate and infant survival in the mouse lemur colony. Of these by far the most important was the apparent failure of the females to become pregnant (due to various reasons). Other mechanisms of reproductive suppression, such as abortion, premature birth combined with foetal loss, and inadequate maternal care only occurred in particularly subordinate females or under exceptionally stressful conditions. The pseudomale behaviour recorded in two females appears to be a particularly aberrant occurrence associated with the more extreme conditions of the Observation Room; as manifested through sudden noise, firebells, higher levels of background noise, a lack of temperature variation and a possible unbalanced sex ratio. Such behaviour could result from an increased level of adrenal androgens which in turn could be due to stress. Such pseudomale behaviour could act to reduce reproduction in two ways. Firstly, the reproductive potential of the females concerned is reduced and secondly the reproduction of their "normal" neighbours is affected due to the inhibition of potential breeding males. It is interesting to note that van Holst (1974) noted that pseudomale behaviour in female tree shrews increased with social stress.

If the theory of reproductive suppression in captivity arising from stress is accepted, the question arises as to whether the effects of stress are irreversible or whether the animals adapt over time to the stresses of captivity and begin to breed with greater success. Reference to table 44 will indicate that some females produced a few litters shortly after the colony was established and have failed to do so since whilst with other females the converse is true. From this observation it can be seen that suppression of reproduction is not irreversible but rather that suppression alters over time possibly in response to alterations in captive conditions.

Observations also indicate that there has been a steady improvement in the reproductive success of the colony over time: during the period prior to the onset of the study (1971 - 1973) 73.8% of oestruses recorded resulted in no apparent pregnancy; during the study period (1973 - 1976), 62.5% of oestruses resulted in no apparent pregnancy; and since the conslusion of the main study only 43% of oestruses have resulted in no pregnancy among the portion of the colony housed in Rotterdam Zoo. Observations on testis suppression in the males have shown similar trends. Reference to table 39 will demonstrate that the difference in testis size between the breeding male and many of the non-breeding males was becoming less marked by the last year of the study (1976). This trend has continued since the animals have been transferred to Rotterdam Zoo, resulting recently in two different grey males, which were housed in the same room, siring litters during the same breeding season.

From these apparent improvements in fertility over time it could be argued that the animals are becoming adapted to the conditions of captivity. However, as these improvements have all been noted in captive born animals which have obviously descended from those few wild caught specimens which could adapt sufficiently to captivity to be able to breed, it is suggested that this apparent improvement in fertility is the result of inadvertant selection on the part of the people maintaining the colony. The initial conditions of captivity were such that only a few specimens could breed regularly, that is those individuals with the greatest predisposition to adapt. The second generation, having descended for the most part from these regularly breeding animals, might be anticipated to have the same predisposition towards adaptability and should this process be continued over time a particular strain of mouse lemur would result, namely one well adapted to the conditions of captivity. Thus, it can be seen that the effects of stress apparently diminish over time as the captive strain emerges.

A final note must be made on the occurrence of reproductive suppression in wild mouse lemurs. It has been suggested that the periodic dramatic decline in population size of some species arises as a result of social stress (Christian, 1950, 1971, and Chitty, 1952). It has also been suggested that social stress may act to regulate reproduction in non-cyclic species so as to maintain their numbers below the carrying capacity of the environment. However, the evidence for this is based mostly on captive studies of artificial populations (Barnett, 1964). Therefore, it would be of interest to know whether similar mechanisms of reproductive suppression are observed in wild mouse lemurs. The data collected by R.D. Martin, cited earlier, indicated that testis suppression does in fact occur in wild populations of mouse lemurs. This might be supposed to be a selection mechanism which ensured that only the largest, healthiest males could pass their genes on to future generations. The actual mechanism through which this selection could act is beyond the scope of this thesis and will not be discussed here.

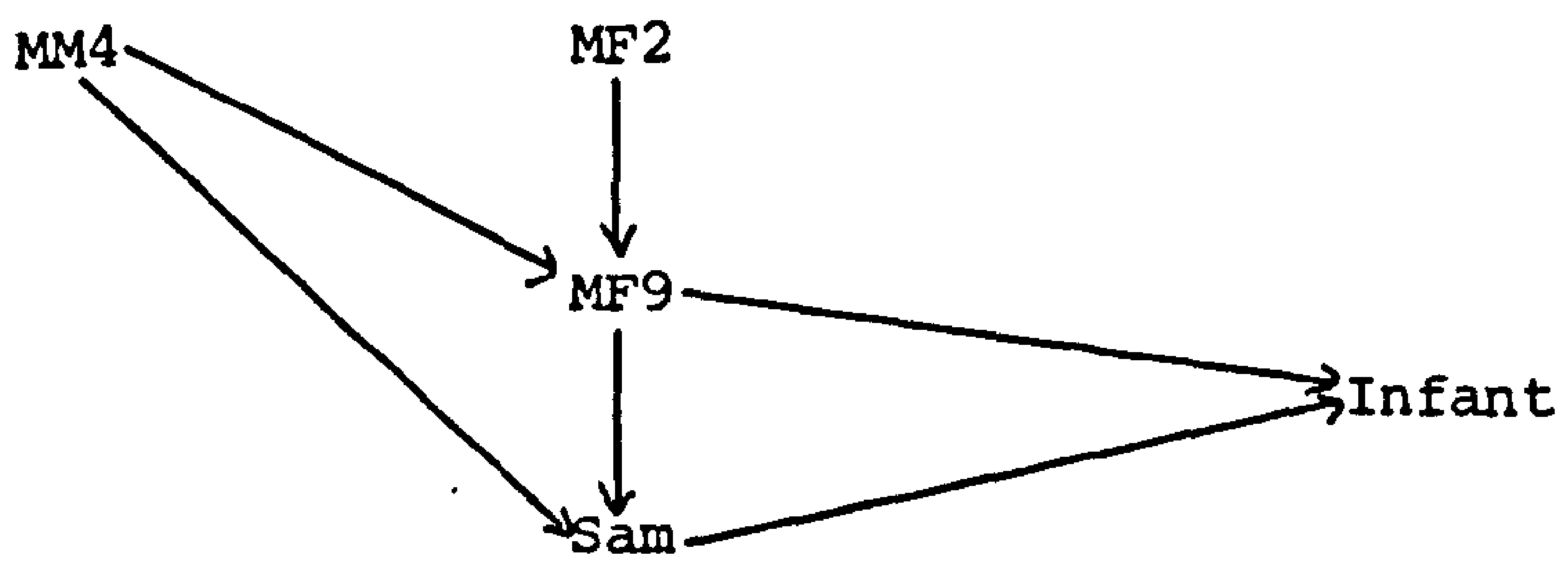
To date there is no evidence as to whether the mechanisms of reproductive suppression observed in the study colony actually act in the wild population. However, it must be noted that the most prevalent mechanism of reproductive suppression in the study colony, that of the non-occurrence of pregnancy, would be the most suitable mechanism of population regulation in wild mouse lemurs as it requires the minimum loss of maternal investment. Obviously, abortion or the loss of full-term young, means a far greater loss in maternal investment.

Thus, at least in the case of the male, it can be said that the suppression of testicular development observed in captive mouse lemurs represents a natural mechanism for the selection of the best males for breeding purposes which misfires in captivity. In general, the degree of testicular suppression observed in the study colony of mouse lemurs was more severe than that observed in the wild (in captivity some males were found to have only size E testes whereas the smallest found in the wild, 25 x 15 mm, approximates to a size C-D). In captivity, testicular suppression can also lead to problems of inbreeding. Whilst the animals were at University College, one male was the sire of all the grey infants born. After the animals were transferred to the Wellcome Institute, a second male in a separate room also fathered some infants. Unfortunately he was an offspring of the original breeding male and this has also led to very high inbreeding coefficients in the colony (as high as 43.75% in some cases - see table 45).

Thus it would seem that captive conditions adversely affect the reproductive potential of the mouse lemur. The reduction of the reproductive output of the female follows a similar pattern to that described in other species at high population density and therefore it would appear that stress resulting from overcrowding is one of the prime causes of this phenomenon.

Table 45

Table Calculating Inbreeding Coefficients



The inbreeding coefficient of the infant produced from the union of MF9 and Sam is given by the equation:-

$$Fx = (1/2)^{n+1}(1+Fa)$$

Where Fx is the inbreeding coefficient of the infant

is the sum of all independent paths of inheritance which connect the sire and dam of the infant

n is the number of segregations in a specific path between sire and dam of infant

Fa is the inbreeding coefficient of the common ancestor for each path

Common ancestor	n	Contributions
MM4 as sire MF9 and Sam	2	$(1/2)^3 = 0.125$
MM4 as sire MF9 and Sam	3	$(1/2)^4 = 0.0625$
MF9 as dam of Sam	1	$(1/2)^2 = 0.25$
		Sum = 0.4375
		= 43.75% inbred

MM4 is listed twice as the same gene may have been transmitted by two routes, via MF9 and Sam or via MF9 to Sam.

SUMMARY

1) The reproductive output of the study colony of mouse lemurs was well below maximum. This reduction of reproductive output was the result of reproductive suppression; primarily in the female although the reproductive potential of many of the males was also affected as was the survival of the infants.

2) Reproductive suppression in the males took the form of the suppression of testicular development during the breeding season. The result was that only one male of each sub-species in each cage room was capable of reproduction. In some cases the maximal testis size and the duration of testis development was related to body-weight (this is similar to the situation in the wild). However, this relationship between body-weight and testis size was complicated by the presence in the study colony of overweight males which never attained maximum testis size.

3) As the suppression of testicular development occurs in males housed in separate cages, it is suggested that the trill vocalisation, a call given only by the breeding grey males, is instrumental in testis suppression in the grey sub-species. No corresponding vocalisation was found in the red mouse lemurs and the mediator of testis suppression remains undetermined in these animals.

4) Reproductive suppression in the female mouse lemur can be divided into five categories; uterine abnormalities, oestrous abnormalities, apparent failure to become pregnant, failure of the pregnant individual to produce full-term young and behavioural abnormalities. These categories are not mutually exclusive. Of all these mechanisms of suppression, the failure of the female to become pregnant was the most common.

5) A final problem encountered was the production of low birth-weight infants. It was found that infants with a birth-weight lower than 4.5 grams always failed to survive.

6) In the course of the discussion the reproductive problems encountered during the study are evaluated in terms of stress. As little data are available in the literature on the effects of stress in the mouse lemur, the observations made on the study colony animals are compared with observations made on other species subjected to conditions of stress. In many cases these findings are found to be similar. It is therefore suggested that stress resulting from the conditions of captivity and the presence of too many conspecifics is the cause of many of the phenomena reported. However, it is emphasised that some of the problems observed may result directly from problems in the management techniques employed: for example, the feeding of a poor or inadequate diet.

7) Data indicate that the animals may become acclimatised to the stresses of captivity over time. However, it is suggested that this apparent acclimatisation may in part be due to the inadvertent selection by the people managing the colony for a special captivity-adapted strain of mouse lemurs.

CHAPTER IX

PATHOLOGY

This chapter deals with the pathology of animals maintained in the study colony of mouse lemurs and also reviews the findings for both the London and Jersey Zoo colonies. This work was undertaken to establish what, if any, similarities there were in the pathological findings in different individual mouse lemurs. From these data it is possible to determine what evidence there is to support the hypothesis of a captivity-induced disease syndrome occurring in the mouse lemur and whether this syndrome, if it does occur, is related to stress. To this end, adrenal weights and adrenal cortical nuclear diameters were also obtained and these data used to correlate adrenal activity with *post-mortem* findings.

The study of pathology in any colony of animals has many potential benefits to the well-being of that colony. The presence of bacteriological and parasitic infection can be determined and the appropriate preventive action taken. Pathological information is of a relevance when determining the appropriate treatment of sick animals and finally it can also be of great importance to the interpretation of behavioural data. In the instance of strepsirhine primates, pathological studies are particularly useful as so little has been published about their pathology and thus any contribution to the knowledge of pathology in these animals is important.

1. Adrenals

In order to evaluate a possible connection between stress and disease in the mouse lemur, data on adrenal weight and histology have been included in this section.

1.1. Adrenal Weights

Adrenal weights have been obtained from thirteen animals from the study colony, three animals from the London Zoo, one from Jersey Zoo, one from a colony maintained in South Africa and from three females collected in the field by Grandidier in 1932.

As the various adrenals were fixed differently it was first necessary to test whether the different fixatives used affected the weight of the tissues. This was achieved by comparing the weights of the kidneys of captive mouse lemurs which had been fixed in Bouin solution or Formalin (these were the two most commonly used fixatives). The variance ratio test indicated that there was no significant difference between the weight ranges of the two sets of kidneys (see table 46).

Adrenal weights varied between 0.007 and 0.041 grams (fig.70). The adrenal weights of six "normal" individuals (i.e. three which were collected from the field, two which were sacrificed with no obvious pathological symptoms and one which was accidentally killed) ranged between 0.005 and 0.010 grams; whereas weights of adrenals from sick animals generally lay outside this range (fig.70). The weight range of 0.005-0.010 grams covers most of the annual cycle of adrenal hypertrophy and atrophy (see Chapter III, section 3) and, as there appears to be no difference in adrenal weight between the sexes (Perret, 1972), is probably a good approximation of the normal weight range of the adrenals found in healthy mouse lemurs. When the weights of both the left and right adrenal are plotted on a histogram there appears to be a continuous weight distribution for the adrenal weight of both sick and healthy individuals, although a t-test indicates that the difference between the mean adrenal weights of "normal" and sick individuals is significant ($p \leqslant 0.05$, see table 47). If only the weight of the heavier adrenal is plotted on the histogram the difference between sick and healthy groups is clearly differentiated. This is due to the fact that in all

Table 46

Variance Ratio test comparing the weight range of kidneys preserved in two different fixatives, Bouin and Formalin.

	Bouin	Formalin
Variance (6^2)	0.0023	0.0022
Number of cases	9	11

The variance ratio: F = 1.045

This is not significant.

Table 47

t-test comparing the adrenal weights of sick and healthy specimens

	Sick	Healthy
Mean weights (mgs)	16.524	7.875
Variance (6^2)	56.867	2.982
Number of cases	21	8

$t = 4.928$

This result is significant, $p \leqslant 0.05$

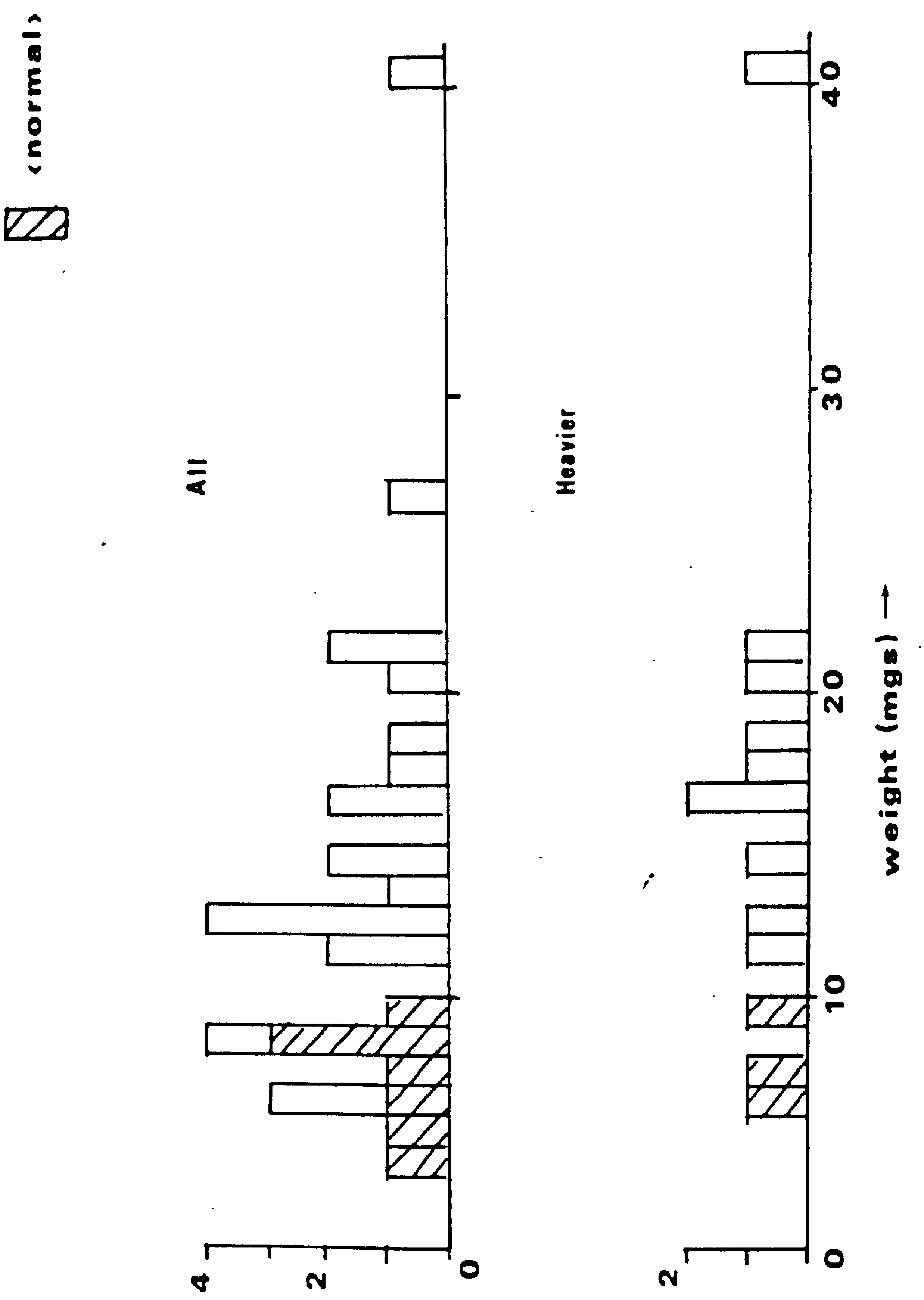


Figure 70

Histogram of adrenal weights

"Normal"indicates that adrenals came from apparently healthy specimens.

individuals one adrenal is enlarged (usually the left: 8 cases out of 11); thus when the weights of all adrenals are plotted, the weights of the smaller adrenal in the sick animals overlap with the weights of the heavier adrenal in the healthy ones. Figure 71 demonstrates the size difference between the adrenals of a sick and a healthy individual.

1.2. Adrenal Histology

Measurements have been obtained for adrenal cortical width and cortical nuclear diameter. For comparative measurements nuclear diameter is the more useful. Not only is the diameter of cortical nuclei a good indicator of adrenal activity but, as cortical nuclei do not vary in size according to their position in the adrenal, their measurements are more reliable than cortical width which varies according to the position in the adrenal from which the section was taken.

Nuclear diameters were very variable. In one individual nuclei varying in size between 3 and 5μ could be found. The mean nuclear diameter in the two "normal" individuals was found to be 4.6±0.9μ and 4.7±0.9μ whereas that in the chronically ill individuals varied between 5.9 and 6.3μ (see table 48). One notable exception to this was observed in the case of MF10. This individual had enormously enlarged adrenals (about 4 times normal size) but the nuclear diameter was only 4.8±1.1 μ. However, these nuclei were all noted to be irregular in shape and of a collapsed appearance.

1.3. The Adrenal Cycle and the Onset of Disease

The annual cycle of adrenal hypertrophy and atrophy has already been discussed (see Chapter III, section 3). The adrenals increase in size and become more active with increasing daylength and reach maximal activity shortly after the longest day. Adrenal size and activity diminish again with decreasing photoperiod.

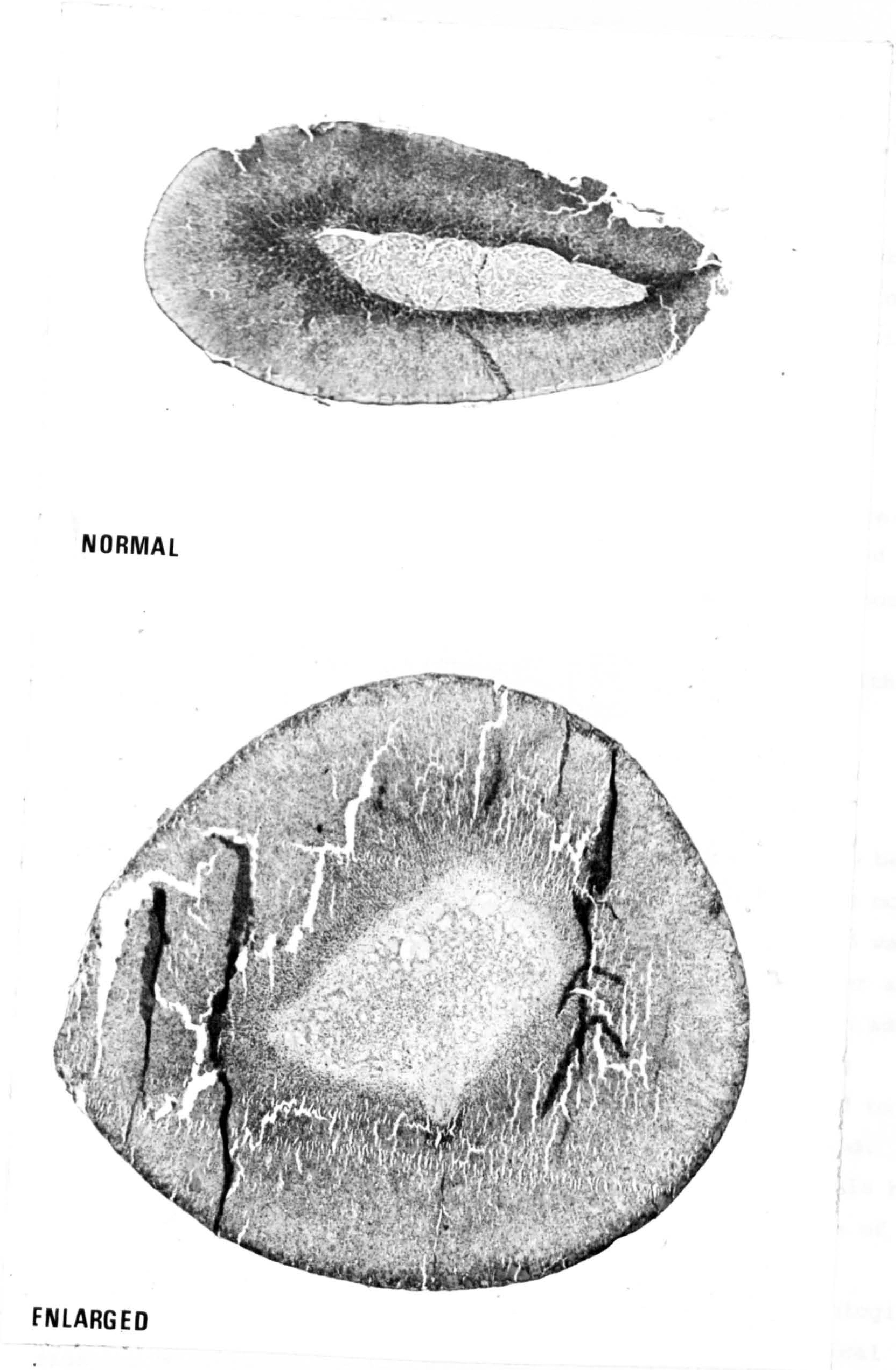


Figure 71

Adrenal size; normal and enlarged

Magnification x30.

During the 8 years that the study colony of mouse lemurs has been in existence, 12 animals have died as a result of sickness. It has been noted that of these 12 individuals, 10 died during the periods of increasing or maximum daylenghth.

2. General post-mortem findings

The most common postmortem findings in the mouse lemurs were:- lung lesions, kidney lesions, injuries from conspecifics and neoplastic disease. All the deaths in the study colony, those reported in the pathology records of London Zoo and those occurring in the Jersey Zoo collection can be associated with one or more of the above problems (see table 49).

2.1. Respiratory Problems

In the study colony respiratory distress was often found to be the first indication that an individual was sick. It was a more reliable indication of ill health than loss of appetite and was very easy to observe. Respiration was very rapid, irregular and gasping. Animals suffering from this condition would often adopt a head-down posture whilst inactive, sometimes even hanging upside down from a branch. In addition, the animals tended to sleep outside their nest-boxes during the white light period.

Histological investigation of the lungs of these animals has shown, in most cases, thickening of the septa and breakdown of the alveoli occurring in focal areas (fig.72). As these individuals had all been treated with antibiotics, bacteriological investigation yielded no results. However, these histological findings on study colony individuals were consistent with the frequent observations of congestion of the lungs recorded at gross postmortem examination of the zoo mouse lemurs by the London Zoo pathologist.

These lung lesions in the mouse lemur rarely occur on their own, but are usually found in conjunction with a variety of other

Table 48

Adrenocortical Nuclear Widths

Animal	Condition	Nuclear Diameter in Microns
RMZoo	Healthy	4.7±0.9
MF4	Old, Sacrificed (winter)	4.6±0.9
RF8	Acute illness (winter)	4.5±0.8
MF1	Chronic illness (X)	6.0±0.8
RF2	Old, Uterine hyperplasia (X)	5.9±0.7
RF1	Leucaemia (X)	6.3±0.7
MM5	Chronic illness	6.1±1.4
MF10	Chronic illness grossly enlarged adrenal	4.8±1.1
Young Male	unknown	5.9±0.6

X indicates that the animal died during the period of increasing daylength.

n, the number of observations = 50 in all cases

Table 49

Distribution of Post-mortem Findings

Animal	Age (years)	Sex	Source	Lung Lesions	Kidney Lesions	Trauma	Neoplastic Disease	Other
RF1	8+	F	Colony wild	+			+ Leucaemia	lymphocyte infiltration in heart
RF2	6+	F	Colony wild	? terminal				uterine hyperplasia
RF7	5+	F	Colony wild	+				uterine hyperplasia
RF8	3	F	Captive born	+				
RFZOO	?	F	Jersey Zoo		+	+ bite from a diff.sp. kept open by conspecifics		lymphocyte infiltration in heart
RM2	5+	M	Colony wild	+	+ Abcess			
RM5	3	M	Captive born	+				
MF1	8+	F	Colony wild	+	+			Adhesions in gut
MF3	5+ (sacrificed)	F	Colony wild					uterine hyperplasia
MF10	2½	F	Colony born given to L. Zoo	+				Adhesions in gut Thickened bladder
MM5	2½	M	Colony born given to L. Zoo	+	+ haemhorrage			Thickened bladder
Male Murinus	?	M	South African Colony				mesothelioma	
Murinus seven animals	?	F M	L.Zoo path. records 69-70	4 cases	2 cases	4 cases	1 case cancer of uterus	

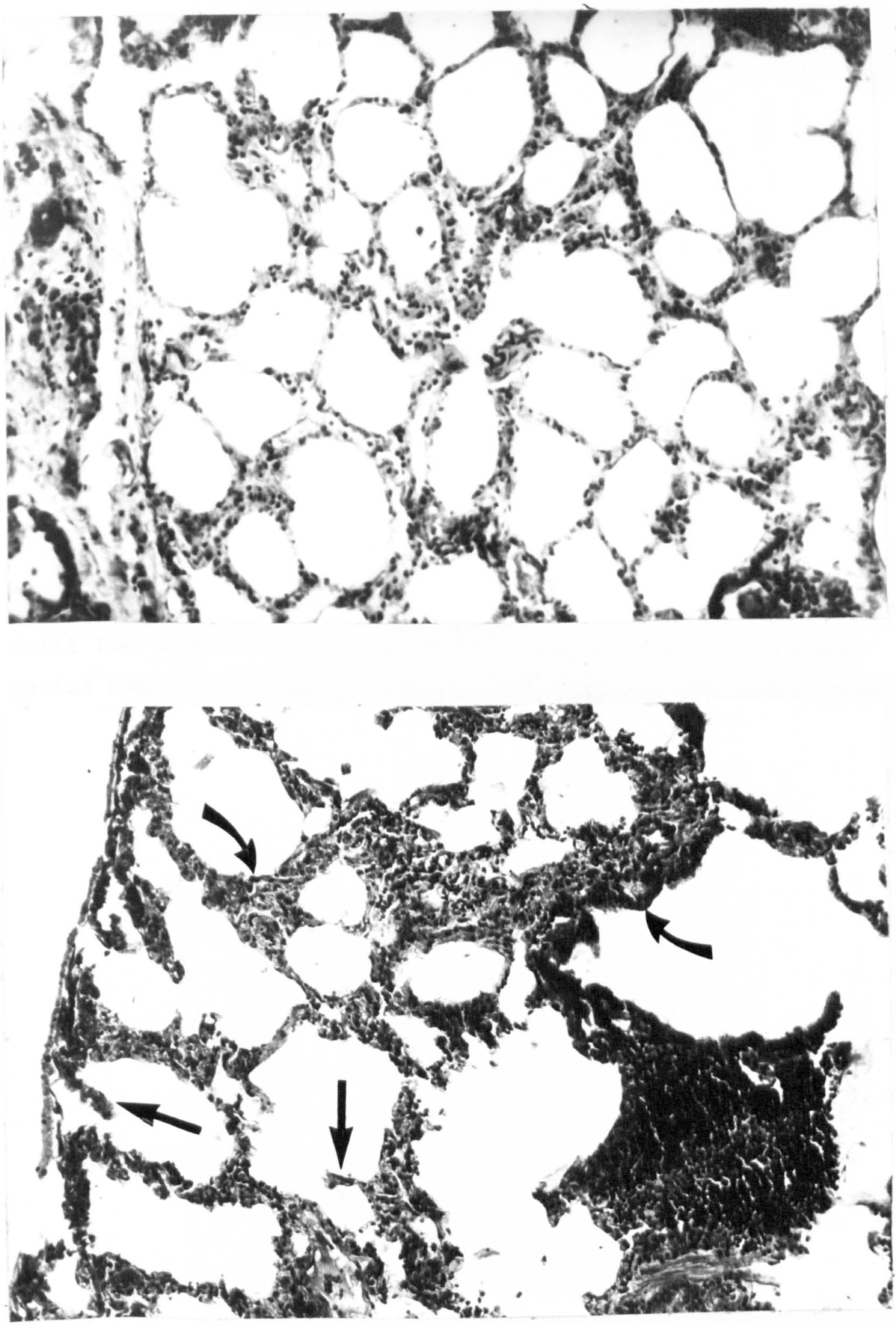


Figure 72

Lung lesions

(x160)

Top figure shows a micrograph of the lung of a healthy animal (a cat); lower figure shows a micrograph of a sick animal (mouse lemur), straight arrows indicate broken-down septa, curved arrows indicate thickened alveolar walls.

problems; for example cystic kidneys, debility and gangrene due to wounding, Klebsiella septicaemia and cancer of the uterus (see table 49).

2.2. Injuries from Conspecifics

Severe wounding by conspecifics has not been observed in the study colony. However, permanent groups have not included more than three individuals. Postmortem records from the London Zoo mouse lemur colony between 1968 and 1970 include many instances of traumatic wounding, some of which led to debility and death. This group of mouse lemurs was established in 1960 and flourished until 1967 when it was transferred to new housing. During the period 1968-70, no further infants were born and the adults died. A maximum of nine adult individuals lived concurrently in this group during 1968 and part of 1969 before the first death occurred.

Observations have been made on two injured female mouse lemurs, one of which was maintained in Jersey Zoo and the other in the study colony. These observations indicated that, not only the wound itself, but also the subsequent behaviour of the injured animal and its cage-mates were important to the recovery of the individual. The wound received by the Jersey female was the result of a bite by a hutia, Geocapromys brownii, housed in the same enclosure. The origin of the wound on the study colony female is unknown. In both these cases it was the subordinate female which was injured. Although in neither case was the original wound thought to be serious, both wounds failed to heal normally. This failure to heal appeared to be related to the status of the female. In the London group, the status of the wounded female appeared to be lowered; she was restricted to one part of the cage by the dominant female and was chased whenever she left it. A similar situation was observed in Jersey, although the degree of subordination to injury was unknown. In both cases restricted movement led to an increase in grooming behaviour with much attention being paid to the wound itself. Also, in both groups, the wound appeared to be the focus for social grooming attempts by other group members. This attention was not welcomed by the injured animal which would frequently retreat into a nest-box at the approach of a prospective grooming partner.

On removal from the social situation, the London female recovered rapidly. The Jersey female was not removed from her cage until three months after the initial injury and by this time the wound had a markedly abnormal appearance. This female died sometime later of general debility.

Chronic self-grooming has also been observed on other occasions, particularly in association with cage transfers. One female in particular removed all the fur from her abdomen which remained completely bald for several months. Chronic tail grooming has also been observed in two individuals.

2.3. Kidney Lesions

Kidney lesions are a common postmortem finding in the mouse lemur. Nephritis occurs particularly frequently, but kidney cysts have also been recorded on two occasions. Postmortem records from the London Zoo include two instances of nephritis. In several cases, histological examination of the kidneys of animals in the study colony, showed glomerular collapse and focal areas of inflammatory cell infiltration (see figs. 73, 74).

In addition to the above mentioned lesions, the kidneys from animals in the study colony appeared to be much heavier than those collected from wild specimens. The mean kidney weight obtained from wild mouse lemurs was 0.075 grams (range, 0.06 - 0.09 grams, n = 3) and 0.254 ± 0.01 grams (range, 0.17 - 0.34 grams, n = 26) was the mean weight of those kidneys originating from captive specimens.

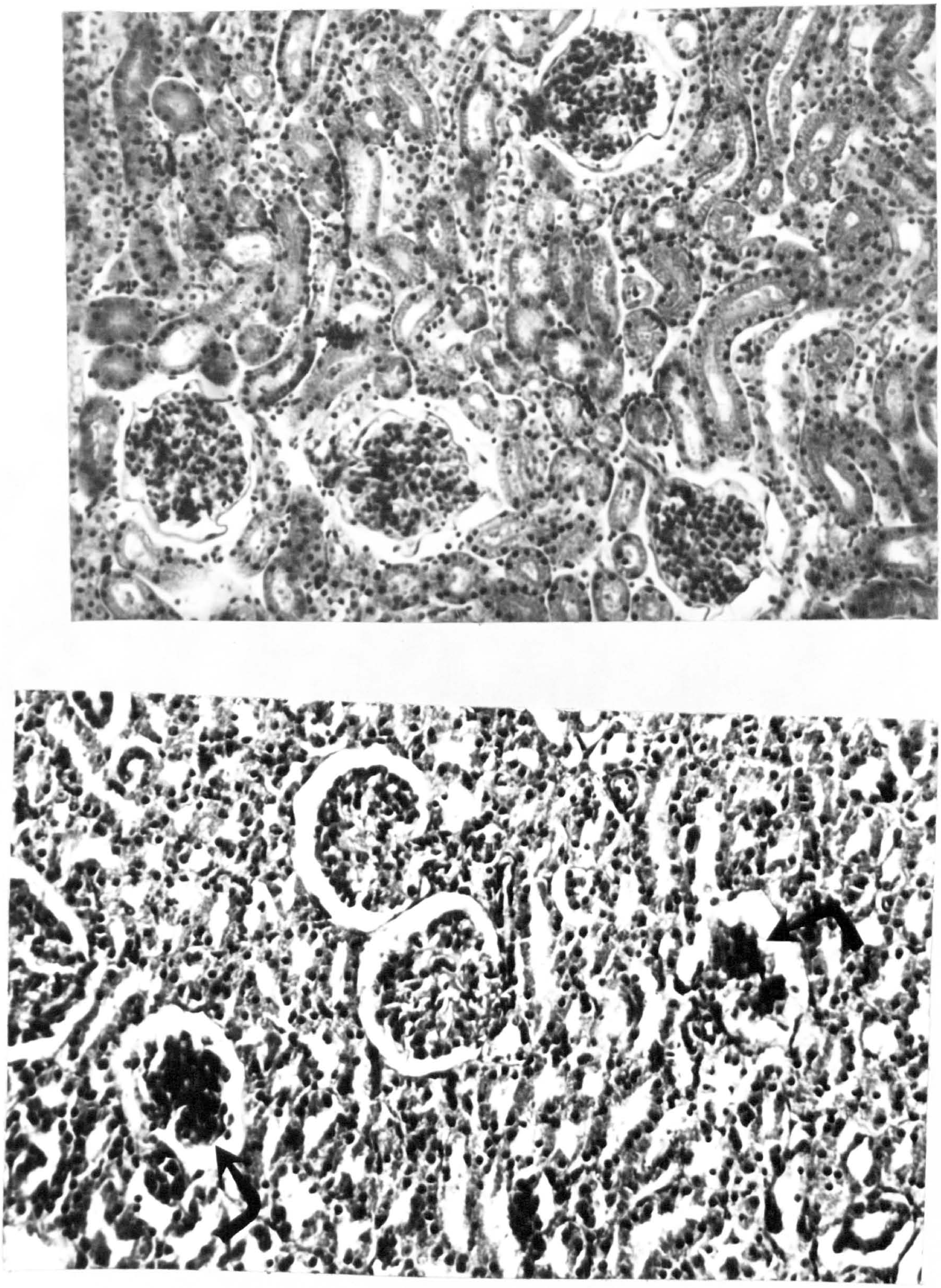


Figure 73

Kidney lesions; collapsed glomeruli
(x160)

Top figure shows a micrograph of the kidney of a healthy animal; lower figure shows a micrograph from a sick animal, arrows indicate the collapsed glomeruli.

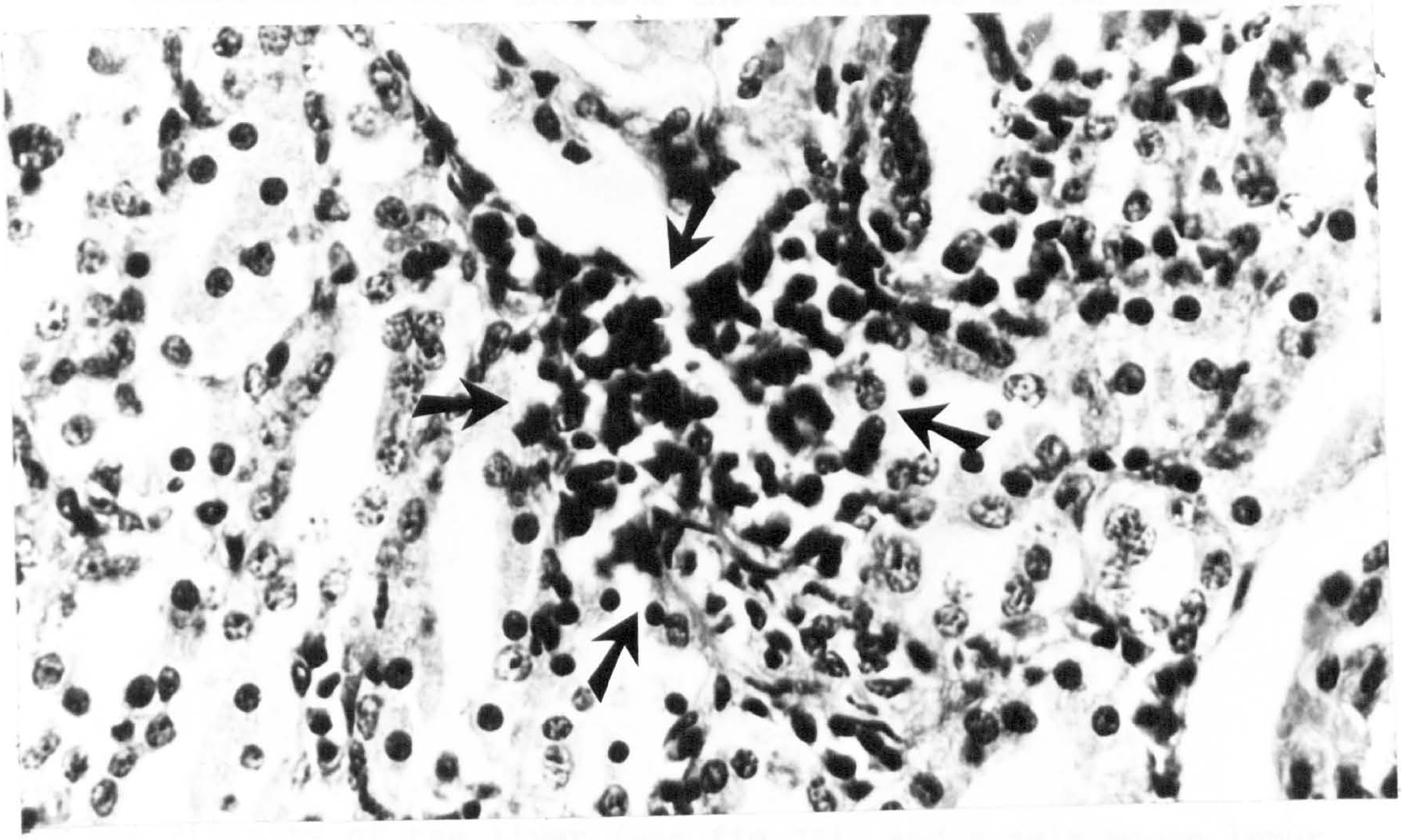


Figure 74

Kidney lesions; focal inflammatory cell infiltration (x400)

Area of infiltration ringed by arrows

2.4. Neoplastic Diseases

All colonies of mouse lemurs would appear, either from the records or from the literature, to have one or two cases of neoplastic disease occurring in them.

London Zoo records indicate the occurrence of two cases, one being cancer of the uterus and the other an individual exhibiting a tumour in the gut.

Petter & Petter-Rousseaux (1966) report two cases arising in their mouse lemur colony, one of these was a fibroblastic sarcoma in the region of the shoulder of one specimen, and the other individual had a tubulo-papillary adenocarcinoma in the uterine horns.

In the study colony of mouse lemurs one case of acute lymphoblastic leucaemia was diagnosed and a second female in the study colony, on loan from the Jersey Zoo collection, also developed leucaemia.

In addition, a histological specimen received from Doyle's mouse lemur group in South Africa exhibited a large mesothelioma in the vicinity of the liver (see fig.75), and a male mouse lemur which died in the Duke Primate Centre was observed to have an apparently tumorous growth of the testis (K. Boskoff, pers.comm.).

2.5. Other Problems in Mouse Lemurs

Other postmortem findings in the mouse lemur were varied. They included:-

Klebsiella septicaemia, spondylosis and chronic cystitis.

Cystic hyperplasia of the endometrium was also recorded in three females (see Chapter VIII, section 2.1). Two problems frequently encountered in living members of the mouse lemur colony were obesity and cataracts.

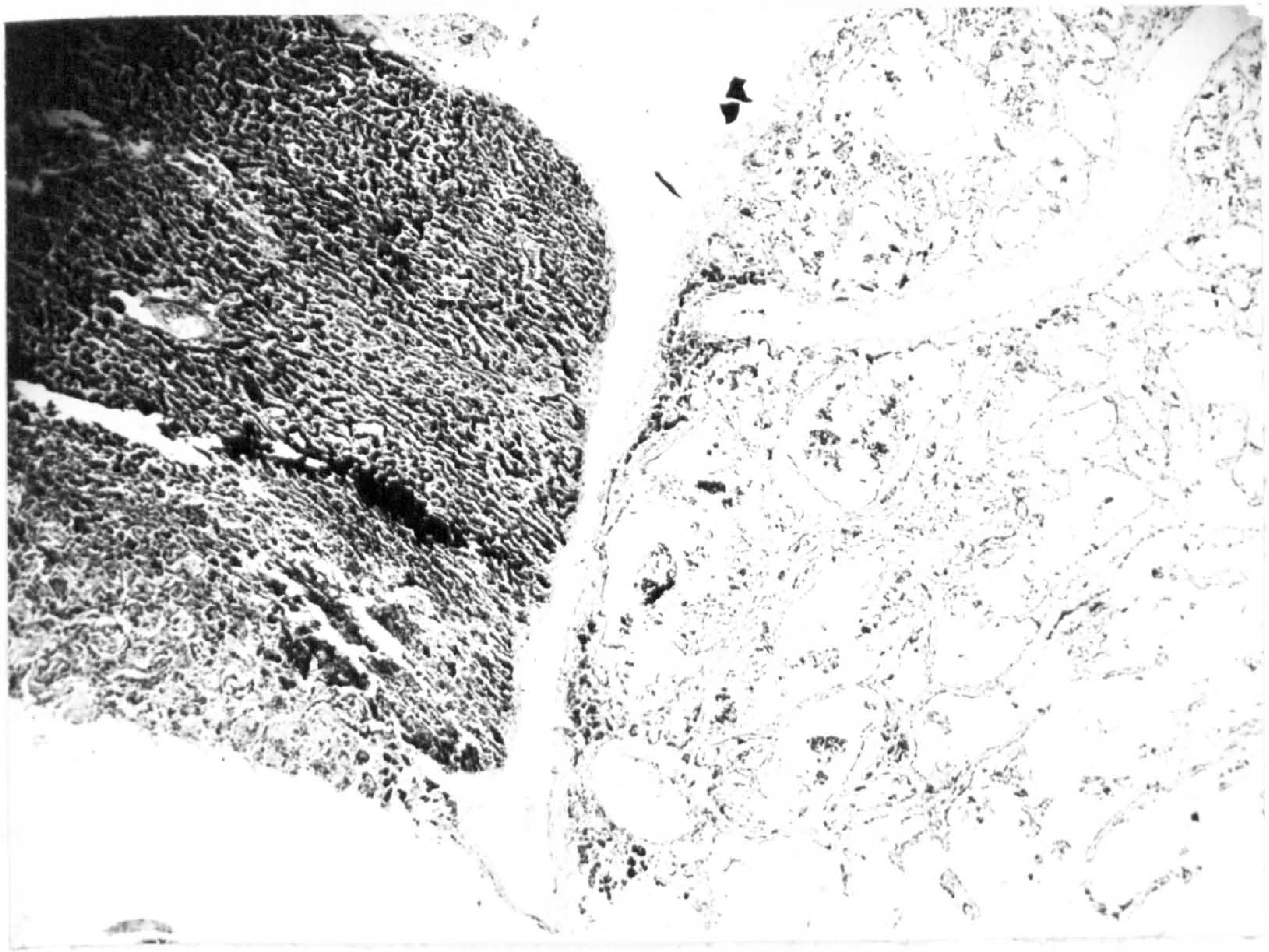


Figure 75

Mesothelioma (x40)

Normal liver tissue can be seen on the left of the micrograph, the cancer is on the right.

2.5.1. Obesity

On average, all animals in the study colony had a heavier body weight than wild-caught specimens. The mean body weight during the breeding season was:-

non-pregnant grey females 78.6±13.0 grams (n=17)
grey males 78.7±16.1 grams (n=16).

The mean body weight of wild individuals during the breeding season was estimated from the histograms published by Martin (1972a) and was 62.9±9.1 grams (n=65) for non-pregnant females and 67.6±10.6 grams (n=17) for males.

In addition, there were four particularly obese animals in the colony. These were the three animals housed in the observation room and one old wild-caught male MM1. The body weight of MM1 rarely fell below 100 grams even during the breeding season and in spite of rigorous dieting. Although the animals in the observation room were never weighed, all appeared to be very large and all had a tail-size of C/D or larger (see fig. 8 and Chapter III, section 2).

2.5.2. Cataracts

During the two years that the mouse lemurs were maintained in the Wellcome Institute, three individuals developed bilateral cataracts and since that time a further three cases have also arisen. During the five year period that the mouse lemurs were housed in University College no cataracts were observed and there appear to be no reports of this phenomenon in the literature.

2.6. Causes of Infant Mortality

Infant mortality during the pre-weaning stage poses a major problem to most captive breeding projects. Not only are the causes of death difficult to diagnose but even where diagnosis is possible treatment of the problem is itself difficult. Some nine infant mouse lemurs died at this stage during the course of the study. Three of these were eaten by the mother and thus no cause of death was ascertained and a further three had decomposed too far for any worthwhile postmortem examination to be undertaken. However, it was possible to determine that five of the nine deaths occurred in low birth weight infants. This is a problem which has been dealt with in detail in an earlier chapter (see Chapter VIII, section 3.1). It suffices here to say that in no case has a low birth weight infant in the study colony survived past weaning.

Postmortem examinations were undertaken on three infants, the ages at death being 4, 9 and 14 days respectively. The fourteen day old infant was a low birth weight infant which had been hand-reared. The remaining two infants were mother-reared twins of which the longer surviving was also a low birth weight infant. The third sibling of these latter infants survived into adulthood. Histological appearances were remarkably similar in all cases. There were apparently no particular congenital abnormalities associated with the low birth weight condition. In all cases pneumonia and enteritis were observed and inflammatory cell infiltration was present in most of the major organs.

3. Discussion

This chapter sets out to evaluate what evidence there is to support the hypothesis of a specific disease syndrome arising in captive populations of mouse lemurs, and to determine the extent to which this syndrome, if indeed it does exist, is related to stress. Snyder & Ratcliffe (1969) postulated that any species, if held in a defined environment throughout life, would develop a pattern of disease which is characteristic of that species in that environment. They went on to state that while this disease pattern might be predicted from diseases encountered in the wild, the full pattern would only be seen in captivity. To date nothing is known about the incidence of disease in wild populations of mouse lemurs. However, if the hypothesis of a captive disease syndrome is to stand, one would expect a repetitive pattern of postmortem findings to emerge from the data obtained from the study colony animals. In addition, one would anticipate that this pattern of post-mortem results would be paralleled by similar findings in other colonies of captive mouse lemurs. Table 49 (page 355) summarises the post-mortem findings on 19 mouse lemurs originating either in the study colony, the London Zoo colony, the Jersey Zoo colony and Doyle's South African colony. Twelve cases of lung lesions, six cases of kidney lesions, five cases of wounding and three of neoplastic disease were recorded in these nineteen specimens. In addition to these post-mortem findings, other frequently occurring pathological conditions were obesity (this involved all animals in the study colony to varying degrees), cataracts (six cases) and uterine cystic hyperplasia (three cases). In consideration of the very small numbers of animals studied during the course of this work, it is felt that the repetitive occurrence of all the above mentioned pathological problems is significant, particularly as Perret (1972, 1974) has reported similar problems of lung and kidney lesions and obesity in her colony of mouse lemurs and Petter & Petter-Rousseaux (1966) have reported neoplastic disease. Thus it is felt that there is sufficient evidence available to support the hypothesis of a disease syndrome arising in captive mouse lemurs. The most characteristic symptoms of this syndrome are thought to be lung and kidney lesions with chronic obesity, and neoplastic disease arising only in some cases. The other problems listed above may well arise in association with the syndrome but are not felt to be diagnostic.

Perret (1972, 1974) attributed many of the pathological problems encountered in her colony to stress and thus it was decided to evaluate the problems arising in the study colony in terms of this concept. According to Selye's hypothesis of a General Adaptation Syndrome arising in response to stress (this concept will be discussed in greater detail in the final discussion chapter, Chapter X), stress can alter the body's response to disease in two ways: firstly, adrenal stimulation, which is the corner-stone of his concept, results in a lowered resistance to disease due to the inhibition of the immune system and secondly, increased output of adrenal hormones leads to metabolic problems and the so-called "Diseases of Adaptation".

The approach to the evaluation of the disease syndrome arising in the study colony was two-fold. In the first instance all the adrenals of animals dying in the study colony were weighed and histologically examined in order to assess what indication there was of adrenal activation. Secondly, evidence from the literature was used to evaluate the association between stress and the various pathological conditions arising in the study animals. The problem concomitant with such an approach is that it is impossible to tell whether any adrenal activation which is discovered is the result of, or the cause of the observed pathological problems. As disease itself is stressful it can also lead to adrenal stimulation. In addition, if one takes Snyder's (1975) view that stress, by suppression of the immune system, renders the individual susceptible to infectious diseases, parasitic infection and neoplastic diseases of viral origin, then such a wide range of pathological conditions are linked with stress that almost every eventuality is covered by the concept, which as a result becomes of very little value when considering pathology. As a consequence only that evidence which links the observed lesions directly with adrenal activity will be considered here. However, this must not be taken to indicate that Snyder's view is invalid but rather that its inclusion makes analysis of the role of stress too complex.

The adrenals obtained from animals dying of illness in the study colony were both heavier and had larger cortical nuclei than those obtained from healthy specimens. This indicates a greater degree of adrenal stimulation present in ill animals. This agrees with Perret's (1972, 1974) findings. However, she found that nuclear diameter varied between 5.5 and 6.5μ in healthy individuals, depending on the time of year, and between 5.6 and 8.0μ in chronically ill specimens. These figures are somewhat higher than those obtained in the study colony. This difference probably reflects a real difference in adrenal activity between the two colonies rather than an artifact due to different histological technique, as one of the adrenals included in this investigation was prepared by Dr. Perret.

Kidney and lung lesions similar to those found in the mouse lemur were predicted by Selye (1950) to occur in association with stress. He found that congestion and oedema of the lungs were typical manifestations of the alarm reaction phase of the General Adaptation Syndrome. However, lung lesions, notably pneumonia, are common postmortem findings in small prosimians: for example Manley (1966), reporting on the morbid pathology of his own colony and reviewing the pathology reports of the London Zoo since 1951, noted that pneumonia claimed a fair number of these animals. Thus the frequent occurrence of lung lesions in these animals may well not be stress related but rather an indication of the susceptibility of small bodied animals to pneumonia arising possibly as a result of cold shock due to sudden draughts and the like. However, the observation in the study colony that lung lesions were usually found in association with other problems could indicate that these lesions are a secondary problem arising from stress due to disease and debility from other causes.

Similar kidney lesions to those found in the study colony of mouse lemurs were found by Perret (1972, 1974) in her colony. She linked these lesions to stress. Similar lesions, notably glomerular hypercellularity and collapse and nephritis, have also been reported in other species in situations of overcrowding and other social stressors; for example in the tree shrew, _Tupaia belangeri_ (von Holst, 1972), the rat, _Rattus villosimus_ (Barnett et al.,1975) and in the woodchuck, _Marmota monax_ (Chritian, 1963). This accords with Selye (1950) who reported that cortisone treatment led to glomerular disintegration in the rat and predicted nephritis as a disease of adaptation.

The increase in kidney weight in the study colony over that found in the wild could possibly also be interpreted in this light. Selye (1950) reported that treatment with D.C.A. and thyroxin led to increased kidney size in rats and predicted that enlarged kidneys would arise as a result of stress. Christian (1964) found that ACTH treatment stimulated kidney enlargement in adrenalectomised, corticosteroid maintained, female mice. This is contrary to what von Holst (1972) found to occur in captive stressed tree shrews, Tupaia belangeri, where kidney weight was lost in stressed animals. If one were to accept the stress hypothesis in this case the dichotomy could be explained by postulating an increase in kidney weight during the adaptation phase of the General Adaptation Syndrome and a decrease in weight during the exhaustion phase. This would agree with the available information as von Holst's animals were subjected to particularly severe stress. However, the possibility of a direct relationship between husbandry and increased kidney weight should not be neglected. Diet is undoubtedly of importance in this context. In addition, Barnett et al. (1974) have reported that, in captive mice, all internal organs are heavier than those of their wild counterparts. Although no reasons for this phenomenon are given, it could be that the greater weight of the kidneys in the captive mouse lemurs is a reflection of this.

The kind of problems arising in the determination of the relationship between the above mentioned pathological conditions and stress do not occur in association with wounding. This problem which, according to Snyder (1975), is one of the most frequent causes of mortality in captive animal populations, is clearly linked to social stress. In the case of the mouse lemurs, this is exemplified by the observations of the London Zoo colony where no problems with fighting arose until the colony numbered nine individuals. After this time reproduction ceased and indications of wounding appeared in the pathological records. The colony never recovered and all members died within three years. This relationship between wounding and overcrowding is further emphasised by the fact that wounding was negligible in the study colony groups. It is also well known in other species that in captivity aggression increases with increased population: for example in mice (Southwick, 1955) and in voles, Microtus agrestis, (Chitty, 1956). However, the role of social interactions and social status in keeping these wounds open and thus increasing the risk of debility and death, as observed in both the London and Jersey groups of mouse lemurs, has not received attention from other authors. It is not a phenomenon solely confined to mouse lemurs; similar observations have been made on the subordinate female of a group of lesser pandas, Ailurus fulgens, (pers.obs.). Delayed wound healing is not merely a result of overgrooming, ACTH stimulation leads to decreased protein synthesis which in turn leads to a slower rate of healing. Thus, social organisation deserves to be taken into consideration when deciding on the best way to deal with the problem of wounding in any group of animals.

The three remaining major pathological findings in the mouse lemur namely, cataracts, obesity and neoplastic disease, also have tenuous links with stress. For example, Selye (1950) links the occurrence of some forms of cataract with stress; Snyder (1975) links the occurrence of some forms of neoplastic disease with stress and Perret (1975) links obesity to hypothyroidism and stress. However, it is felt that in the case of study colony mouse lemurs, these findings are more probably directly related to problems in husbandry techniques. If the case of cataracts is considered first, those forms found by Selye (1950) to be related to stress were firstly associated with a diabetic condition and usually reversible. This was not the case with the cataracts in the mouse lemurs. It is felt in this case that this problem was related to the method of lighting. This could also explain the absence of cataracts in University College and their occurrence in the Wellcome Institute. Although red light was used to simulate night conditions in both places, the light bulbs used in the Wellcome Institute were the more powerful(40 watt bulbs only were used in University College whereas a combination of 60 and 100 watt bulbs were used in the Wellcome Institute). Duke-Elder (1959) reported that certain types of lighting can lead to traumatic cataracts in humans, this is particularly true of light at the infra-red end of the spectrum, similar observations have also been made in laboratory animals (Hogan & Zimmerman, 1962). Red lighting was chosen for the mouse lemurs as they appear to be insensitive to it, their pupils being maximally open under red lighting conditions. Therefore it is possible that the high intensity of red light falling onto the lens caused this problem.

Snyder (1975) links the occurrence of neoplastic disease of viral origin to stress. However, the cause of cancer in the mouse lemur colony is unknown and although a viral cause cannot be ruled out it is possible that the animals were exposed to low levels of various carcinogenic agents in the course of routine husbandry. In view of the fact that neoplastic disease arose separately in four colonies this is felt to be unlikely.

Obesity in the case of the chronically fat animals is probably linked to hypothyroidism, although whether this condition is in fact related to stress in these animals remains unproved. Perret (1975, 1977) has indicated that there are other causes of hypothyroidism in captive mouse lemurs, notably the absence of a circadian variation in ambient temperature which is reported to be related to the regression of the thyroid. In this context it is interesting to note that the Observation Room in which three of the four chronically obese animals were housed, was not subjected to any variation of temperature between the red and white light periods as were the other cage rooms. In addition, the general elevation of body weight in the colony as a whole is probably closely related to husbandry techniques. Such factors as inadequate diet and absence of any annual variation in the food offered are probably of great importance. Under natural conditions mouse lemurs are subjected to seasonal variation in diet, both the quantity and types of food available vary seasonally. Andriantsiferana & Rahandraha(1973a) have reported seasonal variation in food preference in captive mouse lemurs. This seasonal variation was not catered for in the study colony of mouse lemurs. This may have resulted in the animals consuming too much of the wrong kind of food thus causing obesity. However, restricting the animals to a confined area may result in a lack of exercise and the relationship between this factor and obesity must not be forgotten.

Thus, as can be seen from the preceding discussion, the precise role of stress in the aetiology of disease in the captive mouse lemur is difficult to ascertain and remains unproved at this time. However, circumstantial evidence in the form of elevated adrenal weight and the increase in the size of the adrenal cortical nuclei, plus the reports in the literature of similar pathological findings in other animal species under conditions of stress, would tend to support the hypothesis that stress is involved. The relationship between adrenal activity and disease in the study colony is also indicated by the observation that most deaths in the colony have occurred during the period of increasing or maximal daylength, that is to say during the period of greatest adrenal activity (see Chapter III, section 3). However, the possibility that the enlarged adrenals found in the chronically ill individuals may have been the result of the stress of disease cannot be ignored. It is impossible to distinguish, at this stage, whether the observed stress was the cause or the result of disease. It may be possible to do this sometime in the future by carefully monitoring all individuals on a regular basis for the occurrence of various physiological parameters commonly associated with stress.

Thus, although the role of stress remains obscure, as a concept it may still be proved useful, particularly if it leads to a critical evaluation of captive conditions and husbandry techniques in terms of potential stressors. A general alteration of the conditions of captivity towards those of the natural environment for which the animal is adapted, which could result from such an evaluation, might also eliminate many of the aspects of captivity which are directly related to disease.

SUMMARY

1) Investigation of adrenal weight has indicated that adrenals taken from individuals which died exhibiting no obvious pathological symptoms are lower than those obtained from individuals which died exhibiting such symptoms. In addition, the cortical nuclear diameters of adrenals obtained from chronically ill animals are larger than those obtained from animals which died without showing the symptoms of disease. This indication that adrenal activity is associated with disease is supported by the observation that most individuals dying in the study colony did so during the period of increasing or maximal daylength (i.e. the period of increased adrenal activity).

2) From the data obtained from various mouse lemur colonies a pattern of common post-mortem findings has emerged: lung lesions, kidney lesions, neoplastic disease and trauma. Other frequent pathological findings in mouse lemurs include chronic obesity, cataracts and uterine cystic hyperplasia.

3) From these data it is postulated that a disease syndrome arises in captive mouse lemurs. This syndrome is characterised by lung lesions and kidney lesions, with chronic obesity and neoplastic disease arising in some cases.

4) An attempt was made to evaluate this disease syndrome and other pathological findings in terms of stress. This was undertaken by evaluating adrenal activity in the animals dying from these problems, by reference to adrenal weight and adrenocortical nuclear width and also by comparison with data available in the literature on stress-related diseases. Some problems, such as wounding by conspecifics, are clearly related to crowding and social stress. The pathological findings (with the possible exception of neoplastic disease) which form part of the postulated disease syndrome could

all be related to stress. All occurred in association with signs of adrenal activity and similar findings are reported in the literature as occurring in association with stress. However, as disease itself is known to be a stressor, the problem remains as to whether the pathological conditions observed were the result of or the source of the postulated stress.

SECTION D

DISCUSSION OF FINDINGS

CHAPTER X

DISCUSSION

This study sets out to investigate the reproduction and behaviour of the lesser mouse lemur in captivity. To this end data have been collected from a study colony of mouse lemurs over a three year period. These data have been supplemented by: unpublished data collected from the same colony of mouse lemurs during a three year period prior to the study, by data collected during two years after the completion of the main study, by data collected in Jersey Zoo, by data available in the records of the London and Jersey Zoos, by data obtained from histological specimens from wild mouse lemurs and by recourse to the literature.

The approach to the research topic has been a general one in which data have been collected on a wider range of topics than usual. The reasons for adopting this approach are twofold: firstly the aim of the study was to provide a broad understanding of the behavioural and reproductive biology of the mouse lemur (a species relatively new to captivity). This was felt to be necessary by virtue of the paucity of information available on these topics in the literature. Secondly, the method of approach was dictated by the species concerned. In this study one was dealing with an exotic species and thus, very rightly, fewer animals were available than would be the case in studies dealing with laboratory animals or more familiar species. If the mouse lemur were a familiar inhabitant of research colonies of zoos, which bred regularly in captivity, a study such as this would be unnecessary. The paucity of subjects may therefore be seen as an integral factor relating to studies on unusual exotic species generally. Insufficient numbers of subject animals obviously lead to the limitation of the quantity of data available on each aspect of a research topic. In the case of the mouse lemur the availability of data on certain aspects of the study is restricted still further to certain times of the year, by virtue of the seasonal nature of the species. Thus a broad approach was dictated by the paucity of data available on some individual topics. However, such an approach is advantageous in that it provides a coordinated overall picture of the biology of the subject species in captivity.

By virtue of the method of approach adopted comprehensive baseline data have been collected on all reproductive parameters: seasonality, testicular growth, inter-oestrus periods, gestation lengths, litter size and sex ratio, development of the young and sexual maturity. Once these basic parameters had been established and became familiar certain deviations from the expected "norm" became apparent: infertility, failure of pregnancy, the production of non-viable young and inadequate maternal care were observed. As a result of the broad approach adopted, a wide range of behavioural data were also collected on such diverse topics as activity rythms and social behaviour. These have then been correlated with breeding success or the lack of it to provide indications as to precisely which factors influence reproductive potential in captivity. The result of this study, in addition to the collection of basic reproductive and behavioural data, has been to give a picture of the ways in which diverse facets of the captive environment can affect animals and to suggest ways in which the most detrimental effects can either be eliminated or reduced.

As such, the data presented here should be of use to future field and laboratory studies on the lesser mouse lemur. The reproductive parameters provide the basic information essential for analysing both trends in and potentials of wild populations, data essential for ecological studies and conservation plans. In the laboratory situation such data allow for the long-term planning of a colony and provide a baseline against which a colony's success or failure can be evaluated. The behavioural data should provide an aid to the interpretation of behaviour observed in the field situation and provide a base from which future laboratory studies on mouse lemur behaviour can begin. It is also intended that the information presented here should have some bearing on the husbandry and management techniques employed, not only for the mouse lemur but for exotic species generally. The issues raised concerning the influence of the captive environment on both reproduction and behaviour should concern all those who are seriously trying to breed exotic species in captivity.

As a result of the diversity of topics presented it has been decided that, in the interest of clarity, this discussion will essentially be subdivided into two sections. In the first section the normative data will be discussed: basic reproductive parameters and behavioural data will be viewed in terms of adaptation and evolutionary importance. The data discussed are those dealing with the grey mouse lemur and adaptations are interpreted through a combination of field and laboratory information. The second section will deal with deviations from the norm arising in captivity. These deviations will be evaluated in terms of stress. The behaviour of the study species is then examined both as a source of possible stressors and as a diagnostic tool by means of which stressors can be determined and those individuals most likely to suffer from stress can be identified. From these data an attempt will be made to establish guidelines of a more general nature as an aid to those attempting to breed exotic animals.

If we begin by considering the normative data, it is apparent that the biology of the mouse lemur is influenced primarily by two factors, climatic variability and limitations imposed upon it by body size and nocturnality. The first of these factors is of particular importance to any discussion of the differences between red and grey mouse lemurs. Before continuing some mention must be made of the climatic factors which shape the mouse lemurs' existence.

The mouse lemur, in common with all other living members of the Infraorder Lemuriformes, is an inhabitant of the island of Madagascar;

a large island lying off the east coast of Africa. Madagascar lies almost entirely with the tropics, it stretches from 12° south of the Equator at its northernmost limit to 25°30' S at its southern tip, and consequently its climate is essentially tropical. However, there is a considerable variation in climatic types over the island. This variation is in part due to the longitudinal extension of the island, some 1450 km from its subequatorial northern part to its subtropical southern region. In addition to its length, Madagascar has sufficient width (450 km on average) to display the characteristics of a small continent, which in turn are reflected in its climate. These variations in climate are important when considering the mouse lemur. The rufous mouse lemurs are confined to the eastern regions, whereas the grey mouse lemurs are found in the west and south of the island. In addition, there is a region of overlap between the two sub-species in the north (Martin 1972a). If these distributions are considered in climatic terms it can be seen that the rufous mouse lemurs are generally confined to those regions of least climatic variation. The east is a region of high humidity and abundant precipitation. The humidity does not fall below 60% and as such there is no marked dry season. The high atmospheric humidity also makes for relatively uniform temperatures throughout the eastern region over the whole year. In addition, the regions where the rufous and grey mouse lemurs overlap, which according to Martin's map (1972a) appear to be the Massif d'Ambre and Tsaratana, are also areas of more moderate and uniform temperature, where the dry season is less marked. In contrast, the grey mouse lemurs come from areas of marked climatic variability. With the exception of the region of overlap with the rufous mouse lemurs, the areas inhabitied by the grey mouse lemurs exhibit a marked dry season and the variation in temperature experienced during the course of the year is also evident.(Donque, 1972).

As this thesis deals mainly with the grey mouse lemur, it is the influence of climatic variability, in particular the effects of the dry season, which is of immediate concern. As we have seen, the biology of the mouse lemur is strongly influenced by photoperiodic change in that both endocrine and behavioural cycles are synchronised by daylength change. The function of these various cycles seems to be geared to reducing the animals' demands on their environment during the winter, short daylength period, which corresponds with the dry season. The ways in which the animal does this are numerous and complex but are essentially based on the annual cycles of endocrine activity. Not only is reproductive activity affected, but the cycles exhibited by the adrenal and thyroid glands have effects ramifying throughout the mouse lemurs' biology. Essentially, all endocrine functions, in the adult animals at least, are reduced to a minimum during the dry season. This results in a general lowering in metabolic activity, behavioural activity and feeding frequency, and there is also a lowered tendency towards antagonistic interactions with conspecifics. The reduction of metabolic activity and behavioural activity, together with a decrease in the efficiency of the homeothermic mechanism and the concomitant reduction of body temperature, ensures that energy, stored as fat at the end of the rainy season, is consumed slowly. The consumption of energy in agonistic encounters is reduced both by the lack of aggressive responses to conspecifics and by an increase in allogrooming behaviour which functions to reduce aggressive tendencies (Andrew, 1964). The endocrine activities increase again with increasing daylength in order to prepare the animal for exploitation of the resources of the rainy season to the maximum. The development of testicular function in the males and the onset of oestrus in the females are so timed that the first young are born early in the rainy season, the period of maximum food availability. In addition, those females which are successful in producing young early in the rainy season have sufficient time to produce a second litter before the dry season sets in. This ability to maximise the use of food availability during the rainy season is furthered by the surge in feeding activity recorded just after longest daylength, and thus at the end of the rainy season. This burst of feeding behaviour which appears, from Perret's (1974) results, to occur at a time when the activity of the thyroid is decreasing, allows for energy to be stored as fat which helps to see the animal through the dry season.

The second factor which is of importance to the behaviour of the mouse lemur is constituted by the limitations imposed upon it both by body size and by nocturnality. Denham (1971) has indicated that the lives of primates (and indeed of all animals) are governed by two sets of responses: those related to the acquisition of energy from sources at lower trophic levels, and those related to the prevention of being used as an energy source by other animals. In other words to eat without being eaten is of prime importance to all animals. There would appear to be three mechanisms by which animals can avoid a predator: hiding, fleeing or confrontation (Denham, 1971). The mouse lemur is obviously too small to adopt a confrontation strategy whereas its natural habitat, which Martin (1972a) terms the fine branch niche, offers ample opportunity for crypsis. Such a tendency towards concealment would dictate a solitary or semi-solitary life style as well as imposing limitations on modes of communication. Nocturnality, as Charles-Dominique (1977) has pointed out, is an important consideration when looking at the biology of a given species. It has important effects on the sense organs, notably the eye, as well as having a profound effect on modes of communication and methods of collecting food. In addition, it has been noted that most nocturnal species are solitary, presumably because their nocturnal existence lends itself to concealment as a method of predator avoidance.

This need for concealment is reflected in the social organization of the mouse lemur. Each individual spends most of the night foraging alone, but joins together with other individuals of the same sex to form sleeping groups over the day. The females tend to nest together in groups which are thought to be matriarchal lineages. These females also tend to share the same home range. Males on the other hand tend to sleep either alone or in pairs. These males have individual ranges which are larger than those of the females and overlap with those of several females. Obviously, with this kind of social organisation the dissemination of information over a relatively large area is essential. Information on the whereabouts of oestrous females is essential as is the need to warn other individuals, particularly those which are related, as to the proximity of a predator. However, this information must be dispersed without recourse to close contact and without attracting a predator. Visual signals are by necessity very much restricted by nocturnality and by the dense foliage cover of the habitat. Vocal signals on the other hand must be carefully structured so that the caller is not easily located. As such they also tend to be high frequency calls which attenuate rapidly. Olfactory communication is obviously the ideal solution to the communication problem of the mouse lemur. It allows for communication both over distance and over time, thus not necessitating any contact between signaller and recipient whilst providing opportunity for the communication of diverse messages by variation in the type of scent mark used or by variation of the scent produced in an individual mark.

One important fact which has emerged by virtue of the collection of this normative data is evidence of the tremendous similarities which exist between the mouse lemur and the galagos. Such similarity is apparent in reproductive behaviour, communicative behaviour and social interactions. It has been noted that mating behaviour, parturient behaviour and early maternal care are very similar to those described for _Galago senegalensis_ (Doyle _et al._, 1967, 1969). Visual communication signals are in many instances indistinguishable from those described for _Galago senegalensis_ (Andersson, 1969), and the modes of olfactory communication employed by the mouse lemur are observed throughout the galago and loris groups, for example by Andersson, 1969; Charles-Dominique, 1977; Ilse, 1955; Clarke, 1976. Charles-Dominique & Martin (1970) have noted that, although actual vocalisations differ between the mouse lemur and the galagos, the categories of vocalisation are similar in both these groups. In addition, the social organisation of mouse lemurs housed together in groups is similar to that described for *Galago crassicaudatus* (Roberts, 1971, Drews, 1973) and the behavioural patterns seen during introduction experiments are similar to those described for *Galago senegalensis* in similar situations (Bearder & Doyle, 1969).

The identification of such shared characteristics is important as they can be used to build up a picture of the ancestral prosimian or indeed the ancestral primate form. Such shared characteristics can either be attributed to convergence or to the retention of primitive characteristics. It has already been postulated by Charles-Dominique & Martin (1970) that many of the similarities between the Cheirogaleinae and the Galaginae are likely to be ancestral characteristics and as such the similarities listed above can be used to build up our view of the ancestral form still further.

In addition to these shared features there are two other mouse lemur characters which would seem to be essentially primitive, and thus possibly ancestral: marking behaviour and the prolongation of the inter-oestrus period. It has already been postulated that an olfactory communication system based on natural bodily products enhanced by the secretions of simple scent glands must be essentially primitive. Similarly, one plausible explanation of the prolonged inter-oestrus periods found in the mouse lemur is that it is a primitive retention. It has been postulated by Perry (1971) that the prolonged inter-oestrus period of the dog, *Canis familiaris*, is the result of being reproductively intermediate between a polyoestrous species and a mono-oestrous one with a prolonged period of receptivity.

Therefore, the mouse lemur may also be reproductively intermediate between its polyoestrous forebears, with their short cycles and short periods of receptivity, and the galagos and lorises with their longer cycles and longer periods of receptivity.

Two other features of mouse lemur behaviour which merit discussion here are female dominance and territotiality. Female dominance is a feature which has been reported throughout the prosimian group. Jolly (1966) has reported it in Lemur catta and Lemur macaco, Andersson (1969) has reported it in Galago senegalensis and both Roberts (1971) and Drews (1973) have reported it in Galago crassicaudatus. It has been suggested to me (S. Bearder, pers. com.) that female dominance must have a profound influence on social behaviour, and thus it could be suggested that it is a prime feature of prosimian social organisation and one which is very different from the simian primates. However, female dominance, in the case of the mouse lemur at least, would not appear to have a profound influence on behaviour. Under natural conditions males and females live separately, coming together only to mate. Thus, their relative status would have very little impact on their everyday existence, although Martin (1972a) did observe that the female mouse lemurs may have the better habitat under natural conditions. The female ranges usually incorporate the preferred feeding plants. In view of the smaller home ranges held by the females a tendency towards female dominance may be dictated by the need of the female to be able to protect the food resources of her range from the depredations of the male.

In captivity, dominance relationships do not seem very marked, except in introductions. This situation is not normal and thus may only be an indication of a female's response to a strange intruder and not her normal reaction to a familiar neighbouring male. This suggestion finds some support in the reduction of aggressive tendencies in the female after familiarisation with the male.

In the case of the observations in the group situation, the apparent dominance of the female may have been an artifact resulting from the small sample size or it might be related to observer bias. As Còlin Blakemore pointed out in the 1976 Reith lectures, "Perception is a hypothesis about the world", thus the predisposition to observe male or female dominance may be attributed to the observer and such a predisposition would have ample scope for action in a species where hierarchical structures are not very marked. This may explain dichotomies in the literature; for example, Andersson (1969) reports that in some instances female galagos were dominant whereas Bearder & Doyle (1969) report that this was never the case, although they were presumably observing the same group of galagos. Petter-Rousseaux (1964) has reported that male mouse lemurs chase oestrous females, while such behaviour was never observed by the present author. Therefore, considering all these factors it may be more appropriate at this stage to deduce that the hierarchical structure of mouse lemurs is not very marked and that, though there may be some predisposition toward female dominance, it cannot be assumed that female dominance is the rule.

Territoriality would seem to be a feature of behaviour which is apparently absent in the mouse lemur in captivity. From observations of groups of mouse lemurs it would appear that although each individual had its own preferred areas each individual would use all areas of the cage. No aggression was apparent when one individual entered the preferred area of another. In addition, there is no indication of territorial marking. Also in the introduction experiments, the location where an encounter took place, (home cage or otherwise) had no influence on the outcome of the encounter. However, no firm conclusions can be drawn on the territoriality, or absence of it, of the mouse lemur in the field. The constrictions imposed on the animals in captivity, small cage size and the close proximity of many conspecifics, may be such as to eliminate normal territotial behaviour patterns.

A final point of importance which has emerged from these normative data is the fact that there are differences between the red and grey mouse lemurs. These include differences in body weight, gestation length and duration of the breeding season. As we have seen earlier, the grey mouse lemurs encompass a greater range of climatic variability within their range than do the red mouse lemurs. This greater adaptability of the greys is reflected in their relative success in captivity. The study colony, which originated from seven females and three males of each subspecies, numbered ten greys and nine reds by the start of the study and twenty-three greys and six reds by the end. However, the most important difference between the two forms is probably that in vocalisations. In addition to vocalising less frequently than the greys, the red form produces a different mating call. This difference in mating calls, the soft squeak in the reds and the trill in the greys, is particularly important when considering the affect it could have as a species isolation mechanism. It is worthwhile noting in this context that no successful cross matings between the two forms have been obtained in captivity nor do they appear to interbreed in the wild in those areas where their ranges overlap (Martin, 1973). These data together with Martin's (1973) observations on the differences in cranial and external characteristics between the two forms indicate the need for reassessment of the taxonomic status of the two forms of mouse lemur, a need which has already been indicated by Martin (1973).

As has been stressed on numerous occasions in the preceding text, the collection of comprehensive baseline data is not only important in its own right in establishing the reproductive parameters of the study species but is also of immense value when establishing occurrence of deviations from the norm. Data collected during the course of this study would indicate that deviations from the normal pattern of reproduction do occur and take two forms: reproductive suppression and reproductive stimulation. In connection with the former abnormality recourse has to be made to Selye's (1959) theory of a General Adaptation Syndrome arising in response to stress.

This theory is of particular relevance here, as its cornerstone is the supposition that under adverse conditions an organism will sacrifice those physiological functions of less immediate importance to its well-being (for example reproduction or resistance to disease) in order to maintain homeostasis. In the case of the mouse lemur one could attribute the adverse stimulation to the conditions of captivity per se or to the influence of the close proximity of numerous conspecifics on a normally semi-solitary species. Before continuing further it is necessary to discuss the concept of stress and its history in greater detail.

The concept of the General Adaptation Syndrome, that is the concept of a unitary physiological response to a variety of adverse stimuli, was first developed by Hans Selye in the late 1940's. However, Charles Darwin (1872) was following a similar line of reasoning when he noted that a variety of stimuli: fear, anger, cold, injury and disease, gave rise to similar external signs; thus people turn pale with fear or illness and also go white with anger.

The first major step towards Selye's theory was made in the middle of the last century by the French physiologist Claud Bernard (1859). He realised the importance of internal stability or the constancy of the "milieu interieur" (Selye, 1950, Barnett, 1964) to the maintenance of life.

In this century William Cannon (1929), the American physiologist, first coined the term "homeostasis". He worked on the physiology of the adrenal system and its role in preparing an individual for "fight or flight". In addition, he directed attention to the role ot adrenaline and the automatic nervous system in bringing about the internal adjustments necessary for the maintenance of homeostasis (Selye, 1950, Barnett, 1964).

Selye was first motivated to undertake research in this field while working in hospitals. He noted that patients suffering from a variety of complaints exhibited many of the same symptoms.

The basis of Selye's hypothesis is that many forms of adverse stimulation, that is agents which tend to disrupt bodily equilibrium (e.g. illness, heat, cold and wounding), elicit a similar pattern of physiological response. Selye termed these agents "stressors" and the bodily response "stress". As many of the responses to stressors are similar, Selye postulated that a syndrome arises in response to systematic stress. He called this syndrome "General Adaptation Syndrome" and he defined it as "the sum of all the non-specific systemic reactions of the body which ensure upon long term exposure to systemic stress". He emphasised that the General Adaptation Syndrome (G.A.S.) is not a transitory emergency adjustment but is provoked by prolonged environmental changes and that it is an adaptive reaction necessary for the survival of the organism. It is also intimately related to adrenal stimulation and it has been observed that adrenalectomised, corticosteroid-maintained animals cannot adapt to stress (Barnett, 1964). It is the increased production of corticosteroids which enables the animal to adapt. Thus, most of the readily identified changes associated with adaptation are related to the functioning of the adrenal and pituitary glands (Barnett, 1964).

The G.A.S. results in physiological functions which are of less immediate importance to the organism (for example, growth, reproduction and resistance to disease) being temporarily sacrificed in order to maintain homeostasis.

Selye recognised three stages in his adaptation syndrome:-

The alarm reaction; is the response to which the individual is not yet adapted,

The stage of resistance; is the sum of reactions elicited by prolonged exposure to a stressor to which the individual is adapted. Many of the changes observed in the alarm reaction disappear. However, tolerance of other stressors is reduced.

The stage of exhaustion; is the sum of responses which develop as a result of prolonged over-exposure to stressors to which adaptation was developed but could no longer be maintained. Many of the lesions associated with the alarm reaction return during this stage. Selye also reported that all individuals reach this stage if stressed for a long enough period.

Therefore, as Barnett (1964) has noted the ultimate result of prolonged stress is death. Although the G.A.S. is necessary for the survival of the individual, the process of adaptation may itself become the immediate cause of disease or produce favourable conditions for the development of certain diseases.

The hypothesis of a General Adaptation Syndrome is still highly controversial. As Fraser et al. (1975) have pointed out, many recent studies indicate that different stressors elicit different reactions as well as similar ones. In fact, different stressors may even lead to different adrenal responses. For example, extreme cold stimulates the secretion of glucocorticoids while extreme heat does not (Hellman, 1956, cited by Fraser et al., 1975). Therefore, Fraser et al. challenge the unitary response concept of the G.A.S. and emphasise the importance of differences in response.

Selye's concept of a General Adaptation Syndrome, particularly his suggestion that a reciprocal relationship may exist between the formation and secretion of ACTH and the gonadotrophins, formed the basis of Christian's hypothesis of a self-regulatory mechanism which controls the population density of rodents (Brain, 1971). There then followed a series of studies on the physiology of animals under social stress: for example, those by Christian (1950, 1955a, 1955b, 1956), Clarke (1955), Barnett (1964), von Holst (1972, 1974) and Southwick (1955). These studies indicated that animals suffering from social stress, either due to overcrowding or exposure to a dominant individual, exhibit many of the responses predicted by Selye's G.A.S. hypothesis, namely adrenal stimulation paralleled by loss of weight, suppression of reproduction, suppression of growth, decline in resistance to disease and parasites and, in extreme cases, death. However, as Brain (1971) has indicated, much of the evidence on which the relationship between ACTH and the gonadotrophins is based is only circumstantial: for example, ACTH when given to young female mice suppresses maturation of the reproductive tract; castration of males results in an increase of adrenal weight and testosterone administration reverses this effect.

The problems arising out of Selye's hypothesis are further complicated by the broadening of the contexts in which stress is said to arise (Fraser et al., 1975). Psychologists talk of stress in terms of work, noise level and urbanisation whereas veterinarians may talk of it in terms of weaning, overfeeding or change of diet. Another complication involves the question as to whether the maintenance of an animal in an environment where stressors are completely absent is itself stressful.

Before considering whether the concept of stress can be applied to the problems arising in the mouse lemur in captivity, it must first be decided how valid the concept is in its own right given the objections raised against it. Some of the objections can be overruled when considering Selye's original hypothesis, which is based on the occurrence of many similar responses to stressors and not on the absence of numerous different responses. The broadening of the contexts in which stress is said to arise also does not alter the original theory, as long as these stressors are prolonged and evoke the responses predicted in the G.A.S.

The argument that the theory on the relationship between ACTH and the gonadotrophins is based on circumstantial evidence is valid, but in view of the overwhelming support it gets from laboratory studies on stress it may well be argued that this evidence is acceptable. The resolution of the problem as to whether boredom or absence of stressors can be considered stressful depends on whether adrenal stimulation is involved. The suppression of reproduction found in bored animals might be related to the absence of adequate adrenal function. However, there is evidence that chronic absence of stressors results in adrenal stimulation in some laboratory rodents (cited Fraser et al., 1975).

Therefore, this thesis takes the view that observations on the affects of population density and other social stressors would tend to support Selye's hypothesis; therefore, as a concept it is both valid and useful in oonsidering problems relating to reproduction and diseases in captive animals.

As the mouse lemur is by nature a solitary species, it might perhaps be expected to be more adversely affected by proximity of its fellows than would more social species. Thus, despite the fact that the mouse lemurs were mostly housed individually and, in some cases, out of visual contact with their fellows, many of the phenomena associated with overcrowding were observed. The phenomena, which included abnormal oestrus, failure of conception, intrauterine loss, inadequate maternal care and suppression of testis development, resulted in the suppression of reproductive potential.

However, the influence of stressors of environmental origin must not be discounted. It is difficult to ascertain whether the stressors acting on the mouse lemur were environmental or social in origin. In fact, it is probable that both environmental and social stressors impinged on them. Evidence exists that the environmental conditions in which the mouse lemurs were maintained were stressful; fire bells and inadequate temperature variation had their effects. However, captive conditions and husbandry techniques can act directly on the animals as well as being mediated by the G.A.S.; for example, a poorly balanced diet can lead directly to nutritional deficiencies, obesity or vitamin A poisoning. These resulting conditions probably also act as stressors. Social stress factors were also undoubtedly operating. This is evinced by the observations that the reproductive problems were most prevalent in socially subordinate animals or in those housed in group conditions.

However, it could also be argued that the greater degree of stress in these individuals made them more vulnerable to stressors of other origins. Thus, it is reasonable to conclude that stressors of both physical and social origin were acting on the mouse lemurs and that it is very difficult to separate the effects of the two.

Due to housing conditions, social stress must have been mediated by scent or sound as even animals which had no visual contact with conspecifics developed problems. Pheromones have been reported as transmitting information about social stressors in other species (Carr et al., 1970, cited by Brain, 1971). This does not necessarily imply that the pheromones themselves are stressors but it is rather the information they provide, such as indicating the close proximity of a dominant conspecific which is stressful. In the mouse lemur vocalisations are also considered to be important potential stressors and it has been noted that both the fear and disturbance calls disrupt maternal behaviour. In addition there is strong evidence to suggest that the trill call is an important factor in the mediation of testis suppression in the grey males.

In the group situation, social behaviour is another important mediator of social stress. In the two groups observed during this study, no reproductive success was recorded. This was not caused by the same factors in both groups. In Jersey the male's testes developed with increasing daylength to maximal size and mating was observed with both females, but no pregnancy ensued. During the first year the situation observed in the London group was similar. However, in the second year the testicular development of the male was suppressed, presumably by the behaviour of the females. Pseudo-male behaviour of the females was particularly relevant in this context and was probably associated with the rise in agonistic behaviour observed. Aggression is itself associated with adrenal stimulation and thus a self perpetuated situation may be envisaged as ensuing with stress stimulating aggression and aggression causing further stress.

The group situation per se does not necessarily suppress reproduction in the mouse lemur. The records of both the London and Jersey Zoos indicate that successful reproduction can occur in mouse lemurs housed in groups. Observations in the Rotterdam Zoo and data published on a colony maintained in Madagascar (Andriantsiferana et al., 1974) indicate similar successes. The lack of breeding success encountered in the study groups may therefore have been associated with the structure of the groups or with the presence of external stressors; for example noise. However, in view of the fact that the successfully breeding mouse lemur groups cited above were not reproducing optimally, the apparent lack of success in the study groups may be an artifact of small sample size: number of groups or number of breeding seasons. London Zoo's records extend over ten breeding seasons while the colony studied by Andriantsiferana comprised 5-7 groups of between 3-9 individuals over two breeding seasons.

In addition to reproductive disorders, post-mortem results exhibited many lesions which Selye attributed to diseases of adaptation. These lesions were similar to those which have been described in tree shrews, Tupaia belangeri (von Holst, 1972) and woodchucks (Christian, 1963) suffering from social stress. The finding of such lesions in the mouse lemurs may be taken as an indication of stress. Further support for the hypothesis that stress led to the observed lesions in the mouse lemurs was then sought and data were collected on adrenal weights to this end. Although adrenal weights on their own are not valid indices of adrenal function (Christian, 1971) unless certain precautions are observed, it is considered here that adrenal weight provides a strong indication that many of the mouse lemurs were suffering from stress, particularly as the other findings give support to this contention.

It is suggested, therefore, that the mouse lemur suffers from stress of both social and environmental origins in captivity.

The mouse lemurs which survive in captivity can be considered to be those which are adapted to the stressors and, therefore, in the resistance phase of the syndrome. Those individuals which are best adapted have a certain amount of reproductive success even if not reproducing at their full potential (2 litters per season were postulated by Martin, 1972a). However, as they are in the resistance stage of the syndrome they exhibit reduced tolerance to additional stressors. The relationship between further adrenal stimulation and death is emphasised by the observation that most deaths occur in the increasing daylength phase of the light cycle, that is to say during the period of natural adrenal stimulation.

The stimulation of reproduction by stress is a separate phenomenon. The observations do not contradict Selye's hypothesis as acute and, not chronic stress is involved in this case. The stimulation of reproduction by stress has been reported in several species and although it is a phenomenon which could have considerable impact on animal husbandry it has stimulated very little research. Reports exist in the literature which indicate that very slight differences in treatment stimulate reproduction in one case and inhibit it in another; for example, Sieck & Ramley (1975) reported that handling delayed the onset of puberty in mice whereas Morton et al. (1962) reported the reverse. Thomas & Terman (1975) reported that deer-mice housed in groups bred better than those housed in pairs but Terman (1968) reported contrary findings. Paris & Ramaley (1973) have reported that in some instances heat stress advances the age of puberty of mice while in others it delays it (see Chapter VII, section 4). These results indicate that small differences in treatment can produce diametrically opposite effects; these differences may be magnified by the differences in susceptibility to stress of the individuals involved. This study on mouse lemurs is parcticularly interesting in that both stimulatory and inhibitory results of stressful stimuli have been observed in the same individuals.

The postulated difference between acute and chronic stress could also explain why van Bers (1973) reported that stress inhibited feeding behaviour, while findings reported in this thesis would indicate the converse to be true. In this thesis the effects of acute stress are noted while van Bers (1973) was reporting on the effects of chronic stress.

As stated above, it is postulated that this difference results from the difference between chronic and acute stressors. Selye's work and that of the population biologists has dealt with chronic stress. It is a basic premise of evolutionary theory that advantageous characteristics are selected for. Given that an animal fleeing from a predator undergoes acute stress during its flight, it would be disadvantageous to that individual if it experienced reproductive suppression as a result. In fact it may be selectively advantageous to be reproductively stimulated by acute stress; thus, acute stress after a natural catastrophe, resulting in the loss of a large part of population, would give rise to rapid reproduction and an advantageous rise in population. It is possible that the burst of population growth which has been observed in marmots after the removal of a large percentage of their population (Snyder, 1961) was due not only to the removal of constraints on reproduction induced by maximum population density but also to the stimulatory affect of acute stress.

The stimulatory effects of stress may be most readily apparent in seasonal animals such as the mouse lemur or the deer-mouse. In these species there is little adrenal activity during the non-breeding season and therefore stressors acting at this time possibly lead to a level of adrenal stimulation which is little higher than that encountered during the normal breeding season. In contrast, similar stress in the breeding season may cause too high a level of adrenal stimulation. This over-stimulation possibly accounts for the inhibitory reactions which arise from what are apparently acute stressors. The affect of fire bells on mice (Martin, 1976) and tree shrews (D'Souza & Martin, 1974) could result from such adrenal over-stimulation.

In addition to aiding in the identification of reproductive abnormalities, normative data can give important insights into the factors leading to such deviations as well as assisting in their diagnosis. The importance of behavioural data is two-fold: firstly a full understanding of behaviour is important when trying to identify stressful factors or mediators of stress and, secondly, deviations from the normal anticipated patterns of behaviour are symptomatic of stress. Thus the ability to recognise unusual behaviour patterns is an asset when trying to diagnose stressed animals. The role that behaviour (social interaction, scent production and vocalisation) can play in reproductive suppression has already been discussed. The way in which behaviour may be diagnostic of stress is exemplified by the relationship between feeding behaviour and adrenal activity, where simple observation of feeding frequency can be used to estimate adrenal activity. It has also been suggested that certain ear positions, notably the horned ear position, may be used as an indication of nervousness and that monitoring the occurrence of this ear position may provide a quantitative base for assessing the stressfulness of a particular situation, in a similar way to that in which von Holst (1974) used piloerection on the tails of tree shrews. A third way in which behavioural observations may be used diagnostically is when the indicated increased levels of aggression in group situations or variations in the daily activity patterns which may be associated with stress. The effect of stressors on these two parameters was apparent in the Jersey mouse lemurs where disturbance, due to being on public display, apparently led both to increased aggression and to variation of the expected activity pattern so that the animals were more active when the zoo was closed, i.e. when they were subject to reduced disturbance. The final way in which behavioural observation can aid in the study of reproductive abnormality is when it is used to indicate those individuals which are most likely, in a given situation, to suffer from stress related problems. It has generally been found that subordinate individuals are more likely to suffer from stress than their more dominant fellows. Social status can obviously be determined from behavioural studies.

It is appropriate at this point to examine the implications this kind of study can have on animal management techniques. In the case of the mouse lemur it is felt that the following factors deduced from this study should be taken into consideration by all people holding groups of mouse lemurs in captivity:

1) Daylength must be varied over the year and it must be remembered that variation both in the timing of dawn and dusk are important.

2) The dark period should be cooler than the light period.

3) The animals are best maintained separately. However, as this may not always be convenient or desirable, care should be taken in establishing groups. Mother-daughter units are probably best housed together with at least two males. The literature suggests groups of 3.2 are successful (Andriantsiferana et al., 1974).

4) It must be remembered that female groups tend to fragment when the young are born and that both female-female and female-male aggression is high at this time.

5) It is best to establish a new group in a new cage. Due to the absence of territorial behaviour nothing is gained by introducing two individuals in the cage of the more subordinate animal.

6) Reduction of the annual cycle of daylength change appears to be without advantage.

7) Groups of mouse lemurs, even those housed separately, should be effectively isolated into small groups in terms of olfaction and vocalisation.

8) Hand-reared individuals become normal adults in reproductive and behavioural terms.

9) Red lighting should be avoided.

Thus, one can say that this kind of study can provide a considerable number of factors to be taken into consideration when planning a captive breeding colony for the study species. These considerations can then be generalised to incorporate if not all species at least a considerable number. For example, if animals are to be maintained indoors, not only should the type of lighting used receive considerable attention, but, at least in the case of those species inhabiting non-equatorial regions, some variation in daylength should be provided. Temperature variation between night and day is another important consideration for those animals maintained indoors and, in addition, some annual variation in ambient temperature and humidity should be provided over the course of the year if at all possible. All these factors are easy to incorporate into a captive environment but are usually not considered by zoos or many research establishments.

Obviously the optimum captive environment is one which mimics the conditions of the field in as far as possible. Thus, animals which originate from a forest habitat should not be placed in a bare enclosure with no cover provided. This would again seem an obvious consideration but even the most cursory visit to a zoo will indicate how rarely it is considered. However, a complete duplication of natural conditions is obviously impossible in captivity; for example, many of the types of food which an animal consumes in the field are not readily available in captivity. In captivity one has to attempt to deduce those facets of an animal's natural environment which are most important and to try to duplicate as many of them as possible. This is obviously difficult because, as has already been indicated, very little is known about many exotic species in the wild. However, certain salient points should be known, such as the area and thus the climatic zone from which the animals originate and habitat type; for example, whether they are forest or savannah dwellers. In addition, it is usually known whether these animals live alone or in small or large groups. By considering these data a substantial improvement could be made on most captive environments. In the case of solitary or semi-solitary species such as the mouse lemur, special considerations must be incorporated in the planning of their husbandry techniques. Obviously it is not always convenient to maintain the animals separately, introducing them only for breeding purposes. However, when housing them together, cage design should include features which facilitate both hiding and easy escape. For example, one large cage should be subdivided into several smaller units: each of these units should be visually isolated and access and egress should be by means of several small doors which can be closed off should the animals begin to fight.

Another important consideration applies mainly to nocturnal species or to those species in which olfactory communication is of particular importance. If a large number of such animals are to be maintained in a restricted area, elimination of visual contact alone will not be sufficient to protect the animals from the effects of overcrowding. Each individual or each small group of these animals has to be effectively isolated, both olfactorily and vocally in order to eliminate the effects of "psychological" overcrowding.

Having said this one must consider whether the effort of implementing such considerations is really necessary. The answer to this depends on the purpose of the captive breeding project in question. Generally if members of a particular species are kept in captivity, even in quite inappropriate conditions, a few of them will breed. This is to be expected. If there is a wide range of genetic variation in a wild population it is probable that some of these genotypes, perhaps those at the fringe of the range encountered in the wild, will be able to adapt to most captive conditions and will breed successfully. Their offspring will be of a similar genotype and thus will also breed successfully in captivity until over time a particular captive race of the original species will have been inadvertently selected for such an end product to a captive breeding scheme is not desirable to any one interested in studying behaviour or in conservation although it may be an ideal system for medical research.

For those interested in behaviour or conservation the goal of a captive breeding project is to ensure that most of the animals breed, and in this way to preserve the genetic variability of the parent population. Captive conditions should therefore be manipulated to this end. The problem most frequently encountered is the erroneous belief that is one pair of animals breed the conditions in which they are maintained are suitable. This belief is held tenaciously even if x more pairs housed in identical conditions do not breed and the failure of the remaining animals to breed is held to be a problem of their infertility. The relationship between this phenomenon of only a small percentage of captive animals breeding and genetic variability has been described above. Even without this, the assumption that most animals held in captivity are naturally infertile is obviously questionable. In zoos the belief that the captive conditions used are satisfactory is coupled with an inherent conservatism and a reluctance to alter what they see as a "winning team" even if it only "wins" in a very small percentage of cases. This is an attitude which will obviously have to change if zoos are seriously to think of themselves as a force for conservation. They will have to consider new husbandry techniques and experiment more until optimum conditions are found as well as implementing techniques which have been found to be successful in research establishments or in other zoos. It is only by these means that they will be able to encourage a maximum number of specimens of each species to breed, thus maintaining a captive population of that species which is largely indistinguishable from the wild population. Should they choose to remain true to their conservative traditions the result will be the selective breeding of those genotypes most adaptable to captive conditions with the result that semi-domesticated or zoo races of the original species will be developed.

It is hoped that this study, with its bias towards techniques of animal husbandry, will have a positive influence on the thinking of zoo management. The ways in which this influence could act are twofold: firstly, by illustrating the ways in which research can improve captive breeding results and secondly, by stimulating similar studies on other exotic species. In this way it is hoped that a scientific basis for decision-making regarding animal management techniques will be promoted.

APPENDIX I

BEHAVIOURAL TAXONOMY

In order to facilitate the understanding of all the terms used in this thesis to describe particular behaviours, all terms used are listed here together with full descriptions.

Allogroom - one animal grooms its social partner using its tongue and tooth scraper. During this behaviour the hands are only used to position the partner and never for removing objects from the fur. Allogrooming may be solicited by the animal in question extending its head towards a partner's face. During bouts of allogrooming either one partner may exclusively groom the other, the partners may alternate grooming bouts or simultaneous allogrooming may occur.

Anogenital-rubbing - this is a mode of scent deposition in which the animal drags its anogenital region along the substrate.

Approach-retreat - when one individual approaches another only to depart immediately.

Associate - (two or more animals) sitting together in a huddle, often seen interspersed with bouts of allogrooming.

Avoid - when one animal moves out of the way of an approaching individual.

Back and folded ear position - one of the predominant ear positions adopted as a mode of visual communication, see page 212.

Chase - self explanatory. Chases were often terminated when the subordinate animal entered a nest-box. Sometimes the dominant animal would also enter and a period of nest-box sharing would ensue.

Clear whistle - one of the whistle-like vocalisations, see page 240.

Click - constituent part of the purr vocalisation, see page 234.

Cringe - body posture adopted by a subordinate animal, see page 211.

Defensive attack - body posture, see page 210.

Defensive threat - body posture, see page 210.

Distress call - infant vocalisation, given when separated from mother, see page 234.

Disturbance call - vocalisation given in response to disturbance, see page 237.

Ear positions - three prime ear positions are listed: spread, horned and back and folded, see page 212.

Eliminate - urination or defaecation.

Fear call - scream given by frightened individual, probably also given in response to pain, see page

Feed - all those behaviours associated with the collection and consumption of food.

Fight - this was generally not serious but rather a brief wrestling match accompanied by threat calls from both participants. The exception to this was when serious fighting accompanied by biting was observed during introductions between females, see page 171.

Follow - one individual following another. Often seen in the follow-my-leader type of play of juveniles. Also occurred in association with tussling in the London group. It is often succeeded by nest-box sharing.

Food-take - this is when one individual approaches another which is feeding, sniffs the food, removes all or part of the food object and runs away. The mouth and not the hands are used to steal the food. Threat behaviour is only rarely elicited from the recipient of this behaviour.

Gathering call - a vocalisation given just prior to "lights-on", see page 245.

Groom - this includes the licking and combing of the fur using the tooth scraper and toilet claw. In addition, the mouse lemurs were observed to clean their faces by first licking their hands and forearms and then rubbing these simultaneously over the face from ears to muzzle. A similar pattern of face washing has also been described in *Galago crassicaudatus* by Pinto *et al.*, (1974).

Groom other - see allogroom.

Horned ear position - one of the predominant ear positions adopted as a mode of visual communication, see page 212.

Intermediate whistle - one of the whistle vocalisations, see page 240.

Long whistle - one of the whistle vocalisations, see page 240.

Marking behaviour - one of those behaviours assumed to be related to the deposition of scent, viz. anogenital-rubbing, mouth-wiping and urine-marking.

Mouth-wiping - the rubbing of the corner of the mouth, face and sometimes head along a branch.

Move - one of the categories of behaviour used in scoring activity, used to indicate that the animal was involved in some form of locomotion.

Nest-box - one of the categories of behaviour used in scoring activity used to indicate the animal was in the nest-box.

Nest-box share - when two or more individuals occupy the same nest-box.

Nesting behaviour - this behaviour is commonly seen preceding birth. It involves carrying pieces of twig, cherry stones, wood chips etc. into the nest-box. This debris is then arranged and rearranged within the confines of the nest-box without any obvious nest-like structure resulting.

Nosing - on entering the nest-box, the mother mouse lemur will push her snout under one or both her infants and roll it back over her head.

Play - various forms of play are seen in young mouse lemurs. However, wrestling, pouncing on another individual's tail or hands and follow-my-leader type games are most prevalent.

Proximity - one of the categories of behaviour used when scoring activity, used to indicate that the subject animals were within one lemur body length of each other.

Purr - vocalisation similar in both sound and situation of elicitation to a cat's purr, given by infant mouse lemurs when stroked and fed by foster-parent, by hand-reared adults when stroked and probably by other adult mouse lemurs during amicable interactions.

Rhythmic micturation- one of the mechanisms of urine deposition. This tern was first coined by Ilse (1955) for this behaviour in *Loris tardigradus*. During this behaviour the body is lowered to the substrate and the animal progresses forwards with a wriggling motion depositing a thin trail of urine.

Sniff - this category includes the sniffing of the face, flanks or anogenital area of a partner.

Social grooming - see allogrooming.

Spread ears- one of the three predominant ear positions observed in the mouse lemur, see page 212.

Swaying - body posture observed when the animal is peering at a novel object, see page 210.

Threat - a vocalisation which occurs in association with the characteristic body posture of defensive threat, see above and page 245. It is normally given by the subordinate animal in any situation, although, occasionally both animals will be observed to threaten.

Trill - vocalisation generally given only by the male grey mouse lemurs, see page 240.

Tussle - an unusual behaviour only observed between the two females in the Observation Room. It would be preceded by repeated following behaviour. Each female alternately following the other and sniffing her anogenital region. Both females would grapple with each other giving continuous trill calls (see above). The appeared to be oblivious of the observer, if they fell from the branch they would continue with this activity grappling and rolling on the floor.

Urine mark - all those mechanisms of olfactory communication which involve the deposition of urine, urine-washing, rhythmic micturation and deliberate urination, see page 215.

Urine-washing - this involves the raising of the hand and foot on the same side of the body. The hand is cupped below the penis or the clitoris and a few drops of urine deposited in it. The foot is then rubbed over the hand several times in rapid succession and both are replaced on the substrate. This procedure may then be repeated several times using alternate hands and feet, while the animal proceeds along a branch. This pattern has been reported in other prosimians; e.g. Galago senegalensis (Bearder, 1969, Andersson, 1969), Galago demidovii (Charles-Dominique, 1977), Loris tardigradus (Ilse, 1955) and Galago crassicaudatus (Clarke, 1976), and in some cebids; e.g. Aotus trivirgatus and Saimiri sciureus (Andrew & Klopman, 1974).

Whitter - soft call given on encountering a familiar animal and by mothers to their infants, see page 240.

APPENDIX II

NOTES ON HAND-REARING MOUSE LEMURS

During the course of this study attempts were made to hand-rear eight infant mouse lemurs. Five of these infants, four murinus and one rufus, were successfully reared. The three infants which failed to survive were all low birth-weight infants (see Chapter VIII, section 3).

The infants were fed with Babymilk 2. This is a dried milk product designed for human infants, manufactured by Cow and Gate. The milk was made according to the instructions on the packet but omitting the sugar.

During their first three weeks of life or until the infants weighed 20 grams they were given a total of nine feeds each day:- 04.30, 08.30, 11.30, 13.30, 16.30, 18.30, 20.30, 22.30, 24.00. Thereafter the night feed was eliminated. Milk was administered using a small pipette and the infants would take between 0.5 and 1 ml of milk each feed.

In addition to the milk one drop of Abidec, (a multi-vitamin preparation) was added to one feed each day.

Under natural conditions the mother licks the perineal area of the infants to elicit urination and defaecation. In the hand-reared infants this procedure was simulated by rubbing the perineal area with a damp Kleenex tissue. Elimination was stimulated in this fashion after each feed.

Temperature is very important to infant mouse lemurs and it was found that infants kept at 29-30° C developed most rapidly. To maintain this temperature the infants were housed in glass beakers suspended in a water-bath. In order to prevent accidents the mouth of the beaker was covered with a piece of nylon, for example, part of a stocking. This was secured with an elastic band.

Nesting material was provided for the infants using pieces of linen handkerchiefs. Sections of paper tissue were tried but these led to problems as the infants would swallow pieces of the tissue.

When the infants were either 28 days old or weighed about 28 grams they were transferred to small cages. At this time they were also offered small pieces of fruit or mealworm to try.

Once the infants started to eat some solid food the number of milk feeds was gradually reduced to two feeds per day, morning and evening. Water was provided in the cages. This regime was maintained until the infants were returned to the colony at an age of 50-55 days.

The most frequent problem in these infants was diarrhoea: this was treated with 0.5 ml of Diocalm solution. The solution was prepared by dissolving ¼ of a Diocalm tablet in 50 ml of boiled water.

APPENDIX III

THE SYSTEM OF REFERENCE CODES AND NAMES USED FOR THE MOUSE LEMURS

All of the original colony specimens were given reference codes. The first letter of the code, R or M, indicates whether the specimen is rufus or murinus, the second letter, M or F, indicates the sex of the specimen. The males and females of each subspecies were then allocated a number sequentially in order of their acquisition or birth. Thus the 3 male and 7 female grey mouse lemurs used to establish the colony were numbered MM1-MM3 and MF1-MF7 respectively. The first male infant born to these individuals was then coded MM4 and the first female MF8. The four individuals obtained from Jersey Zoo and added to the study colony were given the letter J instead of a number as there was only one pair of each subspecies.

However, since the colony was established several specimens have been hand-reared and these have been given names in addition to reference codes and are referred to by these names in the text. In addition, the infants of one of the hand-reared females, Bonnie, have also been given names, these are Branwen and Attila.

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