ENDOCRINE AND BEHAVIOURAL COMPARISONS OF MALE MICE UNDER SEMI-NATURAL AND CAGED CONDITIONS

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Teach me your way, O Lord,
and I will walk in your truth;
give me an undivided heart,
that I may fear your name.
I will praise you, O Lord my God, with
all my heart;
I will glorify your name for ever.

Psalm 86: 11-12

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FOR ANGUS

ABSTRACT

The work of this thesis was based on investigations from previous conventional cage studies which examined the characteristics of dominant and subordinate male mice. It was predicted that high status males would have high testosterone output and heavy sex accessory glands but would also have low corticosterone output and adrenal weights. By contrast, it was expected that subordinate mice, by virtue of their social position, would be chronically stressed due to continual threat of attack and injury, and adrenal activity would be raised in this group. Raised blood urea levels and pain thesholds as further evidence of stress, were also predicted.

Mackintosh (1970) demonstrated that given adequate space, male mice could establish territory areas under laboratory conditions. As a consequence, the predictions above were tested on mice housed in both traditional laboratory cages and within the more open environment of a free range room. It was also predicted that high status mice from both these forms of housing would possess an aversive urinary odour cue and a urine marking pattern of dense spots, neither of which would be characteristics of low status males.

Among subordinate animals, evidence was found for acute but not chronic social stress so that the predictions for these males were not upheld. Other results showed that in high status males gonadal activity was not always raised over time, particularly among free range territory holders. Testosterone levels in this group notably decreased over experimental periods. It was concluded that the role of this androgen is largely permissive in maintaining status and territory with only low levels are required. Results are discussed in terms of the advantages this might have for mice in colonies under

more natural but confined conditions.

The existence of an aversive cue did not always correlate positively with a particular marking pattern, nor with testosterone output, although it did correlate with territorial status. Overall, the results suggest that too strong a correspondence may have previously been drawn between androgen output and these urinary traits.

CONTENTS

CHAPTER 1

INTRODUCTION

Dominant-subordinate relations amopg small mammals have been investigated over many years under a variety of conditions that have employed the use of caged or penned animals. The central focus of the work in this thesis has been on this type of relationship in male mice living free on the floor of a large, free range room, and on their behavioural and physiological characteristics. Other males were kept in traditional laboratory cages and served as controls. The work examined differences between mice of high and low social status and the ways in which these environments were associated with endocrine changes in relation to status and social stress, by measuring steroid hormone levels in blood plasma. Other features of social status such as urine odour and urine marking patterns were also studied.

The opening section of this discussion is concerned with the body of knowledge that has been built up in the literature in connection with the behavioural and physiological traits associated with dominance and subordination principally through the large number of cage studies. The discussion then extends to the work that has been done in the more open environments of pens and enclosures. Based on the findings from previous studies done in cages and enclosures, a number of predictions are stated which were tested in this study.

The house mouse, particularly in its domesticated, laboratory form has been studied intensively over the past fifty years and a large amount of information has been accumulated relating behavioural factors to physiology, population control, genetics and communication. By reason of its size and ease of management, the

popularity of the mouse is understandable and its only rival in the league table of most studied small mammals is the white rat. Despite the intense research, there nevertheless remain important areas where information is either scarce or merely inferential. Mackintosh (1981) has argued that this is a result of the individual interests of research workers, the success of the mouse as a laboratory animal and indeed, the difficulty that exists in studying this animal under natural conditions. As a consequence the number of laboratory studies outnumbers those from more naturalistic habitats by several hundred to one and it seems reasonable therefore, for doubts to be expressed as to the total validity of behaviour studies under such confined conditions. In a recent statement, Bronson (1979) has commented that "as a generality, we may be spending considerable time in the laboratory studying artefacts of utopian and constant conditions" but in addition, extrapolation from laboratory studies to "natural environments" must be made with caution.

Nevertheless, from the cage studies, information gathered shows that a number of behavioural and physiological features are associated with the social status of an animal. Many of the properties of dominance status appear to be androgen related. Socially dominant male mice have been shown to be aggressive towards unfamiliar male conspecifics (eg: Mainardi et al. 1977, Parmigiani and Pasquali,1979) as well as towards lower status males (Ely and Henry, 1978). Gonadal secretion is relatively high (eg: McKinney and Desjardins 1973, Ely 1981) and these males are also reported as having heavy sex accessory glands and low adrenal weights (eg: Chapman et al. 1979, Benton et al. 1978) together with low corticosterone output (Louch and Higginbotham 1967). However, in response to defence behaviour or intrusion to the territory of another dominant male, levels of adrenal tyrosine hydroxylase, the

enzyme controlling norepinephrine synthesis, are known to rise (Ely and Henry 1978). In contrast, levels of adrenal medullary epinephrine are low in dominants, as is the turnover of brain catecholamines (Welch and Welch 1971, Modigh 1973). It is also known that the urine of dominant male mice differs from that of subordinates. Jones and Nowell (1973a) found that male mice tended to stay out of an area spotted with dominant male urine whereas subordinate male urine had little effect on choice of area. Female mice will spend more time in an area of dominant male urine than in a clean area and subordinate male urine is less attractive to them (Jones and Nowell 1974b). These factors appear to be androgen dependent. Urine from castrates produces little aversion but this property is restored by treatment with testosterone (Jones and Nowell 1974a). Dominant male mice also produce urine marking patterns of small spots spread widely and densely over a given area (Desjardins et al. 1973), a feature which appears also to be influenced by androgen levels (Bronson 1976). The findings from studies which have examined traits associated with high status such as aggression, androgen levels, sex accessory gland weights, urine odour and marking patterns, are discussed in greater detail in later chapters in conjunction with the experiments that were carried out.

Dominant males are also associated with high levels of sexual behaviour and compared with other social categories of mice (Benton et al. 1980), are known to investigate a receptive female for longer. However, the link between high status and reproductive success has been disputed in monkeys (Rowell 1974) as well as in mice (Oakeshott 1974). In this latter study which employed different coat colour to determine paternity, the mating advantage of social dominance was considered to be negligible. Dominant mice were successful in 69 of the 134 matings scored (52%). Data from different studies is somewhat

conflicting however, as in an earlier study by Defries and McClearn (1970), a clear association between social dominance and Darwinian fitness was made. This work investigated the ability of dominant males to sire offspring and the unlikelihood of subordinates to participate in mating behaviour. Again, paternity was ascertained by the coat colour of the offspring. Three males each from one of six different strains were placed in three interconnecting cages along with three females of the BALB/c strain which is known to be recessive for two colour coat loci. Animals lived in these groups of six for two weeks. A clearly dominant mouse was always seen to emerge from each triad with wounding observed on the other two males who tended to reside close to one another in one of the cages. Of 61 litters obtained, 56 (92%) were sired by the dominant male in five out of the six strains tested; one strain consistently showed subordinate status in the presence of mice from other strains. These workers have concluded that social dominance, at least in mice, is a vital component of Darwinian fitness and that certainly under controlled cage conditions, subordinate males contribute very little to the gene pool. It is appreciated that in a natural environment, migration of subordinate animals may well and probably does occur, resulting in their establishment of breeding territories elsewhere. However, these workers have argued that the net effect on reproductive success within a group will essentially be the same. Because of the competitive disadvantages of a stranger in another male's territory, (Reimer and Petras 1967), they believe that the effective rate of migration among established demes may be less than 3%.

In itself, dominance plays a vital role in the lives of social animals, and may arise because of certain advantages to an animal. Among other factors, it provides access to certain resources such as

food or a territory area as well as to females. As a consequence, the chances of passing on the genetic material from a particular animal to the next generation are considerably increased. In the present study, it was necessary to assertain dominance among the male mice used. Klopfer (1974) has said that "dominance is inferred whenever one individual is able to chastise another with impunity", and Ginsburg and Allee (1942) found that social organisation based on dominance-subordinate relations was determined in the last analysis by the ability of their mice to win fights against all other group members, a measure of dominance used in subsequent studies by many other workers (eg: Benton and Brain, 1979). This was the criterion for determining dominance among the mice of the present experiments.

Many of the characteristics associated with dominance are similar to, though not necessarily the same, to those of mice housed individually in cages. A number of workers have pointed to the similarities between these two groups (Welch and Welch 1971, Brain 1975, Brain and Benton 1977). The aggressive behaviour demonstrated by socially dominant mice has also been found to exist in males that are housed on their own. Male mice kept under these conditions were used by Ginsburg and Allee (1942) in their early study which demonstrated that these animals could be conditioned to be more or less aggressive due to either a series of wins or defeats and they made the point that "isolation" is an important prerequisite for conditioning mice upwards, in other words, for the stimulation of aggression in order to improve the chances of winning fights.

It can be argued that the term "isolation" is itself misleading because whether mice that are singly caged can be considered as being in isolation is questionable. These animals, besides lacking social contact with cage mates, are also deprived of visual contact and certain olfactory information and are prevented from establishing

social patterns of behaviour. However, unless they are not only caged alone but also housed in separate rooms (which of course, is impractical), some communication does inevitably take place - auditory, and to a limited extent, olfactory. As a consequence, the terms "single caged" or "singly housed" have been adopted both for use in this discussion and in other chapters as it is felt that the term "isolation" is somewhat inaccurate.

Singly caged adult male mice are generally more aggressive towards other mice than their group counterparts (Brain 1975). Housing them individually for a period of three weeks is sufficiently long for this behaviour trait to become established, and although maintaining this over increasingly long periods (14-16 days up to 287-289 days) produces a rise in aggression levels, an asymptote is reached at 56- 58 days (Goldsmith et al. 1976). Brain has gone to some length to point out that aggression is not a unitary concept and that different physiological changes influenced by differing circumstances form the bases for a number of models of aggression. Rank-related aggression - attack on a conspecific induced by individual housing or the establishment of a dominance hierarchy - is therefore regarded as a quite separate event from maternal aggression or pain-induced aggression.

Because both singly caged and dominant mice are known to attack conspecifics, this has led to the idea that these animals may share a number of other similarities. A number of studies have shown that when compared with subordinates and group housed individuals, dominants and single housed males have heavier sex accessory glands and lower adrenal weights (Benton and Brain 1979) as well as lower levels of norepinephrine and a lower turnover in brain catecholamines (Welch and Welch 1971, Modigh 1973). Gonadal output is also higher in these two groups (Brain and Nowell 1969, McKinney and Desjardins

1973). Some of the data are however, conflicting. Among the studies which have examined adrenal activity as a measured response to stress, heavier adrenal glands and higher corticosterone levels have been recorded in single housed mice compared with control groups (Sigg 1969) although weights of sex accessory glands were unaffected. Decreased sexual activity has also been described among this category of mice (Lagerspetz 1969) but it is of interest to note that changes in levels of sexual behaviour were not dependent on sex accessory gland weight variations.

The view that singly housed mice are similar to dominant animals has not gained universal acceptance. Valzelli (1973) has described the aggressiveness associated with single housing as an "isolation syndrome" due to hyperirritability, a concept which has been used by other workers and is sometimes referred to as the "isolation stress syndrome" (Hatch et al. 1963, Schwartz et al. 1974), the features of which have been described in both rats and mice. Brain (1975) has pointed out that often studies centred on isolation stress have used female rodents as subjects and in these animals, the production of oestrogens is stimulated by individual housing and these steroids produce adrenal hypertrophy which may not be related to stress. Nevertheless, the genesis of "isolation stress" coupled with aggressiveness has been linked to the notion that these animals may suffer frustration due to social deprivation (Dollard et al. 1939), an idea extended to rodents from the social studies carried out on primates by Harlow and Harlow (1962). Whereas this approach equates single housing with stress, an opposing viewpoint (Brain 1975) regards the increased fighting to be a consequence of low stress and the removal of an inhibition to fight imposed by the effect of grouping animals. Certainly dominants are seen to be less stressed than subordinates, as measured by comparison of adrenal weights in

small stable rodent groups and Brain (1975, 1972a) has even suggested that single housing may be more naturally associated with territoriality than with social deprivation. The use of the former term cannot of course be strictly applied to individually housed mice as these animals do not defend boundaries but neither are they challenged or subjected to defeat by conspecifics in their home cages. Indeed Brain (1975) has argued that regarding these single animals as territory holders without intruders or as dominants without subordinates may provide a more accurate reason for their increased fighting potential rather than simply regarding the behaviour as a consequence of increased irritability.

Several hypotheses have been advanced to account for "isolation-induced" aggression including the development of frustration through being alone (Eleftheriou and Church 1968) and an increased or decreased sensitivity to environmental stimuli (Welch and Welch 1969). Olfactory cues which may contribute to the suppression of aggressive behaviour among groups of mice (Brain and Nowell 1970, Lagerspetz and Lagens 1971, Haug 1970) may be missing in single housed animals. In an experiment by Harmatz et al. (1975), single caged mice were chronically subjected either to soiled bedding from group housed males, from isolated males or to fresh bedding, for four weeks. The results showed that fighting with a test male was significantly reduced in the single males that had been exposed to soiled group-housed bedding when compared with the other two groups. These workers have suggested that "post-isolation" aggression in mice may result from the gradual disinhibition from a primer pheromone, present in groups of mice, which acts to suppress or diminish aggressive attack.

Because of the common characteristics that some workers have noted between this group and dominant animals, examination of the

features of single housed mice is important. Indeed, a central prediction in the work of this thesis was that because of known similarities between them, territory holders in the free range and singly housed mice, together with dominant males from groups in cages, would share similar endocrine characteristics in terms of adrenal and gonadal output as well as having similar urine odour qualities and urine marking patterns. This prediction formed the foundation upon which the experiments on levels of testosterone were subsequently carried out, full details of which are given in chapter five with a further discussion of the previous studies on dominant and single caged animals.

In contrast to the attributes of dominant, and to some extent single housed mice, subordinate animals have been shown to be distinguishable by a number of opposite features. These include low androgen output (Smelik 1985) with lower sex accessory gland weights and a lack of aggression towards other mice (Benton and Brain 1979). Epinephrine levels are raised in this group (Welch and Welch 1971) and these animals also show evidence of social stress in the presence of aggressive, high status males, having raised corticosterone output and high adrenal weights (Henry and Stephens 1977) which may suppress androgen output as well as androgen related characteristics. For instance, as mentioned above, the urine of subordinates differs in quality from that of dominant male mice (Jones and Nowell 1974a,b,) and subordinate mice are known to produce a urine marking pattern of few and large peripheral pools in a test arena (Desjardins et al. 1973).

Definitions of the mainly physiological characteristics of domininant and subordinate mice have been established largely through studies carried out on caged animals. However, many of the social habits have been known for about fifty years. As early as 1938,

Uhrich showed that if small groups of laboratory mice were housed together, they were capable of organising themselves into a social group in which one male was dominant and all the others submissive to him. This form of despotic dominance has since been the finding of many other workers (e.g. Brain and Benton 1977, Brain 1980a,b) although Poole and Morgan (1973) found that the stability of this arrangement was dependent upon group size and that when group numbers of four, five, nine and twelve were investigated, changes of dominance were more likely to occur in the larger two groups. Poole and Morgan (1976) also found that if groups were derived from litter mates, little fighting took place with a resultant lack of hierarchy formation. It would appear therefore, that a critial period for socialisation may begin prior to weaning. The mice used in the study in this thesis, although grouped from non-litter mates, grew up together from weaning at the age of three weeks and later formed stable social hierarchies. Group integrity may well be maintained by individual recognition. It has been shown that mice can distinguish between two males on the basis of olfactory cues (Bowers and Alexander 1967) and Kalkowski (1967) in a separate study, showed how his mice could separate eighteen mice into nine pairs, also on the basis of odour.

Again, these findings are derived from observations made on caged animals and the problem therefore remains about whether the results can be said to apply to either truly wild mice or even mice housed under semi-natural conditions. The difficulties however, of studying an animal of such small size which is also nocturnal and timid in temperament in more natural surroundings, should not be underestimated and when investigations go beyond observation and the recording of behavioural data into an area involving the study of physiological parameters, the practical problems of experimental

design and procedure may be immense. As a consequence, a number of workers have compromised and abandoned the laboratory cage in favour of pens or enclosures for a variety of studies in an attempt to approximate to the wild state.

Results from this approach have shown that mice are capable of not only organising themselves into social groups or hierarchies, but also establishing and defending areas resulting in clearly evident territorial behaviour. This is one of the striking results of enlarging the space available to a group of animals. The characteristics of this behaviour were first detailed by Crowcroft (1954, 1955a,b, 1966) and Crowcroft and Rowe (1957, 1961, 1963) using large, enclosed areas under semi-natural conditons. The observations from this work have been extended by others and in particular, by Mackintosh (1970, 1973, 1981) who not only replicated and confirmed much of Crowcroft's work that mice were indeed capable of establishing social hierarchies within recognisable territory areas but that this effect could be demonstrated using smaller enclosures under laboratory conditions.

The findings of Mackintosh, together with previous physiological data gathered from the cage studies on dominant and subordinate animals, prompted the work that forms the body of this thesis and on which a central question was based: were the results from studies on mice, which under these confined conditions, had demonstrated a number of physiological differences between dominant and subordinate animals, applicable to mice housed under the more open conditions of an environment such as the free range room where the increase in space available permitted not only the formation of social hierarchies but also, as Mackintosh showed, the establishment and defence of territory areas?

As the arrangement of the free range room used in this study was

closely modelled on the enclosures used by Mackintosh (1970, 1973), it was therefore predicted that the resident male mice would, as both he and Crowcroft found, set up and defend territories and establish stable social hierarchies comprised of a small number of territory holders with other mice subordinate to them. In parallel with the mice housed in the free range room, other males were also housed both singly and in groups of five in traditional laboratory cages to serve as controls. It was anticipated that among the group caged mice, dominance hierarchies would also be established although the cage area was not considered to be a territory as no defence against outsiders could take place.

The use of the free range room provided scope for a wide variety of both behaviour and physiological tests. However, for a number of reasons, mainly time limitation, traditional behaviour tests to examine sexual behaviour, forms of aggression, levels of attention etc. were not undertaken. Instead, the study presented here focused on three main areas of investigation: social stress, androgen levels and urinary odour and marking patterns. Within each of these study areas, a number of predictions were made in relation to the findings of previous workers.

From other studies made on social stress and in particular, the work done on mice in population cages by Henry and Stephens, one of the main predictions was that subordinate mice, particularly those in the free range room, would show physical evidence of exposure to such stress. To determine this, measurements of plasma corticosterone and adrenal weights were made together with blood urea levels. In one test, pain threshold levels were examined as an indication of stress induced analgesia. Full details of these experiments are given in chapter four.

The expectations concerning the territory holders together with

the single housed mice and group housed dominants have been discussed above but are restated here in the prediction that these three categories of mice would have high levels of testosterone together with high sex accessory gland weights when compared with all subordinates. The experiments that were carried out to test these predictions are given in chapter five.

Although no formal behaviour tests were carried out, a study was made of urine odour and urinary marking patterns. From the work of Jones and Nowell briefly outlined already, it was predicted that the free range territory holders, together with the mice from single cages and the group housed dominants, would possess in their urine an odour factor which would serve to deter other mice from prolonged investigation. In addition, it was predicted that the three categories of high status mice in this study would have marking patterns which would compare well with the results from Desjardins' study and which would be seen to contrast with the urine patterns of lower status animals. The details of both previous studies and the experiments that were carried out are given in chapter six.

Abandoning the cage in favour of environments that provide scope for a wider range of behaviours has been helpful in extending knowledge of the social organisation of the house mouse. Crowcroft (1955a,b,1966) observed that social hierarchies developed in small colonies of mice under certain conditions such as when fighting dispersed the population so that the animals then lived in spatially distinct areas or territories. Once divided up in this way, individuals tended to remain in the same social groupings; a number of the males living in these areas were seen to attack other mice that intruded into the nesting boxes or the space surrounding them. For certain mice, there was always a strong connection between locality and success in fighting and these animals were considered to

hold dominance status relative to a particular territory area. In Crowcroft's studies, territory boundaries were determined by four factors: observing the places where the presence of a mouse elicited attack, where a chase was broken off by the attacker, where patrolling or "sentry".activity was seen or where a retreating mouse turned, reared up and drove its attacker away. Dominant males could be seen to win fights on their own territories but not on the territory occupied by another dominant. These observations served to extend an earlier definition by Noble (1939) who defined a territory as "any defended area".

For the purposes of his work, Crowcroft used enclosures varying in size from $1.3m^2$ to $6.0m^2$ and in these, he demonstrated that within a population, only a few males hold territories and that provided there was sufficient space, there were small areas of "no-man's land" between them. Other males were non-territorial subordinates, restricted in their movements due to the risk of attack from dominants, and they tended to crowd together in nest boxes. A remarkable feature of these social groupings in Crowcroft's experiments was the length of time that the stability of the colonies could be maintained without changes of status taking place - a feature remarked on also by researchers in later studies.

Following Crowcroft, a number of other workers have employed the use of enclosures to develop a further understanding of the social structure of the house mouse. The work of Mackintosh (1965, 1970, 1973, 1981) has done much to point out that the extensive use of the cage in mouse studies has had the serious effect of vastly oversimplifying the environment in which the animal lives and in particular, that the reduction of space necessarily precludes the formation of territories. In two experiments, Mackintosh (1970, 1973) examined the factors affecting territory formation. His

territories were established by first separating two groups of mice by a partition in an enclosure, $1.8m^2$. Removal of this barrier one week after the animals first entered the pen produced similar findings to those of Crowcroft (1966) with the formation of two distinct, defended areas. Unlike Crowcroft however, no area of "no-man's land" was discernable between the occupied parts of the arena. Mackintosh also found that the existance of the boundaries, although restricting the movement of males, exerted no restraint on the mobility of females and juveniles who moved freely about the enclosure. Crowcroft (1966) recorded that females in his groups that were either pregnant or lactating, were highly aggressive and were also capable of defending territory areas where nests of young were being cared for, observations that were confirmed by Mackintosh. A notable finding of Mackintosh (1970, 1973) was that boundaries tended to form in places which had prominent physical features and he investigated how clues for their recognition were picked up by the mice. He did this by moving around landmarks or reference objects and found that the boundaries moved accordingly. He concluded the relevant information as to their whereabouts was mainly visual and in particular, close objects took preference over distant ones. Although he did not discount the importance of olfactory cues, Mackintosh argued that they were useful only when they did not conflict with visual cues. This last point has been criticised by Bronson (1976) who has argued that mice, being nocturnal, are far more likely to depend upon their olfactory sense when discriminating between objects than on their eyesight particularly as the eye is more sensitive to variation in light intensity and change of pattern (Berry 1970) and it cannot perceive form very clearly (Waugh 1910). Generally speaking, the mouse eye appears to be better designed for low-light vision than high visual acuity (Walls 1942).

Of further interest in Mackintosh's work was the finding that the subordinate mice showed a divisibility into subdominants and at least two classes of subordinate males (Evans and Mackintosh 1976). Subordinates would establish their own territories if they could find an unoccupied space. In agreement with the findings of Crowcroft (1966), subordinate activity generally was maintained at very low levels. Males with adjacent territories appeared to enter activity phases and undertake patrolling activity in such a way as to minimise contact with rivals. Among other workers, Reimer and Petras (1967), using a complex population cage composed of nest boxes inter-connected with tubes which served as runways, demonstrated that using this housing arrangement, mice again could be induced to form territories. In one experiment, the territories established by males within the first two weeks of entering the population cage, remained unchanged and stable throughout the eight month period of the study. During this time, females also were seen to defend their nesting areas and indeed, one group successfully defended a territory for a five week period without the presence of any males. When strange mice were introduced to this environment, 80% (fifteen out of nineteen) were killed by resident males within the first twelve hours. Using a cage and runway structure of this type, Reimer and Petras found that in general a mature family deme was composed of one dominant male, a number of females with immature animals and on occasions, a few badly scarred subordinate males huddled together; these findings compare well with those of Crowcroft (1966) using more open-pen forms of territory arrangement. Anderson and Hill (1965), Lloyd (1975), Poole and Morgan (1976a) and Lidicker (1976) have also achieved similar results to these workers, in their pen studies except that in the first of these three, the territories were not always reliably stable. In general though, these studies are linked

in that they all show that mice are territorial and will form socially stable, mixed-sex groups. They also provide evidence in opposition to the view of Blair (1943) who argued that territoriality in the house mouse was only poorly developed. Davis (1958) and also Eibl-Eibesfeldt (1950) have considered the defence of territory to be a feature associated with all adult members of a family group ("Gross-familie"). This view is challenged by Lloyd (1975) and Anderson and Hill (1965) who found no evidence that females display aggression towards strange mice nor was any form of group defence seen. However, Lidicker (1976) in his study of population growth in enclosures, states clearly that females were observed to take part in group defence. Whether the aggression seen in females was associated with pregnancy or lactation is however, unclear although Crowcroft (1966) noted a positive correlation between these two events and fighting and defence by females.

In more recent work, Bisazza (1981) has offered interesting evidence to show that separate strains of mice differ greatly in their patterns of social structure and spatial division. When placed in enclosures, 60 x 120 x 50cms, males of Swiss outbred strain were strongly intolerant of each other and established individual territories. By contrast, BALB/c males formed hierarchically organised groups in which subordinates neither defended territory nor were sexually active. Males of the C57Bl strain however, were seen to live peacefully together, apparently without rank order although strangers were always attacked and sexual behaviour was evident in all group members.

From a number of other strain comparisons, differences have also been found in aggressive behaviour (Scott 1940, 1966, Van Oortmerssen 1971, Eleftheriou et al. 1974). In a comparative study between wild mice from the Isle of May and laboratory mice (Mackintosh 1981), the

wild mice were found to fall into two categories: aggressive and non-aggressive. Males from the aggressive group were seen to adopt a postural stance during agonistic encounters not previously observed and which was termed "Upright Posture". From this position, the mice were very active, being able to move backwards and forwards and delivering bites to their opponents. The presence of a skeletal variant has been demonstrated in the May population (Clevedon-Brown and Twigg, 1969) which differs from the morphology for wild mice on the mainland as far as is known, and this variant may be influential in the adoption of the fighting posture observed.

In the behaviour genetic study by Van Oortmerssen (1971), the report carries an interesting discussion of the origins of the mouse species. It would seem that the species Mus musculus can be divided into a number of subspecies of which Mus. m domesticus and Mus. m musculus are just two. Mus m. domesticus was used in the studies of Crowcroft (1966) and Crowcroft and Rowe (1963) who described this strain as being strongly territorial, chasing other males out of an occupied area. Males that did not establish territories were subordinate to those that did and hence, social hierarchies were formed. This strain is considered to be more commensal than Mus m. musculus, living mainly in buildings or other environments closely associated with man (Zimmerman 1950). As the opportunity to burrow or dig holes does not generally arise, these animals for the most part, are surface dwellers. Skills for building nests without the support of walls are required and it is likely that these mice experience more predation than those living in holes. Above-ground living may therefore induce the more aggressive, territorial behaviour than is found in Mus. m. musculus which is relatively less aggressive and hole-dwelling. This strain has been studied by Eibl-Eibesfeldt (1950) who found that these mice were good diggers,

making their nests in holes and with a preference for fields (Zimmerman 1950). Eibl-Eibesfeldt described these animals as living in large family groups and although the members quarelled from time to time, they were generally tolerant of each other. Encounters between strange males generally ended with one mouse fleeing. From his own study on the behaviour of three inbred strains, Van Oortmerssen (1971) advanced the theory that laboratory strains of mice derived from these separate wild stock, as a consequence, show differing behavioural adaptations, such as greater or lesser degrees of territoriality. In general the findings from these studies should serve to caution against disregarding hereditary differences in social behaviour and whereas grouping may provide a satisfactory condition for more passive strains, it may also be a highly artificial condition for those types which are mutually intolerant.

Results from cage studies have shown that resident mice are generally intolerant of intruders, (Uhrich 1938, Andrzejewski et al., 1963, Poole and Morgan 1976b), though there is evidence that the prior fighting experience of an intruder may have considerable influence on the outcome of a fight with the territory holding member of a colony (Burg and Slotnick, 1983). In this study, instead of using naive intruders or ones with losing experience who then suffer defeat, strangers with experience of winning were introduced. These animals retaliated when attacked and engaged in vigorous mutual fighting with resident dominants. Indeed these workers found that strangers attacked the residents as many times as they were attacked and that half these mutual fights ended with the resident fleeing or showing a submissive posture. The results are interesting in as much as they question reports by other workers (Crowcroft 1966, Mackintosh 1970, Poole and Morgan 1975, 1976b) that strangers never initiate fights against residents and rarely retaliate if attacked. This has

been called the "home cage effect" (Crowcroft 1966, Uhrich 1938) because the dominant resident generally has good knowledge of its territory plus considerable experience of winning and the intruder by contrast, placed in a novel environment containing unknown territory boundaries and escape routes, is at a disadvantage and then tends to suffer attack and defeat. It would appear though from Burg and Slotnick's study that the prior experience of an intruder should be carefully controlled for in aggression experiments of this kind and restraint should be exercised in making generalisations.

Many of the studies on mice that have used enclosures or other enlarged confined areas, have been undertaken with the mechanisms of population control as the main subject of investigation. Southwick (1955) has argued that a clear link exists between the social structure of a mouse population and density levels, with the former important for determining the latter. Anderson (1961), when reviewing this relationship, showed that the lower limits of density achieved by experimental populations just overlap with those that have been recorded in the field. The basis for this line of thinking was established by Christian (1955, 1956, 1963) in his theory of population control, whereby behaviour-endocrine feedback was believed to act as a mechanism to control and stem population growth in mammals. The effects of crowding, besides influencing the animals' endocrinology, also produced subsequent modification of adult behaviour by the juvenile social environment. The implications of Christian's theory concerning the importance of endocrine factors is discussed in more detail further on but in summary, his hypothesis was that population control involves the activation of the pituitary-adrenocortical axis by "purely behavioural or social interactions" which are density-dependent and closely integrated with varying levels of aggression. An alternative idea of Chitty (1960)

assumed genetic variation for traits affecting social organisation and he suggested a model in which at peak densities, individuals with a predisposition towards aggressiveness, were at an advantage for individual survival but at a disadvantage in terms of reproductive success.

In order to prevent over-crowding for the house mouse, territoriality seems to be the basic spacing-out mechanism. This was found to be the case in a number of the enclosure studies discussed above (Crowcroft 1955a, Anderson and Hill 1965, Reimer and Petras 1967, and Mackintosh 1970), as well as in wild populations (Eibl-Eibesfeldt 1950, Anderson 1961). It is worth noting though that findings from these two types of investigation are not strictly comparable because experimental populations in enclosures or pens are necessarily confined and therefore the effects of high population density may be artificially accentuated due to the lack of possible emigration. Indeed, of the enclosure studies, only in the ones carried out by Crowcroft were there no subordinate males living in the territories. This was probably because his territories occupied less than the total area of the pens (some 250sq. feet), sizes considerably larger than those used by other workers. Thus there could be a few areas in an enclosure where defeated mice were safe from attack. The territories featured in other studies (Mackintosh 1973, 1981, Reimer and Petras 1967) occupied the whole of the areas available so that there was little opportunity for subordinate animals to escape to unoccupied sites although these mice showed a tendency to huddle together in groups where they were able to avoid attack. Formation of such aggregates appears to be associated with previous experience of defeat. In pen studies of high density populations, great numbers of defeated mice were seen to cluster, becoming very inactive as the population increased (Christian 1956,

Lloyd and Christian 1967).

A common response to confinement by mice is an increase in levels of aggression and in the pen experiments with freely-growing populations, continually renewed fighting was seen to occur as young mice matured and old ones died or were defeated, so that aggression was virtually continual throughout the experimental period (Davis 1958, Southwick 1955, Crowcroft and Rowe 1963, Lloyd and Christian 1967). Bronson (1979) has put forward the view that "social plasticity" kept within the framework of intermale aggression appears to provide the basic setting for the social organisation of house mouse populations. In the same review, the point is also made that the habitats of feral mice rapidly change and so it is probable that social instability may well be a common characteristic of most mice colonies with the exception of those based on a plentiful and stable food supply. As a side point, availability of food has not necessarily been found to be a destabilising factor in mice colonies. In population density studies, a variety of intermediary factors appears to affect social stability when the numbers reach high levels even when food is plentiful. Restricted access to food has been shown to have only minor effects on the reproductive development of young males who, despite a stunted appearance, nevertheless developed normal numbers of sperms in their testes and vasa deferentia with an 80% fertility success rate at puberty (Hamilton and Bronson 1985).

Many of the population studies have examined the factors operating to control numbers (Christian 1955, 1970, Davis and Christian 1957, Davis 1958, Christian, Lloyd and Davis 1965, Lloyd and Christian 1967, DeLong 1967, Lloyd 1973, 1975, Oakeshott 1974, Lidicker 1976). In a non-varying physical environment, maximum numbers seem to be mainly determined by the suppression of successful breeding by females rather than by violent death although this

certainly plays some part in population control. From the data on increased density in natural populations however (Newsome 1970, Selander 1970, Berry and Jakobson 1975), Berry (1981) has argued that crowding per se does not normally seem to inhibit reproduction. Regulation was also found to be influenced by a decline in the numbers of live births and in the mortality of infants and young adults; this has been shown to result in the reorganisation of territorial systems. In Lloyd's study (1975), mortality was also associated with a seven-fold increase in aggression and with redistribution of breeding females among dominant males. Although aggression between males did not directly lead to infant mortality in Lloyd and Christian's study (1967), active fighting was very evident as shifts in the social structure took place. Besides this, top ranking males were seen to have high levels of androgen activity as measured by the weights of sexual organs, (Christian 1955) a decrease in androgen output was found in dense circumstances (Lloyd 1973, Lidicker 1976), indeed an individual's response was dependent upon its position in the social hierarchy. Although Oakeshott (1974) has questioned the relationship between social status and relative mating success, in the main, his work supports Christian's in showing that as population density and levels of juvenile crowding increase, so social stability with secure dominance status, and hence territoriality, in many males decrease.

The discussion so far has centred on the studies carried out on the mouse as this was the animal used in the present study. However, a large volume of research has been done in parallel over the years on other small mammal species and this warrants some discussion as many of the studies have widened and clarified findings from mice. There is an awareness though of the dangers involved in cross-species comparisons. The work of Barnett (1955), Calhoun (1952, 1961, 1962,

1963), Robitaille and Bovet (1976) and Adams and Boice (1983) on the rat has provided much useful information about the social organisation of this species. Unlike the mouse, this animal is more colonial in habit but is still organised into social hierarchies within groups. In one of a variety of studies, Calhoun (1962) confined laboratory rats to an environment consisting of four interconnecting pens. Social instability increased as numbers grew to 150 adults in a total area of 100 feet square and fighting was marked in all four pens. The territory holders were the least affected by this disrupted social environment whereas other males became either hyperactive or pathologically withdrawn, exhibiting various abnormalities of sexual behaviour. The increase in social disorder affected female behaviour as well. These rats became progressively less adept at nest building and eventually ceased to show this behaviour altogether. Failure to transport litters was observed and when this did take place, often only a few of the pups were moved with a high degree of scattering of the remaining young or simply dropping them on the floor. This was largely due to frequent interference with the mothers by other rats. Many females were unable to carry pregnancies to full term or to survive delivery of the litters. The general social disruption produced a phenomenon which Calhoun has described as a "behavioural sink" whereby animals would crowd together, often as many as sixty or eighty, in a single pen during feeding periods. Individual rats would rarely eat except in the company of other rats: as Calhoun says, "eating and other biological activities were thereby transformed into social activities in which the principal satisfaction was interaction with other rats" (p.139) as in mice, Calhoun and also Barnett (1958, 1967) observed restricted movement in rats that had adapted themselves to subordinate roles after suffering defeat. Barnett divided his rats

into three social classes: alpha, beta, and omega, and it was the beta rats which formed this subordinate category.

Territory formation and defence has been observed in Meadow voles (Microtus pensylvanicus) kept in pens (Louch 1956) and like rats, Wood voles (Microtus agrestis) are also divisible into three social groupings (Clarke 1955). In this study, one class comprised heavier animals with glossy coats and were wide ranging in their activities; a second group, which made up the bulk of the population, were low weight individuals with ragged fur and numerous bites particularly in the hind quarters; these animals showed restricted movements. The third group were intermediate between the other two.

From studies on different gerbil species using cages (Thiessen 1973, Swanson 1985), enclosures (Gallup and Waite 1970, Agren 1976), and from field observations (Daly and Daly 1975), it appears that this animal may live in a variety of social conditions: solitary, in pairs, or in communities. Generally, the Mongolian gerbil (Meriones unguiculatus) is considered to be communal and shows territorial behaviour under semi-natural conditions. Swanson (1985) points to the family as the basic social unit with the number of individuals in the group dependent on the social relationships within the colony rather than the size of the living area. Where dispersal is inhibited, neuroendocrine mechanisms influence curtailment of population growth. Displays of dominance leads to submission and to the arrest of sexual development in young females and the marking behaviour of young males. As a consequence of this, the social stability of the group is maintained.

In Prairie deermice, Terman (1965) found an increase in aggregation when population numbers were nearing and at a maximum, similar to that observed in mice (Christian 1956, Lloyd and Christian 1967) although caged groups of deermice did not show the high levels

of aggression seen in Mus (Bronson and Eleftheriou 1963). Indeed other factors, such as sexual experience or the presence of females seemed necessary in order to stimulate fighting.

As remarked earlier, the behaviour of confined animals may not necessarily parallel what takes place in the wild and the investigation by Clough (1968) into the behaviour of the Norway lemming (Lemmus lemmus) is therefore important as it is one of the few studies reported to compare events in the wild with those under captive conditions. The high levels of aggression and resulting formation of a social hierarchy were found to be a consequence of confinement and close proximity and were not typical of behaviour in the wild where the lemmings generally were seen to move singly and avoidance was the primary response on meeting a conspecific.

Among rodents, Eisenberg (1967) has postulated that depending on the animals' social organisation under natural conditions, so different species have differing thresholds of sensitivity to captive grouping depending on whether they are solitary, semi-solitary or communal. Confinement of wild rodent populations to pens that normally live in a solitary or semi-solitary state has it seems, pronounced effects on the levels of aggression which are no doubt related to the inability of the animals to maintain their normal dispersal patterns and social habits under these conditions. For rodents that tend to form groups, it has been argued that elements of this social structure may facilitate the adjustment of high group numbers. Dominance hierarchies for example, where individuals know their rank, are thought to result in increased stability and to reduce aggression (Davis 1971). Relevant to this, Henry and Stephens (1977) and Henry (1982) have pointed to the importance of "socially healthy systems" in murine, primate and even human societies in order to protect their members against adverse social changes. They

suggest that systems where social relations and territoriality are in disorder, fail to give this vital support.

In considering the mechanisms involved in social stability, one idea put forward is that fighting often takes place due to the behaviour of subordinate animals and Rowell (1974) working with monkeys, has suggested that a subordination hierarchy may therefore be a more useful working concept than a dominance hierarchy, because aggressive encounters are usually determined and often initiated by the subordinates' behaviour. In captivity, hierarchies may therefore be due to stressful conditions whereas in the wild, they may be tenuous or absent. Rowell (1974) argues against the hypothesis that their formation reduces aggression and indeed, among certain primate groups hierarchies are associated with high levels of aggression (Rowell 1967, Bernstein 1964). There is also in these species inconclusive evidence that high ranking males have greater overall reproductive success.

It was predicted earlier in this chapter that due to the social pressure imposed by dominant mice, in particular the territory holders of the free range, all subordinate animals would be subjected to stress due to their social position together with their inability to avoid attack by more aggressive conspecifics. As well as this, these mice would be under the constant threat of attack, defeat and injury. Support for this hypothesis comes from previous findings. Louch and Higginbotham (1967) using mice housed in cages, found that in social hierarchies where subordinates were under this form of social pressure, values for corticosterone in these animals were twice as high as for dominants where levels were little changed from control, unstressed animals. This finding has been repeated by Ely and Henry (1978) using population cages similar in design to those of Reimer and Petras (1967) which permit not only the formation of

social hierarchies, but also the establishment of defined territory areas. Similar results using rats housed in pens have also been obtained by Dijkstra et al. (1984, 1985). A common link between these studies was that subordinate animals were unable to escape persecution by dominants.

From these findings, it was anticipated that in the experiments here, certain physiological changes would result due to social pressure such as adrenal hypertrophy and high corticosterone levels and in addition, there would be poor general condition among subordinates, with reduced coat quality and slow healing of inflicted wounds from bites. As well as increasing pituitary-adrenal output, persistent social stimulation is also recognised as being influential in increasing sympathetic adrenal medullary activity (Ely and Henry 1978). Raised blood urea levels and brain catecholamine levels are also known to result from chronic stress and there is also evidence for links with a number of pathologies such as gastro-intestinal ulceration, hypertension, cardiovascular disease and renal damage related to reflux nephropathy (Henry et al. 1971, Ely and Henry 1974 and Henry et al. 1982). A more detailed discussion of social stress is found in chapter four. It was not possible within the scope of this work to investigate all these areas, however corticosterone output and adrenal weights were measured together with blood urea levels and although no formal measures for renal damage were carried out, observations were made at autopsy on the external state of individual kidneys and details of colour and any surface pitting were noted.

In conclusion, it is worth briefly mentioning that the effects of social stress also affect the pituitary gonadal axis via subtle and not fully understood interaction with the pituitary-adrenal system. Mice of low social rank show not only increases in adrenal

weight but also decreases in weights of sex accessory glands when compared with dominants (Christian 1955, Davis and Christian 1957, Brain 1972a) together with lowered androgen output (McKinney and Pasley 1973). From these findings, it was therefore expected that reduced testicular function in the form of low testosterone output and lowered preputial and testes weights would also be a feature of subordinate status in this work.

It was recognised that using the free range room necessarily imposed certain restrictions on the animals that lived there and it was also accepted that such an environment provided a closer approximation to the more enclosed habitats occupied by mice than to the true feral state. Despite this, it was anticipated that the scope for investigation would be extended beyond the confines of the cage resulting in the extension of a number of previous findings which had utilised more traditional housing methods.

CHAPTER 2

GENERAL METHODS

This section describes the general methods used for experiments discussed in subsequent chapters. Methods, such as radioimmunoassay procedures, specific to particular work are described in the relevant chapters.

A. Animals and Husbandry.

'TO' strain outbred male albino mice were used in all the experiments. Mice were originally obtained from A. Tuck and Son Ltd., Battlebridge, Essex and bred in our own laboratories. After weaning at three weeks of age, sixty male mice were assigned non-systematically to twelve non-sibling groups of five, where they remained undisturbed except for routine care until ten weeks old. Food (Pilsbury's Ltd., Small Animal Diets, Birmingham) and water were supplied ad libitum. Animals were housed in large plastic cages (Supplier: North Kent Plastics, Ltd.,) (42 x 25 x 11 cms) with stainless steel tops and were maintained on a reverse lighting regime (red lights on 12.00-22.00 hours). The lighting schedule was synchronised between two animal rooms. Temperature variation within and between the rooms was 18-22^{o}C. Cages contained wood shavings for bedding which was changed weekly or when necessary.

At ten weeks of age, the fur of each animal was marked with hair dye to aid in identification. Twenty of the sixty mice were then individualy housed in small cages (30 x 13 x 11 cms); another twenty were rehoused in their existing groups of five in large cages with fresh bedding.

The final twenty mice were placed on the floor of a room measuring 2.4 x 2.8 m. This "free range" room had white walls and a

painted concrete floor which was covered with wood shavings (see Fig. 2:1). A door with a step-over barrier at one end of the room allowed entry for routine care and a pane of glass in it permitted observation. A blackout curtain was kept pulled across the door except during observation sessions.

At the outset of every experiment, the floor area was divided into four quarters by sheet metal barriers, 60 cms high. Mackintosh (1970) investigating territory formation in mice, found that positioning physical barriers for a period at the start of an experiment, resulted in individuals gaining and maintaining control over discrete areas and as a consequence, there was less likelihood of a single "despot" defeating other mice and occupying the whole available space. Each quarter here contained a water bottle, a wire mesh hopper with food, a wooden nestbox with perspex lid (17 x 17 x 11 cms) and two entrances diagonally opposite one another at the corners, and a number of housebricks arranged as shown in Figure 2:1, just inside the metal barriers. A group of five mice was removed from its cage and placed in each of the four quarters. All animals were then left undisturbed for ten days.

After this time, daily observations under red light, lasting 15-30 minutes were made on the group caged and free range animals in order to determine which animal was dominant in each group. This was decided by recording which mouse consistently chased others and initiated and won fights, resulting in submissive behaviour and postures (Grant and Mackintosh 1963) and causing all other mice in a group to retreat. When the same animal was seen to consistently show this behavior over five consecutive days, it was designated as dominant in that group.

Once a dominant mouse was identified for each group in the free range room, the barriers were removed and the four groups then met

one another for the first time. Animals remained in their housing conditions for approximately eight weeks during which time, routine observations were made on caged and free range mice to note any changes in social status. Disturbance for routine husbandry and fur-marking were kept to a minimum. When a series of experiments was finished, the mice were removed and the room was cleared out. The nest boxes and food baskets were scrubbed with a solution of disinfectant which was also used to clean the floor after removing the old bedding. The housebricks were soaked in disinfectant also and then stored for drying. An alternate set was available for the next experimental period.

B. Blood Sampling.

Mice were anaesthetised with diethyl-ether and approximately 300 µl of blood was collected in plastic tubes using the retro-orbital puncture technique (after Riley, 1960). This method allows repeated sampling from an animal. Samples were heparinised, centrifuged for three minutes at approximately 3000 RPM (2640g) and then frozen at -20^{0}C until required.

C. Post Mortem Data.

Animals were killed by cervical dislocation and dissected within one hour of death. A record was made of the wet weights of the left preputial gland (unsqueezed), left adrenal gland and left testis for each animal as well as body weight at the time of death. A dissecting microscope was used to carefully remove fat tissue from the adrenals. The state of the bladder, whether full or empty was noted and any discoloration or pitting of the kidneys also recorded.

D. Territory Formation and Classification of Social Groups in the

Free Range.

Because the dominant animals fought and won fights in their home areas but retreated without defeat from other parts of the room, it was considered justified to regard their areas as territories which they defended and lived in (Mackintosh 1970). Mice in the free range were divided into three categories and the criteria by which they were assigned to these groupings were as follows.

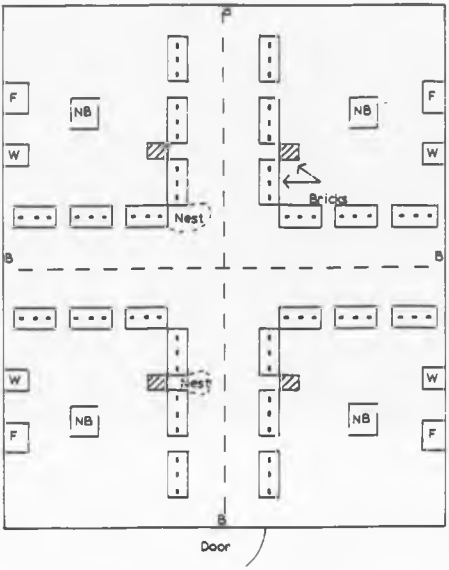
A territory holder (dominant) attacked all other mice on his territory; defeated all other mice in this area; retreated without submission if attacked elsewhere.

A subdominant attacked and defeated subordinates of his group on the area in which he lived; attacked mice from other groups, eliciting from them submission or retreat; did not attack other subdominants or the territory holder but was defeated by the latter when attacked; fled or submitted when threatened by the territory holder. Where two subdominants lived in a territory, neither was ever seen to attack or defeat the other.

A subordinate attacked no other mice of his own group and was defeated by the territory holder and subdominant(s); occasionally attacked mice from other groups whilst in his own home area.

The definition of territory is based on that adopted by Reimer and Petras (1967) which states that a territory is "an area, including nest-boxes, in which a single individual or stable group of individuals is repeatedly found".

Figure 2:1 Diagram of Free Range Room



W = Water bottle NB = Nest Box

F = Food B-B = Barrier

Room dimensions - 2.4 x 2.8 m.

Summary of Experiments

EXPERIMENT	CONTENT	CHAPTER REPORTED IN
FR1	Not reported	-
FR2	Urinary aversive factor tests	6
	Urine marking patterns	6
FR3	Peak corticosterone levels	4
	Testosterone levels	5
	Urinary aversive factor tests	6
	Urine marking patterns	6
	Body weights	4
	Organ weights	4/5
FR4	Not reported	-
FR5	Body weights	4
	Blood urea levels	4
	Pain threshold levels	4
	Testosterone levels	5
	Organ weights	4/5
FR6	Testosterone levels	5
	Organ weights	5
FR7	Not reported	-
FR8	Trough corticosterone levels	4/5
	Testosterone levels	5
	Urinary aversive factor tests	6
	Urine marking patterns	6

EXPERIMENT	CONTENT	CHAPTER REPORTED IN
FR9	Urinary aversive factor tests	6
	Urine marking patterns	6
	Testosterone levels	6
FR10	Testosterone levels - free range males housed with females	5
	Organ weights	5
Expt. 11	Testosterone levels and aggression - caged mice	5
	Organ weights	5
FR12	Testosterone levels - free range mice with females (short exposure only)	5
FR10/12	Comparative measures of agonistic behavior	5

All tests, other than those in experiments FR10, 11, FR12 and FR10/12, used mice housed in both the free range room and laboratory cages.

GENERAL KEY TO FIGURES AND TABLES

TH - Territory Holder

SD - Subdominant

S - Subordinate

D - Dominant

SH - Single Housed

FR - Free Range

GH - Group Housed

Ex Con - Exchange control

CHAPTER 3

OBSERVATIONS ON FREE RANGE MICE

The content of this chapter is a description of the social behaviour that was observed in the free range groups of mice that were used in different experiments.

Apart from a study of the agonistic behaviour which is presented in chapter five, no attempt was made to make a quantitative record of different activities. Despite this, details of events witnessed were kept in a notebook and were systematically written down as observed with attention given to behaviour patterns seen in specific individuals. Observations were made both in the white and the red light periods and, except for when animals were being watched daily in order to determine dominance or for experimental reasons, these observations took place approximately two to three times a week for periods of up to three quarters of an hour. This account therefore, is an attempt to provide a vivid description of some of the events that were witnessed during the many observation periods. In order to do this, a rather less formal style has been adopted. In addition, a number of photographs depicting scenes of the mice housed in the room, are also included in this chapter.

Many of the events that took place were common to all the groups of mice that spent time in the room but where differences arose or circumstances were associated with a particular group, this is indicated with reference to the numbers of the experiments that the mice were a part of.

At the outset of each experiment, the same events were seen each time a new group of five mice was put into each of the quarters in the free range room. There was always an immediate and intensive

investigation of the new area. Movements were not simply random across the floor but followed a particular pattern. Several circuits of the periphery would be made first with the animals remaining close to the walls; then gradually one, then another would venture into central area, darting back to the nearest wall if disturbed. Their pattern of movement could have been influenced by "old trails" of mice who had gone before as no doubt, traces of old occupants still lingered. During this time, group members would largely ignore one another except for an occasional sniff on meeting. Close attention was given to every object an animal encountered so that for instance, all aspects of a nest-box would be thoroughly examined. This behaviour may have lead not just to familiarity but also spatial knowledge about the position of the object relative to others in the area.

It was generally about twenty minutes before the first of the animals gave up the intense search of the room and took a greater interest in the rest of the group. This was when the first fight occurred. What acted as a trigger for this is unknown but one mouse would suddenly turn on another and a fight would follow which ended in one of the two either retreating or showing a submissive posture.

When these events were first seen it was possible to be misled into believing that the animal that attacked and fought first in a group would eventually be the one that would be classified as the dominant. Although this was the case for many of the groups, in several circumstances the mouse that would eventually defeat all others of his group was still not determined.

During the two weeks that led up to the removal of the metal barriers, group members appeared to live peaceably together, although attacks by dominants on subordinates were seen. Great interest was constantly shown in the barriers, probably due to the movements of

mice in the other quarters that were always audible. The barriers did not fit so tight to the floor that the feet and tails of mice could not pass beneath them and a good deal of floor scratching at these areas took place.

On every occasion, the most intense fighting occurred when the barriers were removed and groups of mice met one another for the first time. Because of their intense curiosity and great urge to explore, the enlarging of the area available to them meant that several members of a group would radiate outwards to the other areas from their own. This naturally resulted in their "trespassing" on occupied land and produced sharp retaliation by the residents. Territory holders, subdominants and subordinates could be seen to attack and fight any incoming stranger. Acts of defence resulted in fights where animals would be locked together and roll over and over. The duration could be ten to thirty seconds and when they eventually broke apart, it was the intruder that generally backed down; he would either run back to his area or just retreat a few steps before venturing into the strange area again and being involved in another fight. Many mice, when defeated in these initial attacks, would return immediately to their own areas and remain there for a time before venturing out again. An animal in retreat might turn and try to drive off his pursuer although generally they kept running until they reached safety. Although subordinates were seen to attack and chase away strange mice, it was the dominants who seemed to do most of the attacking in this initial period when the room was opened up. Not only were they involved in fights with neighbouring mice, but they also chased and attacked their own subordinates, who drew attention to themselves very often by their fighting behaviour with others. During this period of high activity, mice tended to attack anything mouselike that moved. As a consequence, errors of identity

resulted and if a subordinate mistook his dominant for an intruder he could pay dearly for his mistake.

The level of fighting gradually reduced within about two hours but it would be during the following four to five days that the number of aggressive encounters between groups would fall to a point where they co-existed in relative harmony. The savagery of the first hours and days inevitably produced some casualties though. Mice sometimes lost bits of their ears and the ends of their tails during the fights, irrespective of status, but bites to the sides and the rump were more commonly found on the subordinates. It seems probable that through winning or losing fights at this time, positions in a social hierarchy became firmly established. This was because mice learnt whether or not to fight back or to initiate attacks. Ginsburg and Allee (1942) examined the effect of fighting in mice by staging a series of "round robin" contests. They found that a series of defeats tended to condition a mouse to submit and accept defeat whereas mice that were victorious continued on to show success in subsequent fights. They also found that it was easier to condition a mouse downwards towards subordination than upwards towards being a winner.

It was also during this early period in the free range that an animal might lose his status as a territory holder. This happened on a limited number of occasions but when it did, the new dominant could be a member of the group or an outsider. In some groups, certain other mice besides the dominant, were seen to attack and defeat subordinates as well as chasing away any strangers. Moreover, these animals never attacked, and were submissive to, the dominant of their area. It was generally one of these "subdominants", as they have been called, who might defeat a dominant and take over the territory. He would then either chase all the other mice out or allow the

ex-dominant and other three subordinates to remain but could make their lives uncomfortable through much harrassment.

In some groups, the subdominant could be seen to engage in more fighting than the dominant, to the point where it was possible to consider that a change of status had occurred. Although highly aggressive, subdominants would from time to time be brought to submission by the dominant mouse. Unlike the dominants, these mice were not seen engaging in "patrolling" behaviour or the intensive investigation of a home area that was very characteristic of the territory holder.

A group might have one or two subdominant mice or none at all. These mice seemed to display great curiousity about other areas of the room and explored widely despite the attacks and chases that this could produce. This behaviour contrasted with that of the territory holders who rarely left their home areas, making only brief forays when they did. Had they been in the wild, it is possible that these subdominants would have sought out territory areas for themselves but of course, the confined space of the room did not allow for any actual emigration. It thus seemed that they had little to lose except fur on their rumps, and all to gain.

Despite this, on a few occasions, certain animals were seen to leave the main territory and live instead in small areas which they defended. This tended to be the area between the lines of brick which formed a cross-shape on the floor and although it was covered with wood shavings, had no other landmarks(see Fig. 2:1). Before the barriers were removed, territory boundaries were formed at the edges by the sheets of metal which were placed in a cross formation in the area of wood-shavings. When the room was opened up, the intense fighting that ensued forced animals to retreat to the two lines of house bricks in each quarter so that these then marked the boundary

to each territory, leaving the central cross area as a no-man's land. It was here that a few sudominants took over small areas. They were seen to build nests by collecting wood-shavings into a cup-shaped structure which they surrounded with food pellets, and from these small holdings, the mice defended ferociously, darting out at any other animal that came too near.

The social hierarchy for every group would be firmly established after four or five days and barring accidents, would then remain the same over the period the mice remained in the room. Once this had happened, it was then impossible to introduce a strange male mouse to the room, should the numbers be reduced due to death, irrespective of his status or previous housing arrangements. He would immediately suffer attack and be defeated without putting up resistence, finding himself driven from one area of the room to another, forced to endure the consequences of entering the territories as a stranger. Unless removed, these new mice would be found dead within two or three days and would be badly wounded before then.

Odour appears to be the important sensory cue that enables mice once established together, to recognise members of their own group and intruders as "foreign". Archer (1968) has shown that mice housed together habituate to the odours of one another and aggressive behaviour is the response to the odour of a male who does not carry the group's "label".

The social arrangements were stable to the point that it was possible to predict where individual mice would be found when observations were made. When removed from the room (e.g. for blood sampling or fur re-marking) their behaviour seemed to correlate with their social position. Down-trodden, bitten subordinates were easily caught whereas dominants resisted capture, ran around at high speed and attempted to bite when handled or characteristically "rattled"

their tails when approached.

Every week, observations were made on the groups to note whether changes if any, had led to altered social status and this was important as the mice were removed from the room periodically for fur-marking and experimental purposes. During the early stages of the project, there was concern about whether removing the mice would severely disrupt their social order, causing experiments to break down, particularly as the designs of certain tests meant that the mice would need to be absent for as long as thirty-six hours at a time. The worry proved to be unfounded. Each time the mice were replaced in the room, they showed a pattern of behaviour in some ways similar to their first entry. The floor would be covered with mice who, completely ignoring one another, would crawl freely all over the room, perhaps re-learning their environment. As they appeared to pick up familiar cues, the pace of their investigations quickened and they would run swiftly from object to object. This period lasted usually from five to twenty minutes depending on the length of time spent out of the room. Scuffles then broke out as ownership was re-asserted and within half to three quarters of an hour, all the mice would be back in their previous areas with the social system as it was before, and to an outsider, it would appear as though the room had never been disturbed.

This pattern was seen over and over again and it seemed remarkable for two reasons in particular. First, the mice demonstrated what seemed like extremely good memories for the odour cues with which they had, no doubt, marked objects in the room and perhaps for the visual landmarks that helped define the areas, which may have included the door and the lights. Secondly, the odour cues if they helped in the re-establishment process, could retain their freshness or at least were still recognisable many hours after being

laid down. This feature of scent marks is of course vital for communication in the wild where a message must remain viable in the absence of the sender, but it was of interest to note the power that these scent cues still possessed in our stock of laboratory mice, bred and usually housed under the confined conditions of cages.

Intense investigation also followed the removal or rearrangement of an object in the room. The two opposite entrances to the nestboxes were frequently plugged up with food pellets and woodshavings by the mice and when trying to capture them, it was generally easier to remove a whole nest box full of mice from the room rather than pick them out one at a time. It was difficult however, not to upset the housework on these occasions and this unfortunately had repercussions. When the box was returned, it was impossible to replace it exactly as it had been before with every pellet perfectly lined up. This would cause immense social disruption and the mice would spend a good while carefully examining the box and replugging the doorways.

As well as recognition of objects, it seems likely that the mice were able to identify one another on the basis of odour. Bowers and Alexander (1967) showed that by means of olfactory cues, mice could recognise individuals of the same strain. This ability would explain why certain individuals in the free range appeared to suffer so much persecution. Subordinates that had been dominant territory holders but had lost their positions, appeared to be subject to very severe attacks. These males quickly fell into very poor condition, with large scabs on their rumps and shabby coats. In experiment FR8, where three of these deposed males were actively sought out for harassment, it was found that despite their changes in status, these mice still produced urine marking patterns and an odour associated with dominance status even two weeks after they had been overthrown.

Another example of individual recognition on one occasion occurred during the period of experiment FR5. A particularly aggressive dominant had driven all the other members of his group out of his territory during the first week when the room was opened up, and so lived alone. These subordinates were forced to take refuge in an adjacent area where they shared the wire food box with a number of other low status males. Several weeks later, one of these males ventured back into his old haunt and finding no opposition (the dominant being asleep in the nest-box) investigated the patch, then had a short feed and a drink before returning to his current home. As he left, the dominant awoke and appeared at one of the doorways. He must have sensed a trespasser as he carefully followed the route taken by the intruder through his area. He then followed it on across his boundary and into the adjacent area where he went straight to the food box. Amongst the subordinates huddled there was the intruder that he was seen to seek out and attack. The other mice fled in all directions to avoid the trouble and the recalcitrant mouse was given a severe beating before the dominant left him alone to return to his own territory.

A group of subordinates that were forced to take up residence in a new area never became completely absorbed into the resident group, and could suffer as a result. This may have been due to their not acquiring fully the new group's odour "label" but instead, may have always retained some of the odour they shared as an original group.

As a rule, subordinates kept together and as well as occupying food and nest boxes, the top of the vertical bricks was very often a spot where they lay, one on top of another. They were also seen to crawl under other mice and this habit may also have served as a means of seeking protection. Although precarious as perches, these raised retreats seemed to be locations that dominants and subdominants

visited rarely. A subordinate soon learned that his pursuer would leave off the attack once he had climbed onto a tall brick. Albino mice are reputed to be particularly short sighted and may not be good at maintaining odour contact at high speed (Crowcroft 1966) so that an animal that took cover or could reach a spot above ground level stood a chance of shaking off his follower. A group of subordinates perched on a brick resulted in single individuals avoiding intense persecution. This "safety in numbers" could mean that aggression directed towards these animals was spread across the group. It may also have been more difficult for a mouse to attack from below, so that being aloft on a brick gave this added form of protection.

Crowcroft (1966) has referred to the term "patrolling" to describe one of the ways dominant mice defend their territories. This behavior was seen constantly among the territory holders of the free range and involved frequent movement along the lines of bricks together with close scrutiny of the rest of the area and the objects in it. Each house brick had three holes in it into which a mouse's body fitted perfectly so while doing the rounds of his patch, a dominant was often to be seen head down in one of these holes with his rump up in the air. A tour could also include a visit to the spot where subordinates would be sitting. Often only a cursory sniff was put in their direction but if the dominant entered a nest box they were occupying, a brief moment of quiet would be followed by pandemonium as attacks occurred and mice fell over one another in their attempts to flee through the two doorways. When attacks occurred on the tops of bricks, subordinates simply tumbled off as quickly as possible to avoid being bitten.

If a dominant climbed onto a brick where subordinates lay, instead of always attacking he could be seen moving over and between the animals as they lay in a pile before leaving them alone. When

the mice were examined after this type of encounter, it was very noticeable that the fur on their backs was wet with urine. Over time, the fur became progressively more yellow and stained. It may have been that a dominant marked the subordinates as he no doubt marked all the other objects in his environment with his own scent, so that even if under more natural circumstances, they would have been driven out of a territory, here in the free range they were tolerated because they may have carried his particular odour.

Paradoxically, it was possible for a dominant to be seen on occasion sleeping with the same subordinates which he would then attack during periods of waking. What served to trigger an attack cannot be stated for certain but an attack on an outsider was undoubtedly influenced by smell and possibly by sight, although a dominant could be attacking one of his subordinates while quite unaware that a stranger had entered the area and was investigating. This intruder might quietly move off without any trouble having arisen. Due to short-sightedness perhaps, it was possible for a strange mouse to pass within inches of a resident and not be noticed by him.

From the data collected on aggression behaviour (Chapter 5), it is evident that most encounters took place between members of a group rather than between neighbours. This is probably because the mice spent most of their time in their home areas. When an animal did explore elsewhere, he could turn and run back without any obvious provocation. When two mice from different areas did meet, a glare or a lunge from the resident was usually sufficient to drive an intruder away. When fights did occur, they were generally of five to ten seconds duration and involved the territory holders and subdominants although subordinates were also seen to attack intruders. Subdominants usually ran away after a fight, often chased by the

resident. The fights that occurred between the territory holders could follow interesting patterns. Encounters in one or another's area normally ended with the intruding dominant running back to his own patch where, from the line of bricks, he would stare at his opponent who stared back. Both animals would typically be seen to rattle their tails. This movement resembled a side to side motion but when seen close up, is in fact a "rattling" caused by rapid waves of the tail from base to tip and is well described by Crowcroft (1966).

Fights also took place in the area of no-man's land between the bricks. A dominant might hurl himself on another if the intruder approached close to the unseen territory boundary. A hard struggle would follow with the animals rearing up and facing one another while making swift darts with the head towards the flanks of the opponent. A full fight could then follow with the combatants rolling over and over one another. The animals would then break apart and hunching their backs, would move around one another in a semi-circle, moving their bodies in an arc-shape using very small steps while vigorously tail-rattling. This would be followed either by another attack and fight or one animal might turn away as though to leave and then about-face and repeat the body movements and tail-rattling procedure. These may have served as warning signals or could have demonstrated conflicting emotions when an animal was undecided about further attack or retreat.

Territory holders always won fights on their home ground or chased an intruder out with perhaps a bite on the rump. If attacked away from their areas they generally retreated or fought back and then retreated as described above. Using cage conditions, Uhrich (1938) found that the tendency of male mice to fight and win depended on whether they were in their own home pen or on an opponent's

familiar ground, a single mouse being capable of defeating an entire group in his own cage but being defeated himself in the home pen of the group.

Much of a dominant's waking time was given to patrolling and checking and re-checking the identity of other mice in the group using nasal and genital sniffing. His movements on the territory were quick and confident but if he ventured to another part of the room, his whole demeanour would change. The further he got from his home, the slower and more cautious, his actions became. His whole body would be almost flattened along the ground as he crept forward with the head and nose stretched out front and his tail straight out behind. These tentative ventures could end with his sudden return to home base with no other animal having been encountered due presumably, to a stimulus the observer could not detect. Coming across a foreign odour, was sufficient to make a dominant return smartly to his home area and on occasions, he would then turn to face the area he had visited and would rattle his tail agitatedly, though no other mouse was to be seen nearby.

Subordinates tended to venture out in search of food and water when the dominant mouse was asleep and they could avoid harassment and on these occasions, their forays could take them into the neighbouring areas. The wire mesh food baskets that contained food were open at the top and the mice scattered food pellets all over the floor area. Despite the easy availability, all the mice were extremely choosy about which pieces they would eat and could be seen carefully selecting the pellets they ate. This exploration for food, despite the excess provided, may be part of the mouse's adaptation for its original way of life, searching on the ground for food such as the seeds of grasses. Some food pellets may of course, have been urinated on or otherwise marked by other mice.

Watching their investigatory behaviour for food, it was evident that knowledge of the geography of the room was excellent. The quick movements of the mice, over objects and around corners showed no hesitation. Mackintosh (1973) has argued that visual as well as olfactory cues are important to mice in their recognition of territorial boundaries. At the end of one experiement (FR3), an attempt was made to investigate this. After tests were concluded, all mice were removed from the free range and housed in their respective groups in large laboratory cages. The bedding from each of the four quarters in the room was then carefully swept up and placed in separate bags. The bricks that delineated each quarter were rotated through ninety degrees so that those from the "A" quarter were moved to the "B" quarter and so on. The bedding was then put back so that it was placed with the bricks it had been taken from and finally, the mice were returned following an absence from the room of approximately half an hour. As olfactory cues are vital for communication in mice, it was predicted that the territory areas would also be rotated through ninety degrees along with the bricks and bedding thereby following the odours deposited prior to leaving the room. It was surprising therefore to find that all the mice except for three subordinates, returned to the geographical areas they originally lived in, following a period of intensive investigation. Two factors may have influenced this result: first, removal of the bedding was not accompanied by washing of the floor beneath and odour from urine impregnated on the floor may have been stronger than that within the bedding and was therefore a greater influence on the behaviour of the animals than the bricks and bedding combined. Secondly, besides the landmarks on the floor, the room also contained the door opening into it together with a number of shelves along one wall and these additional features may have

served as important visual cues for orientation by the mice. Although an interesting test in itself, it is not felt that the result threw any new light on the important roles of odour and vision in this species and hence, too great an emphasis was not placed on the observations made.

Returning to the social relations between the animals, dominant territory holders appeared to vary in their levels of aggressiveness towards the animals with whom they shared an area. Occasionally, a dominant would allow no other male to share his territory with him, chasing any animal away, while some dominants attacked other group members so vigourously and persistently that none of them normally went close to the territory holder at all whilst he was awake. Others were less aggressive and would allow subordinates close without attacking them. Most subordinates though, huddled together often in a pile, seemingly trying to avoid attack whenever the dominant was awake and moving about. Often these mice were inhibited from moving even to urinate. It was this restricted movement which probably gave rise to the very distended, full bladders that were found in these animals at post mortem and which have also been described in subordinate mice by Henry et al. (1982). Grey and pitted kidneys were also a feature of some of these animals and the poor external state of these organs could be linked to the excessively full bladders that were seen. Further discussion relating to this is given in chapters 4 and 6.

Subordinates rarely attempted to defend themselves and would produce loud squeaks if an attacker came near them. A few of them became badly bitten and ragged to the point where they seemed to give up altogether and would be completely ignored by other mice including dominants and subdominants who stopped attacking them. It seems likely that these mice stopped producing any aggression-promoting

signals and were therefore no longer perceived as threatening. Bites on subordinate mice were often found to be particularly bad around the ano-genital region. This hind part of an animal could be extensively damaged and this may in some way have contributed to the urine retention state described above.

Levine et al. (1978) state that a habituated animal has a set of expectancies with which to deal with the environment and that provided changes do not occur, physiological responses related to arousal are no longer shown. In particular, suppression of the pituitary-adrenal system under these conditions, is emphasised and this subject is dealt with in detail in experiments involving subordinate animals which are discussed in Chapter four. In the light of remarks by Levine et al., most subordinates appeared to cope with their situation and despite the ragged state that some fell into, deaths in the free range were uncommon. It was generally towards the end of an experimental period that fatalities, if any, occurred and the badly scarred animals were usually the casualties. Cause of death was difficult to tell although the state of the bladder and kidneys was noted and it was possible that kidney failure might have been responsible although this was never certain. Deaths tended to occur when no one was present to retrieve the dead animal and as the mice were in the habit of covering a body with wood shavings, more often a corpse was not found until the room was being cleared out at the end of the experiment and by then, the body was too decomposed for useful examination.

Over the period of study, three dominants were found dead in the room at different times. For two of them, no physical defects could be found externally and inside, the bladders were empty and the kidneys were dark pink and appeared healthy. In the case of the third mouse however, the stomach was found to be full of white coat

hair and the mucosal lining was red and appeared inflamed. Whether a hair blockage was the cause of death could not be said for sure. Mice that chased subordinates would be seen at times with their teeth clamped firmly onto the rumps of their victims. Subordinates had thin coats anyway and no doubt lost more hair during these episodes. A quantity of hair could therefore have built up in the stomach resulting in the inflamed lining that was seen.

On these three occasions of the death of a dominant, the area of the room that he had held did not remain unclaimed for long. This top social position was taken on by a subdominant already in residence on one occasion and by two from neighbouring areas on the second and third occasion. These animals may be considered potential dominants given the right opportunity. If the new owner-to-be was an outsider and found little resistance, his expectations of overall victory grew with each defeat he achieved and with the retreat of the resident mice as he approached them. These mice were then either driven out or would live in an uneasy association with their new territory holder.

On each of two occasions, a subordinate became dominant in a vacant area. Although these animals were not physically in poor condition, they had been forced to submit to the dominant they lived with and had put up no resistance to attack. As a result, it was surprising to discover that they were nevertheless capable of gaining and defending a territory area. On each occasion, the areas they took over only contained subordinates so they may have encountered little resistance. What seemed interesting though, was that for both these mice, the status of subordination only seemed relative to a particular dominant animal and within a particular geographical location. Once outside these social and physical confines and given the opportunity, they demonstrated their capability for adopting new

social roles which they successfully maintained. Despite the argument for conditioning proposed by Ginsburg and Allee (1942) it appears that social status defined by fighting experiences was not altogether fixed and rigid among the free range mice.

In another free range set up (Experiment FR8), while the groups were establishing themselves after the barriers were removed, a dominant territory holder surrendered to two subdominants and left the area. After a good deal of fighting, these two mice eventually divided up the area between them, a split which ran almost diagonally across the quarter and gave one mouse the food basket and water bottle and the other, ownership of the nestbox. It was impossible to tell exactly where the boundary lay but it was evidently very clear to the two mice. Neither of them would accommodate the two remaining subordinates in the quarter living with them so these animals spent a good deal of time running back and forth between the two small territories, trying to avoid attack.

These two new territory holders patrolled and fiercely defended their ground and because there was no area of unoccupied land between them, encounters between them were inevitable and frequent fights took place. One result of this aggression was that one mouse lost half his tail: an occurrence which made him easy to identify. These two mice continualy investigated each other's areas, resulting in fights and chases which could lead to displaced aggression towards the subordinates. During one period of observation, an episode that can only be described as "tactical decision-making" was seen involving this same pair of animals. One of them was seen crouching on the roof of the nestbox. A strange mouse from another area had entered the area and was investigating the bricks. The owner appeared to be about to move when the neighbouring territory holder stepped across the boundary and also entered the territory. For two

or three seconds, it appeared as though the resident mouse was weighing up the situation as he looked from one intruder to the other and back again, slowly moving his head. He then made a lunge at his opponent who immediately fled, before he rushed at the outsider who received a bite on the rump before also fleeing and emitting loud shrieks. If mice are capable of finding themselves in situations where the consequences of an action have to be weighed up before a decision on what to do is reached, this appeared to be one of them.

These last events conclude the narrative on the social activities of the all-male groups that lived in the room. The short account that now follows describes some of what was seen during the only experiment that was run where eight adult females were present for the whole of the experimental period that the mice were in the room.

For this experiment (FR10), the number of males placed in each quarter remained the same but each group also contained two females which had been put into each of the males' cages one week before the groups entered the free range and had become pregnant.

The presence of the females did not appear to influence the social arrangements that each group set up although the fighting that occurred during the first few days after the mice all met, never reduced to the low point that was reached when only males lived in the room. This may have been partly due to the lack of restriction placed on the females' movements by the existence of territory boundaries. Unlike the males who were confined largely to particular areas, the females were free to wander about the room as they chose. However as they moved through a territory, they inevitably attracted the attention of the residents who would immediately investigate them, sometimes attempting to mount them and also follow which often resulted in these males arriving in the neighbouring, or adjacent

territory. This behaviour produced many more encounters between members of different groups than was usually seen and data recorded about the aggression that took place, indicate much higher levels compared with all-male groups, (Chapter 5). Despite this, however, the social order did not break down.

The births of the first litters began at about the same time that the barriers were removed. During their pregnancies, several of the females appeared highly aggressive and would even attack a dominant who attempted to mount them. The aggression of the females was particularly evident during the nursing of the young. Two or three females shared a nestbox and presumably, shared the care of the pups also. A male that ventured in would be attacked and driven out. However, one quarter was occupied by a particularly aggressive dominant who continually showed aggression towards the subordinate mice. When he attacked, his victim might enter the nest box for safety only to find himself in the nursery. The dominant would also enter and a fight would take place but because of the rather confined space they were in, it could happen that pups would be found dead or dying after the fighting had ceased. Certainly the nest would be upset and young scattered. Three females nested in this box but due to the high levels of aggression, few of the pups reached the age of weaning at about three weeks.

By this time, the females were pregnant again and due to produce their second litters. Both territory holders and subdominants were seen to copulate with the females so the paternity of the young was unknown. In order to keep the numbers of mice in the room fairly constant in case overcrowding led to a breakdown of social order, the first litters were removed from the room just prior to the second ones being born. This was also done to protect young pups who on venturing out from the nests, became the victims of attacks when

fights took place and they happened to get in the way.

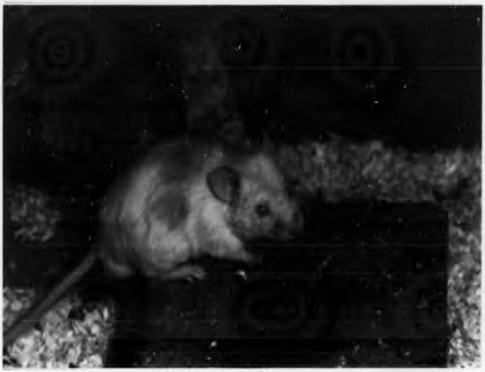
Most of the behaviour that was seen in the free range, whether or not females were present, was undoubtedly influenced by the confines of the room which allowed no emigration and was therefore an unnatural environment if compared with the habitats of wild mice. The free range room was in no way an attempt to reproduce feral conditions. Nevertheless, the big advantage of using this type of housing condition is that it permitted the setting up and defence of discrete areas, so therefore dominants could be regarded as territory holders, a condition which is impossible to achieve in the confines of the laboratory cage. Indeed, observations showed that territoriality could be reproduced under these conditions together with a range of behaviours that were akin to those of more confined, but known habitats of wild mice. Having produced such a system, it was then possible to proceed with a number of physiological measurements and behaviour tests.

The free range room with barriers removed showing territory areas with brick boundaries.

Single territory area with food hopper, water bottle and nest-box housing subordinate mice

Free range subordinate mice with bitten rumps and scarred tails on vertical brick.

Free range subordinate mice with bitten rumps and scarred tails on roof of nestbox



Free range territory holder.

CHAPTER 4

SOCIAL SUBORDINATION AND STRESS RESPONSES

Introduction

The experiments discussed in this chapter tested the prediction that subordinate and subdominant mice would show evidence of prolonged psychosocial stress with continual activation of the pituitary-adrenal system. It was therefore expected that these animals would have raised corticosterone levels together with increased adrenal weights when compared with territory holders and group caged dominants. Previous studies have demonstrated links between stress and other physiological changes such as blood urea levels and kidney damage and between stress and pain thresholds due to the stimulation of endogenous analgesic mechanisms. It was also anticipated therefore, that these animals could show raised urea levels, and also high pain thresholds when given hot-plate tests. It was also thought possible that certain other features of psychosocial stress would be evident. Based on previous work, the prediction here was that subordinates would fall into poor physical condition as shown by coat quality and by the failure of wounds to heal; a number of deaths could result. Longer term effects could include chronic hypertension, arteriosclerosis and renal failure, as has been observed in other studies. The experiments here tested a number of these factors although not all of them, for example, "condition" were measureable. Tests for hypertension, arteriosclerosis and renal failure were not pursued, these effects being longer term than the duration of these experiments.

The adrenal cortex has long been known to play an important part in the organism's adaptation to excessive demands by the environment but as well as this, both medulla and cortex are vital in maintaining homeostasis. Three classes of steroid hormones are influential in

this: the mineralocorticoids, the adrenal sex hormones and the glucocorticoids. It is the latter group that as well as performing a number of regulatory functions in the body, are also a vital part of the stress response. Through their own plasma concentrations, levels of glucocorticoids are controlled by a negative feedback loop involving the release of corticotrophin releasing factor (CRF) from the hypothalamus and adrenocorticotrophin (ACTH) from the anterior lobe of the pituitary. This loop controls circulating basal levels but may be overriden during prolonged stress, by means of a system which is complex and not detailed here. A point to note though, is that the system is sensitive both to the rate of increase of free steroid in plasma as well as to its absolute levels (Jones et al. 1972).

As the work of this thesis was not exclusively concerned with stress and stress effects, a complete over-view of research in this area is considered to be inappropriate here. Rather therefore, than embarking on a full discussion of the theoretical concepts of this topic and the functions of the response, the discussion in this chapter is focused on social stress as a major stimulus to the stress response and in addition, the accompanying physiological changes and disorders that are known to be associated.

The core of the stress response is adrenal activation which consists of two phases: adrenomedullary involvement mediated by the sympathetic nervous system (SNS), and activation of the adrenal cortex mediated by ACTH. The former system invokes the "fight-flight" response which is stimulated by threats from predators, challenges by rivals for access to food, water, shelter, mates etc. and is associated with raised catecholamine output resulting in increased alertness and action-readiness (Henry, 1983). Canon (1929) first showed that hormones of the medulla (the catecholamines, epinephrine

and norepinephrine) cause a rapid response to sudden emergency situations. Later, it was demonstrated that this arousal response was not limited solely to extreme situations but could be activated in response to signals which indicated only potential risk of damage. By using complex population cages to induce chronic psychosocial stress in CBA strain mice, Henry et al. (1971,1977) have shown a doubling to tripling of levels of tyrosine hydroxylase (TYR-OH), the rate-limiting enzyme in the synthesis of norepinephrine, in dominant male CBA mice when their social position is being established or is under threat.

Activation of the medulla is succeeded by the responsive rise of the glucocorticoids. In accord with Selye's General Adaptation Syndrome (1950), these raised levels enhance defence against stress although more recent ideas have expressed doubt on this point as it is not clear why a high corticosterone response should help an animal to defend itself against specific stressors. Under conditions of chronic stress, the system is thought to be activated by a state of helplessness as perceived by the individual and so leads to defensive behaviour and submission, together with a decrease in aggressiveness. Experimental studies have shown that the increased activity of the pituitary-adrenal axis is also associated with enhanced learning of new patterns and the more ready abandonment of old ones (de Wied et al. 1972). As a consequence, a stressed individual may adapt its behaviour and is tolerated within a group rather than being expelled, a mechanism which is vital for a social animal which depends on group effort. Under laboratory conditions, the increase in corticosterone levels is very swift and pronounced in rats that are prevented from coping behaviourally with a fear-provoking situation, a response that is slower in animals that are allowed to cope (Bohus 1975).

The concept of a dual neuroendocrine response to stress is

strongly supported by the work of Henry 1983, Henry and Stephens, (1977) and von Holst (1985) among others. In addition, Henry et al. (1967,1971) have demonstrated that different types of social interaction lead to varying increases in both adrenocortical output and sympathetic activity in adult mice which are associated with a variety of clinical disorders such as hypertension, the accelerated development of arteriosclerosis, degeneration of coronary vessels together with fibrosis and also severe renal tubular damage, a finding which has been substantiated by the work of von Holst (1972) in tree shrews. Henry et al. have argued that the results of their work using population cages, are not related to density of numbers alone as the experimental animals were provided with more space than caged control groups. Instead, they have put forward the view that the critical element is probably the perception that the animals have of one another. Their mice were singly housed for a number of weeks prior to entry to the population cages and they have argued that if prior social experience is vital for providing opportunities to develop adequate coping mechanisms, then the inadequate social experiences of these animals early in life make it impossible for them to develop a stable hierarchy and cope effectively with the pressure from social demands as adults within the colony.

Henry and Stephens (1977,1985) have also provided evidence to show that under certain conditions, the adrenal medullary and adrenocortical responses are correlated with behavioural patterns that move in different directions. For instance, challenges to expectancy result in sympathetic adrenal medullary arousal with accompanying irritable aggressive behaviour. The blood norepinephrine to epinephrine ratio increases as part of the raised catecholamine response. These authors have suggested that such events may be associated with depressed gonadotrophic activity together with a

vulnerability towards disfunction of the alimentary and cardiovascular systems.

A major difficulty that arises in studies of this type, particularly when the stress is chronic, is in determining how much of the observed response is due to the purely physical nature of the stressor, such as pain from being bitten, and how influential is the psychological component. Anticipation of a stressful event has been found to be one of the most effective inducements of psychoendocrine response (Sandman et al. 1973). Using the stressor of chair restraint in a number of studies, Mason has shown that the hormones he examined (glucocorticoids, epinephrine, norepinephrine and thyroxine) showed very similar response patterns to those found during conditioned avoidance response tests, using rhesus monkeys (Mason 1968, 1972, 1974, Mason and Mougey 1972, Mason et al. 1973). As a consequence, more recent work has attempted to separate purely physical stimuli from those of a psychological nature (reviewed in Mason, 1975b). Again using rhesus monkeys, hormonal profiles were determined in response to physical stressors such as restricted food, exposure to heat and cold, and dietary changes. Careful attempts were made to minimize psychological stress. Techniques such as the feeding of non-nutritive, fruit flavoured cellulose pellets were employed together with avoidance of sudden or severe temperature changes. The response profiles obtained differed sharply from those data gathered from the psychologically stressful conditioned-avoidance and chair restraint studies. Mason has argued that hormonal profiles offer powerful evidence that integrated endocrine responses are specific to the physical demands imposed by the environment.

The idea of specificity contrasts with earlier thinking that response to stress, particularly that of the pituitary-adrenal system, was non-specific in character. This was originally deduced

from observations that the pituitary-adrenal axis could be stimulated by a great variety of stressors such as shock, haemorrhage, cold, heat etc. However, such arousal-orientated models of endocrine response are now considered as over simplistic. Studies such as those by Mason which examine multiple response patterns provide evidence that hormonal profiles are integrated and relatively specific to differing types of noxious stimuli. As a consequence, current questions employ a line of enquiry which consider whether endocrine systems exhibit a pattern of response that is specific to the event. Both the endocrine system as well as the autonomic system may exhibit stereotypy relative to a particular situation (Lacey 1967) with differing events evoking distinctive patterns of integrated hormonal responses, an idea supported by Henry (op cit.) as being relevant within the context of the social environment also. Veith-Flanigan and Sandman (1985) have proposed that these different events resulting in varying hormonal response patterns, serve an adaptive function not only by preparing the individual's metabolism for the anticipated event, but by enabling that individual to display adaptive patterns of behaviour as a function of selective action on central and peripheral neural processing.

The importance of psychological stimuli as activators of the stress response has been demonstrated by many workers (eg: Mason 1968, Levine, Goldman and Coover 1972). Under conditions of constant social stimulation, the psychological effects of being attacked or the constant presence of an attacker may be classified as social stressors. However, animals do show the ability to adapt and cope with adverse situations through behaviour modifications that are vital to the stress response. Under natural conditions, where continual interaction with the environment takes place, animals need to maintain the integrity and equilibrium of many forms of internal

regulatory processes, even when extreme circumstances threaten to upset normal functioning (Smelik 1985). Aspects of the studies by Christian (1955, 1970), Christian and Davis (1955,1956) and Christian, Lloyd and Davis (1965) focused on the ways in which the control of numbers in populations of mice, was dependent upon an increase and intensification of aggression and defeat among individuals. Also the ways in which the social stress that this produced was intimately linked to an increase in the activity of the adrenal cortex together with an overall weight gain by the adrenal glands, particularly among defeated mice.

Similar effects of social stress have been found in other rodent species: Clarke (1953) described enlargement of the adrenal glands resulting from fighting in Microtus agrestis and Louch (1956) found evidence of increased adrenocortical activity in dense populations of Microtus pennsylvanicus. Rats subjected to attack by conspecifics showed extreme depletion of lipid from the zona fasciculata of the cortex (Barnett 1958) which indicated high glucocorticoid output. More recently, the sensitivity of the pituitary-adrenal axis to social stress has been demonstrated in studies on tree shrews (von Holst 1972b,c, 1985, von Holst and Buergel-Goodwin 1975, Raab et al. 1982) and on deermice and lemmings (Andrews and Belknap 1979). The severe effects of such stress on these animals of low social status, demonstrate the importance of social rank and aggression in relation to adrenal stimulation.

It has been known for a number of years that forms of behaviour such as aggressive, submissive and defensive responses in competitive situations are affected by ACTH (Scott and Frederickson 1951), and that an individual's position within a social group as well as conflict within the hierarchy, influence pituitary-adrenal function (Brain 1972a). Brain et al. (1971) have shown in addition, that

administration of $ACTH_{1-24}$ reduces isolation-induced aggression, a result that was not achieved when $ACTH_{4-10}$ was given (Brain 1972b). The effect would appear to be extra-adrenal as the influence of ACTH on agonistic behaviour is effective in adrenalectomised rats and mice maintained on corticosterone and is independent of the testes (Leshner et al. 1973, Leshner and Politch, 1979, Leshner 1983). The reduction in aggression through the influence of ACTH has also been demonstrated in sham-operated as well as adrenalectomised mice: these animals were subordinate in an isolation-induced aggression context (Poole and Brain 1974).

Observations on the effects of the adrenocorticoids are also extensive. In mice, isolation aggression is either attenuated (Brain et al. 1971, Leshner et al. 1973) or takes longer to develop (Sigg et al. 1966) in the absence of the adrenals. Burge and Edwards (1971) however, found no difference in the aggressiveness of isolated adrenalectomised mice. This may be due to the use of different methods to measure aggression and to different strains of mice. Differential plasma corticosterone responses to stress have certainly been found in different strains of mice (Levine and Treiman 1964), and the evidence also indicates that the behavioural response and effectiveness of hormone treatment are also strain-dependent (Levine and Levin 1970).

Results from a number of experiments show that the site of action of corticosteroids is localised to some extent in the same brain areas as that of the ACTH fragments. The ACTH receptors are present in the septal area, the hippocampus, amygdala, anterior hypothalamus, medial thalamus and in the mesencephalic reticular formation (Bohus 1970,1973, Endroczi 1972). Putative receptors for corticosteroids have in particular, been located in the hippocampus (McEwen et al. 1969, 1975) and these receptor sites may provide a

molecular basis for the behavioural effects of the corticosteroids.

In his original theory, Christian proposed that overstimulation of the adrenal glands would result in a high death rate among crowded populations, this being the equivalent of the exhaustion phase of Selye's General Adaptation Syndrome. This may certainly occur in some cases but the instances of death may not take place sufficiently often for them to play a vital role in controlling population numbers. Indeed, the reports of Calhoun (1962) that hyperaggressiveness played a substantial role in the death rate of his penned rats was not well substantiated with quantification, nor have the results been replicated by other workers (Lobb and McCain 1978). More recently, the theory has been modified (Christian 1970,1971) so as to emphasise the more subtle effects of crowding such as decreased growth rates, increased susceptibility to disease and decreased reproduction (Lloyd 1973, Christian, Lloyd and Davis 1965, Lidicker 1976). However criticism of the idea that crowding per se is responsible for these effects has come from Henry (1985) and Snyder (1975) who have argued that self-perception within a particular social context is more influential in determining both the behavioural and physiological response that results. Captivity does not necessarily involve crowding and when it does, the results may not be negative or stressful as a consequence. Ad libitum feeding of captive rodents does not produce overcrowding if natural spacing mechanisms, such as burrowing, are permitted (Boice 1977). Wilson (1978) has warned about the pitfalls in forming conclusions based on studies of feral populations about density-related aggressiveness and stress, when there may in fact be nothing unnatural about this form of aggression when there are shortages in resources.

Despite this, from laboratory studies on rodents, most of the evidence supports the view that social pressure in the form of

fighting plays a significant role in the physiological changes that accompany social stress. In experiments using controlled fights, mice subjected to defeats by trained "fighter" mice show an increase in adrenal weight (Bronson and Eleftheriou 1964) and an increase in plasma corticosterone levels (Bronson and Eleftheriou 1965a). These authors have also obtained evidence to show that the psychological effects of defeat are as powerful as the physical effects of attack (Bronson and Eleftheriou 1965b) given a background of defeat, in raising levels of corticosterone in the mouse. In addition, previously defeated mice will show large increases in these levels when placed in close proximity to the trained fighter even though no physical contact takes place between them. This result suggests that the psychological response of fear to a dominant, aggressive animal heightens the purely physical response for the defeated animal and so would greatly accentuate the adrenal response to grouping in such mice where fighting takes place.

In many laboratory studies, the existence of social stress has largely been due to the inability of subordinate animals to emigrate away from aggressive dominants and the oppressive confines of a group and instead, these animals have been forced to stay together and suffer physical attack. These very artificial environments can in no way be said to reflect feral conditions and Christian (1970) has argued that in the context of the wild state at least, social competition pinpoints in particular the role of the subordinate animal in natural selection and evolution. Due to the great numerical preponderance of subordinate migrants over dominant core residents, provision of raw material for formation of new groups by way of natural selection comes from the occasional subordinate migrant that survives in a new habitat.

The hormonal factors influencing subordination appear to differ

from those involved in promoting and sustaining dominance status. The raised output of adrenal hormones during stress may influence the behaviours which lead to social subordination such as avoidance of attack, submission, avoidance learning and resistance to extinction. Leshner (1981,1983) has argued that the most likely response of a subordinate animal to attack is that of flight which of course, is not possible in the cage. As a consequence of this, these animals produce responses which are "appeasement" gestures (Nock and Leshner 1976) to terminate attack (Eibl-Eibesfeldt 1975, Wilson 1975). The only factor that can cause submissiveness is subjection to defeat and injury and Leshner argues that virtually no animal ever submits without this prior experience. Submissive postures may be innate but an animal needs to learn when to produce them and the context and cues that demand this behaviour. As the proportion of defeats following attack builds up, so submission occurs more and more readily. Submissiveness though is not the opposite of aggressiveness as non-aggressive animals may not be particularly submissive and these two traits are dissociable. Using physiological manipulations, separation of the two has been carried out by modifying neural states (Lau and Miczek 1977) and the endocrine characteristics of the individual (Leshner and Moyer 1975, Leshner and Politch 1979, Maruniak et al. 1977).

Although the hormonal state itself will not cause submissive behaviour, it may determine how readily submission occurs following attack and defeat. Leshner (1980) has demonstrated the role of the pituitary-adrenal system in terms of its effects on submissiveness in a number of experiments that have investigated the roles of both ACTH and corticosterone (Leshner and Politch 1979). The castration of mice, although reducing aggressiveness, has no influence on the readiness to surrender following attack by an opponent. However,

increasing the levels of ACTH and corticosterone does. Although ACTH treatment raises the level of submissiveness in intact animals, this is only the case if the animals can respond with an increase in corticosterone also. Mice that have had their levels of corticosterone controlled by fixed dose levels of steroid following adrenalectomy, are unaffected by ACTH treatment. However, increasing the levels of corticosterone leads to increased submissiveness. These results therefore suggest that corticosterone is the crucial hormone in the control of surrender rather than ACTH, and the androgens have little or no role to play (Moyer and Leshner 1976, Leshner, Moyer and Walker 1975). Although many of these results appear conclusive, there is no supporting evidence for Leshner's ideas by other workers and studies have only been carried out on mice so to place too much emphasis on the data would be mistaken.

Mention was made in the earlier part of this discussion of the studies of social relationships within confined groups of animals. More recent work has coupled behavioural investigation with the study of physiological changes associated with group living. Because of the relevance of these various approaches to the predictions made at the beginning of the chapter, a number of these experiments are now discussed in greater detail. Much of this work, using a variety of species, has been carried out on both stable and unstable groups where among males, the status of dominance and subordination may not be fixed and constant over time. Data from population cage studies on mice show that dominant territory holders that co-habit with females may, after a period, eventually be overthrown by younger males from litters that under feral conditions would have been forced to leave the group (Reimer and Petras 1967). The design of the Reimer-Petras population cage, consisting of a series of nest boxes with interconnecting tube runways, was an advancement on the earlier

territory studies where cages proved too small for adequate development of social behaviour or pens were too large to permit efficient monitoring of activity. Their nest boxes contained a good supply of food and nesting materials as well as water. A gate at the approach to each nest box could be dropped and locked, trapping the animals inside to facilitate counting and other measurements. Ten male and twenty four female mice were released into the population cage in each experiment, remaining there for a period of 250 days. An initial period of intense exploration was followed over the ensuing two days by considerable fighting after which time, it decreased and the system stabilised. These workers found that certain territories, established in the first two weeks of an experiment, remained unchanged up to eight months later. Louch and Higginbotham (1967) showed that in short term, stable social hierarchies, the corticosterone levels in the plasma of dominant mice was little changed from single housed, control, unstresssed animals while those of subordinates were almost twice this value. In this experiment, CFW male mice were isolated from weaning before being placed together in groups of four. Levels of corticosterone were determined after six and twenty four hours in the second housing condition and were reflective of an acute stress response to fighting and defeat.

The Reimer-Petras type of population cage has also been used successfully by Henry and co-workers (eg: Henry and Stephens 1977) in many experiments on CBA mice. Their basic design consisted of six "shoe box" laboratory cages interconnected with plastic tubes to form a circle. From each box, a separate tube connected to a central hexagon containing food and water. This system could be further enlarged by the addition of six additional boxes to the original six by more plastic tubes. Again, like the Reimer-Petras design, this set-up facilitated counting and other measurements as well as

providing easy access for maintenance. As a consequence, many of the studies by these workers have been relatively long term, often lasting several months (eg: Henry et al. 1982). Mice were able to set up social groups with one or more territories being defended by one or more dominant males. Again, males were trapped in individual cages with the use of gates to permit the investigator to identify individuals (Ely et al. 1972, Ely and Henry 1974). In a more recent study, Ely and Henry (1978) have confirmed the findings of Louch and Higginbotham (1967) using this type of population cage. Subordinate mice, blood sampled immediately after three hours of immobilisation, showed a significant increase in corticosterone as compared to that of dominant animals. In the same experiment it was also shown that dominant mice respond to social interaction with predominantly sympathetic adrenal-medullary activity and in response to ACTH treatment, produce a lower corticosterone output compared with subordinates. However, these patterns were reversed when the social status was reversed.

Under different conditions, where social aggression has been used to influence the state of the adrenals, weights of these organs were not only higher in defeated mice compared with controls (Archer 1970), but it was found that short bouts of defeat (five minutes of daily fighting over a seven day period), was sufficient to bring about an increase of weight, indicative of the sensitivity of these glands. In Archer's experiment, isolated "fighter" mice that experienced daily victory showed no evidence of increased adrenocortical activity. In another study (Brain 1972a), male TT mice were housed in pairs for seventeen days and were then identified for status based on the degree of wounding incurred, and on behaviour. Each mouse was then placed in an aggression test for five minutes with a "standard opponent" (Brain 1981). On the following day, the

mice were killed and weights of bodies and organs recorded. Although there were no differences between dominants and subordinates on behaviour measures, subordinate mice were characterised by adrenal weights that were significantly higher compared with dominants.

Adrenal activity as measured by corticosterone levels has been shown to be influenced by housing conditions (Benton et al. 1978). Single housed and dominant mice from group cages exhibit lower levels of this activity than do subordinates. Brain (1972d) has also shown that the use of single cage housing results not only in low adrenal weight and output in mice, but there is also an increase in gonadal function. Male hamsters in the same study showed the reverse of this, probably because in this species, testosterone stimulates adrenal function. However, Stern et al. (1960) and Hatch et al. (1963) suggest that differential housing has varying influences on relative adrenal weights, at least in male rats. Dessi-Fulgheri and Lupo di Prisco (1974) have demonstrated increases in adrenal weight in "isolated" female but not male rats. It may be that given the opportunity under more natural conditions, the mouse being a territorial animal may be more accepting of isolation than the hamster which tends to be of a more colonial nature. However, rats whch are also considered to be colonial, appear to be adaptable to single housing without excessive adrenal stimulation. Adrenal activation in mice is known to be responsive to the time spent in this latter form of housing (Goldsmith et al. 1976) and where this type of caged condition is used, it would appear not to be the sole influence on adrenal physiology and possibly status. Indeed, the importance of time length as a separate independent variable cannot be ignored.

Other evidence to support findings that the physiology of dominant and subordinate animals differs under conditions of stress

comes from laboratory studies on tree shrews carried out by von Holst. This animal, which a number of researchers regard as a primitive primate, demonstrates high levels of territoriality (von Holst 1974) and fighting among males has been shown to result in the subjugation of one animal by another after which, the victor shows no further sign of arousal and pays no further attention to the defeated animal. In contrast, the loser is seen to creep away to a hiding place where it remains crouched and still, emerging only to eat and drink.

In a number of experiments, tree shrews of the same sex were paired together in cages for up to twenty-three days (von Holst 1985). Both males and females immediately attacked intruders of the same sex and were seen to defeat them within a few minutes. Victors then paid these animals no further attention. However these defeated tree shrews demonstrated behaviours which divided them into two groups: subordinates which were totally submissive and were seen to enter a state of helplessness as described above, and subdominants which appeared to adapt and cope with their situation. These animals, in contrast to the subordinates, defended themselves if attacked or attempted to avoid possible confrontation by fleeing or giving way, thus showing a great increase in locomotor activity compared with the amount displayed by subordinates. Glucocorticoid levels in these subdominants followed a pattern similar to that of the dominant animals, being raised in the early one to three days of confrontation but then returning to initial values which were maintained from then on, even in situations where fighting took place daily. By contrast, levels in subordinates rose and remained 300% higher than baseline levels. Body weights of dominants were reduced during the first few days of confrontation but then increased steadily, eventually overtaking their initial values. Weights for subdominants remained

fairly constant but for subordinates, a severe drop in the first two days was followed by continual decline until the end of the experiment on day-twenty three.

Rats have also been used to produce a model of social stress by being placed in small groups or colonies (Dijkstra et al. 1984, 1985). In these experiments, a number of subordinates and a dominant rat lived together in an area measuring 2 x 3m, with or without females present and the layout provided nestboxes for retreat. Periodically, strange males were placed in the arena. From a variety of hormones measured, it was shown that an acute stress response occurred due to the presence of strangers which produced rises in corticosterone in all social groups of animals when compared with controls. Resting levels however, showed no differences between social groups although surprisingly, at post mortem, both dominants and subordinates exhibited raised adrenal weights together with lowered thymus weights when compared with control animals.

Other evidence that dominant animals may have raised basal cortisol titres as high as those for subordinates during social unrest or group formation, comes from studies on mixed-sex groups of talapoin monkeys (Keverne et al. 1982). However, after social patterns were established, dominant individuals, in contrast with the rat study, were found to possess the lowest basal titres of corticosteroids. Taken together, the studies cited above as well as those of Dijkstra et al. (1984) and Keverne et al. (1982) suggest that elevated glucocorticoid levels are not only related to social subordination in a group, and indeed, subordination may not necessarily be stressful, but are responses associated with aggression during group formation or during social disturbance. In other studies on primates, elevations of basal cortisol levels have also typically been seen in individuals undergoing sustained stress

of a psychogenic nature (Rose and Sachar 1982, Sapolsky 1983).

In a group context, where animals undergo social pressure, the indication is that the interaction of the environment with hormonal changes is subtle and complex. ACTH is able to stimulate epinephrine production independently of the nervous control mechanism (Henry and Meehan 1981, Henry 1983) and these workers have also shown that instead of showing a "fight or flight" response when confronted with aggression, subordinate mice instead appear to "despair" and submit to the situation. This form of behaviour where an animal appears unable to cope, has been examined in a number of studies. The phenomenon has been termed "learned helplessness" for which a theoretical model has been proposed by Maier and Seligman (1976). This effect has been demonstrated in a number of species including humans (Hiroto 1974), mice (Braud et al. 1969) and rats (Maier et al. 1973, Seligman et al. 1975). Learned helplessness may be an analogue of clinical depression and according to Seligman, depression occurs when individuals learn that they are helpless and cannot control or cope with events in their lives. In general, the term has been used to designate instances in which a behavioural effect is produced by the uncontrollability of the events experienced by the animal rather than the events per se. According to Seligman and Beagley (1975), there are three characteristic types of behaviour at least in the dog which, they argue, should serve as criteria for determining learned helplessness. These are failure to escape shock, failure to escape shock on future trials even when a response occurs and terminates shock, and that these two criteria are produced by inescapable but not equivalent escapable shock. Brown and Dixon (1983) have found that the behaviour of gerbils, given inescapable shock automatically every thirty seconds, met these criteria. It is probable that the mice in Henry's population cages were reduced to a state of

"helplessness" although an alternative explanation is also possible. Glazer and Weiss (1976) have stated that an animal becomes inactive during inescapable shock and that this inactivity continues until shock termination. Animals, such as the gerbils in the above study , when they fail to escape, freeze in a crouching position, remaining in that posture until the shock or threat is removed after which activity is resumed.

Support for this idea comes from von Holst's work with tree shrews. Following defeat, the loser hides and becomes almost totally inactive, emerging only to eat and drink. Further fights are rare or non-existent yet the subordinate remains seemingly "helpless" and may eventually die within twenty days (von Holst 1974, 1977). However, if the tree shrews are separated by a wooden partition following fighting and defeat, the loser recovers and even if subjected to fighting every day for several weeks, it loses little body weight and does not die prematurely (von Holst 1972a). In contrast, if the animals are separated after the first fight by only a wire mesh partition so that the loser cannot be attacked, but has a clear and permanent view of the victor, it again dies within a number of days.

The experiments of von Holst (1972a,b,c,) indicate that death is not caused directly by aggressive social interaction and the resultant physical consequences but instead, is because of CNS processes taking place in the subordinates. Based on the experience of defeat and in learning to recognise the victor, these tree shrews enter a state of learned helplessness which is shortly followed by death, a series of events which have also been observed in mice in the Henry-Stephens population cages (Henry et al. 1967, 1975) and by Barnett with wild rats where the design of his enclosure made privacy and territory formation difficult for an individual so leading to social disorder with high levels of aggression. Barnett (1958) has

suggested that this intense aggression particularly in the presence of females, may be comparable to a displacement response. Behaviour such as aggression can result when attempts at other acts, such as coitus are frustrated, although in itself, fighting may be inappropriate within that situation.

Early work (von Holst 1972a) showed that cause of death in these subordinate animals was due to renal failure, believed to be due to the constant exposure to social stress. Post mortems revealed that the kidneys of these animals were 17% lighter compared with controls. The urea content of blood was raised and haemoglobin was lowered proportionately. Death could occur after only forty-eight hours if the blood urea levels were above 180ng/ml. Kidney failure was believed to be a result of a decrease in renal blood flow following vasoconstriction due to high sympathetic nervous activity in the renal nerves.

Kidney pathology caused by social stress has also been reported by Henry and Ely (1980) and in addition, the subordinate mice from population cages show a behavioural inhibition to urinate with the consequence that they are found to have gross bladder distention at post mortem (Henry et al. 1982) resulting in interstitial nephritis. Although these workers have shown that there is little evidence of vascular involvement in this kidney problem, nevertheless, these animals also have high blood urea levels probably due to urinary reflux.

The final subject to be discussed in this section is that of pain thresholds. The predictions made at the start of this chapter stated that subordinate mice, particularly those housed in the free range, would show various responses to social stress. Raised pain threshold levels were anticipated particularly in this group of animals and also for group housed subordinates when compared with

mice of high status and those housed in single cages.

This perhaps unexpected prediction is drawn from the body of literature which has been built up on "stress-induced analgesia". Developed primarily in the late 1970's, this area of research is concerned with pituitary-adrenal activation and the interaction between ACTH and opioid peptides. The principle findings to emerge from experiments, repeatedly confirmed at least in rodents, is that stressed animals show a marked increase in pain thresholds. Although the main body of work has focused on the effects of electric shock, a number of other stressors have been tested. Test animals show increased latencies to respond to a heat source such as a hot-plate or to a lamp in a tail-flick test. In the light of findings by Miczek et al. (1982) and Rodgers and Hendrie (1983) this phenomenon was considered relevant to the present study. Earlier work (Miczek et al. 1982) showed that the stress of being defeated was important in the induction of SIA and that it was the experience of defeat rather than injury from fighting which was the central component. The work of Rodgers and Hendrie (1983) went on to show that SIA could be linked to whether an animal was of dominant or subordinate status.

The role of ACTH and certain opioid peptides is believed to be as follows. ACTH and β-endorphin are secreted concomittantly by the pituitary gland in response to acute stress by activation of central noradrenergic pathways (Szara 1982). The precursor molecule, pro-opiomelanocortin is, however, present in both the intermediate lobe of the pituitary and the hypothalamus. The peptides are also released long-term after adrenalectomy, an effect which is blocked by dexamethazone (Guillemin et al. 1977). Analgesia controlled via the endorphin/enkephalin systems may be influenced by corticosterone levels. These in turn, are affected by either dexamethazone or metyrapone (Mousa et al. 1981). It can therefore be predicted that

measures to increase or decrease levels of plasma ACTH should result in similar increases or decreases in plasma β-endorphin levels. It has been argued by Bolles and Fanselow (1980) that a stress-activated, endogenous analgesic mechanism could be adaptive in situations in which the perception of pain might otherwise disrupt effective behavioural performance, for instance, in defensive behaviour.

In a study of opioid-like analgesia in defeated mice, animals exposed to repeated attacks by other mice showed decreased nociception in response to radiant heat focused on their tails (Miczek et al. 1982). This form of analgesia was blocked by centrally acting opiate antagonists, naloxone and naltrexone. These authors and others (Mandenoff et al. 1982) suggest that endogenous, opioid-mediated analgesic mechanisms are readily activated by situations involving "biologically significant" forms of stress, such as defeat. Following this, Rodgers and Hendrie (1983) showed that not only does social conflict activate these endogenous analgesic parameters, but as stated above, they may in fact be status dependent. Again using mice, their study shows that potent naloxone-reversible analgesia only occurred in intruder mice that were involved in agonistic behaviour whereas among the residents of a group, there was only a moderate reaction to aggression. For the intruder at any rate, it would seem that conflict may activate pain control mechanisms through the release of these endogenous opioids.

It has also been demonstrated that stress-induced analgesia has two forms: long and short term. The long-term is opioid-mediated whereas the short term is not. The long-term form has been associated with the theory proposed by Maier and Seligman (1976) concerning learned helplessness which was discussed earlier. The work of von Holst (op cit) has shown that subordinate animals accept

a state of helplessness and apparently "give up" and on the basis of this finding, it was anticipated that the subordinates in the current study due to chronic stress which could lead to a similar helpless state, would therefore have raised pain thresholds when given hot-plate tests. The experiments sought to test the idea that status as well as conflict was influential in stimulating endogenous analgesic mechanisms.

The work on stress-induced analgesia has further demonstrated that the consequences of chronic stress are far-reaching. In addition, there are many stress related pathologies which are well-known and diverse including gastro-intestinal ulcers, chronic hypertension and heart disease.

Summary of Experiments

Experiments are reported in the following order: FR3, FR8, FR5.

FR3: Peak levels of corticosterone measured in caged and free range mice at ten, twelve, thirteen and eighteen weeks of age. Significant differences found within groups. Measures of body weight showed differences between caged and free range mice.

FR8: Trough levels of corticosterone measured four times in caged and free range mice at the same age weeks as for FR3. No significant differences found.

FR5: Three separate tests carried out:-

a) Measures of body weight in caged and free range mice at ten, thirteen and seventeen weeks of age. Results showed significant within-group changes over time.

b) Blood urea levels measured in caged and free range mice at seventeen weeks. Levels raised in all social groups of free range mice compared with other groups.

c) Pain threshold levels measured at fifteen weeks of age in caged and free range mice. Results show levels raised in caged subordinate mice only.

Methods

1. **Blood Sampling**

The procedure for this employed retro-orbital puncture and is fully described in Chapter 2. Plasma corticosterone levels obtained by this method do not differ from those obtained by decapitation (Nichols 1980). In order to obtain true resting values of plasma corticosterone, only as many animals as could be sampled within three minutes of disturbance of a cage or the free range room, were sampled at one time (after Levine and Treiman 1969) so that on some occasions, sampling was carried out over a number of days.

2. **Plasma Corticosterone Levels**

Total plasma corticc.terone levels were determined using a modified radioimmunossay procedure developed by Nichols (1980) and based on the method of Gross et al. (1972), so that both plasma samples and standards underwent extraction procedure. Samples of plasma and standards in borate buffer 1% BSA were washed with 2,2,4 trimethylpentane to remove progesterone, before extraction into 1ml of ethyl acetate. Aliquots were removed and dried down under nitrogen. Samples were redissolved then assayed in duplicate by the addition of 0.7ml of binding solution to each tube. This comprised antiserum (rabbit anticorticosterone-21-thyroglobulin, Miles-Yeda, received in freeze-dried form) and 1μCi ^{3}H-corticosterone made up to 70mls with borate buffer containing 1% BSA. Samples were then incubated overnight at 4^{o}C. Free and bound phases were separated by adding dextran coated charcoal in borate buffer followed by centrifugation. 0.5ml aliquots of supernatant were pipetted into 5mls. of Lumagel in liquid scintillation minivials. Tubes were counted for ten minutes on a Packard Tricarb 300 Scintillation Counter. Counting error was less than 2% and counting efficiency was 31-34%. Counts were converted to mass (ng) by computer using a

logit-log transformation of Rodbard et al. (1970). Printout gave details of the slope, intercept, correlation coefficient and test of linearity of the standard curve.

The antiserum used interacts significantly (>20%) only with progesterone and deoxycorticosterone but not with cortisol, testosterone or any other major steroid. Progesterone levels are not only low in male mice but this steroid is removed by washing. Deoxycorticosterone does not occur in significant quantities in mouse plasma (Gross et al. 1972) so that the antiserum has good specificity for corticosterone in this species. Recovery of corticosterone added to mouse plasma in known concentrations averaged 88.12% for fifteen assays over a period of sixteen months. Intra- and inter-assay variations were 8.6% and 13.0% respectively. The relationship between the amount of corticosterone added to a plasma pool to the amount estimated was linear over 20-200 ng/ml. The least detectable concentration was less than 5ng/ml and sensitivity was 50pg.

3. **Animal Weights**

Body weights of the mice were recorded using an electronic top-pan animal balance (Mettler PI200) which had a delay time device of 0.6sec.

4. **Blood Urea levels**

Estimates of levels of blood urea were determined using Azostix One-Minute Test for urea in whole blood (Ames Division, Miles Laboratories Ltd). A drop of fresh blood was placed on the reagent area of a test strip and then washed off under running tap water after sixty seconds. The strip was then matched with a colour chart and the result read off within one to two seconds after washing. Values associated with each colour band were expressed as blood urea in nmol/l but as these were only approximate, the result for each test strip was point scored on a basis of 1-7 based on the values

determined for each reading from the chart as follows:-

nmol/l	3.3 ± 2.1	5.4 ± 2.1	7.5 ± 2.1	10.85 ± 3.3	14.2 ± 3.3	17.9 ± 3.7	21.6 ± 3.7
Point score	1	2	3	4	5	6	7

5. **Pain Thresholds**

These were assessed by using a hot-plate analgesia meter supplied by Technilab Instruments' Inc., New Jersey, U.S.A. This consisted of a platform ($28cm^2$) which could be heated to specific temperatures, with removable clear perspex walls (15.3cm high) and a cover to contain the animal. A timing device that could be operated manually or using a foot pedal was incorporated into the apparatus. The temperature was set at 51^0C and stabilised at 50.4 ± 0.5^0C (after Creighton 1985). An animal was placed on the platform and the latencies to the first and second licking of the paws was recorded. Each animal was tested three times with an interval of twenty minutes between each test. If within sixty seconds of a test beginning, an animal did not produce the required response, it was removed from the apparatus.

6. **Post Mortems**

The left adrenal gland was removed from subjects following cervical dislocation and full details of this are given in Chapter 2.

7. **Statistical Analysis**

Measures of body weight and plasma corticosterone levels together with the data from pain threshold tests, were analysed with parametric statistics; analysis of variance, Student's t-tests and Tukey's Multiple Range Test. Adrenal weights were recorded as a

percentage of total body weight. These data were arcsine transformed and then analysed by parametric tests of difference. All these data are expressed in terms of means and standard errors.

Blood urea scores were analysed using non-parametric statistics; Kruskal-Wallis Analysis of Variance and Mann-Whitney-U tests.

EXPERIMENTS

Experiment FR3 The aim of the tests in this experiment was to examine basal levels of plasma corticosterone at the circadian peak, four times over a period of eight weeks in mice housed both in cages and in the free range room. Levels have been recorded as reaching 98.67 ± 13.5ng/ml at peak time (Creighton, 1985). Blood samples were taken at the same time of day on each occasion beginning two hours prior to red lights on at 13.00 hours. Levels of corticosterone appear to reach their circadian peak approximately two hours prior to onset of this dark period (Nichols 1980). On each occasion, two days after sampling was completed, animals were weighed. After a final set of weights was obtained at twenty weeks, animals were sacrificed and the left adrenal was removed.

The animals used in these tests, together with the age at each sampling time, were as follows:-

1st sampling - age 10 weeks Blood samples were taken from seventy -two mice that were housed in twelve groups of five and two groups of six in large plastic cages. After being weighed two days later, the two groups of six mice were singly housed in small cages, four cages of five were divided between the four quarters of the free range room (metal barriers in place) and the final forty mice were placed in their existing groups of five in clean cages with fresh bedding.

Between weeks eleven and twelve, daily observations were made on the mice in the free range and in the cages in order to determine which was the dominant animal in each group. Details of the method

of these observations are given in Chapter 2.

2nd Sampling - age 12 weeks After the animals were blood sampled and weighed, the metal barriers were removed from the free range room so that the four groups met one another. At the same time, twenty of the forty mice caged in groups of five, were redistributed and mixed between four of the cages. The dominant animal in each cage was not removed but stayed in the home area. However, the subordinates were allocated to different cages in such a way that each one was placed with a strange dominant male and with two or three strange subordinates. The aim of this rearrangement was an attempt to mimic events of the free range room but under the controlled conditions of the cage environment. Since at barrier removal in the free range, most mice encountered "strangers", this was done forcibly in half the caged groups. If encountering strange males proved to be a stressful event, then the physiological effects of this would possibly be followed under caged as well as free range conditions. The redistributed males in the cages are referred to as "exchange control" dominants and subordinates.

3rd Sampling - age 13 weeks All animals were blood sampled and weighed one week after removal of the free range barriers and reallocation of the exchange control mice.

4th Sampling - age 18 weeks A final set of blood samples was taken from all groups six weeks after the free range barriers were removed. Animals were also weighed and then again two weeks later at twenty weeks of age.

A summary of the animal groups and their social status is as follows:-

Condition 1:	Free range	Territory holders	(6)
		Subdominants	(7)
		Subordinates	(7)
Condition 2:	Group caged	Dominants	(4)
		Subordinates	(16)
Condition 3:	Exchange control	Dominants	(4)
		Subordinates	(16)
Condition 4:	Single caged	-	(12)

Results

(a) Corticosterone Levels

Figure 4.1 shows the corticosterone levels in the three social groups of free range mice over the four samplings. Data from the four samplings were analysed using two-way ANOVAS, the results of which are given in Table 4.A. No differences were found between any of the groups but within the groups, levels changed significantly over time ($p<0.03$, ANOVA a Table 4:A). Differences between weeks in the levels were determined by taking the result for each mouse in all three social categories combined and comparing first with second sampling second with third etc. using Student's t-tests, with the following results:-

WEEKS	F-ratio	T-value	df	Probability
10 v. 12	1.602	2.0689	38	$p<0.025$
12 v. 13	2.675	2.0401	38	$p<0.025$
13 v.18	2.984	1.9974	38	$p<0.05$

Peak corticosterone levels in free range mice Fig 4:1

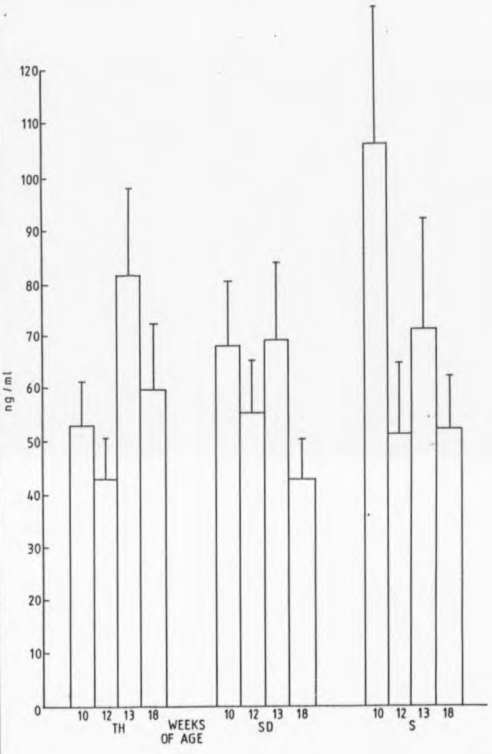


TABLE 4A

Results of Two-way Analysis of Variance on Corticosterone Levels (FR3)

	Source	df	F-ratio	Probability
a) Free range territory holders, subdominants and subordinates	Trt. groups	2+17	0.1894	0.8292
	Time	3+51	3.2334	0.0289
	Trt. x time interaction	6+51	0.8293	0.5076
b) Dominants: free range territory holders, group housed, exchange controls	Trt. Groups	2+11	4.3606	0.0348
	Time	3+33	1.188	0.3294
	Trt. x time interaction	6+33	1.1789	0.3413
c) Dominants and single caged mice	Trt. Groups	3+22	1.8125	0.1743
	Time	3+66	1.1525	0.3346
	Trt. x time interaction	9+66	0.8989	0.5314

TABLE 4A cont...	Source	df	F-ratio	Probability
d) Subordinates: free range, group housed and exchange control	Trt. Groups	2+56	0.5114	0.6039
	Time	3+168	7.6694	0.0005
	Trt. x time interaction	6+168	1.0942	0.3705
e) Exchange - control: dominants and subordinates	Trt. Groups	1+18	0.066	0.8001
	Time	3+54	4.00	0.012
	Trt. x time interaction	3+54	0.067	0.9769
f) Group housed: dominants and subordinates	Trt. Groups	1+18	13.2648	0.0019
	Time	3+54	0.6701	0.5741
	Trt. x time interaction	3+54	1.885	0.3228

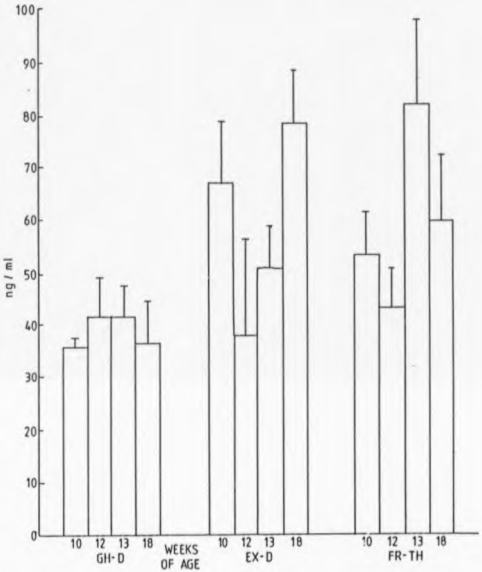
Levels for these groups differed significantly particularly across the first three samplings ($p<0.025$). By week 18, levels were reduced in all three groups, being lowest in the subdominants but still highest in the dominant territory holders.

Figure 4:2 shows the corticosterone levels for the three categories of dominant animals - groups caged, exchange control and free range territory holders. Levels differed significantly between the groups ($p<0.03$ ANOVA b, Table 4:A) and these differences were further examined by taking the means of four results for each animal and comparing them for every group using Student's t-tests, with the following results:-

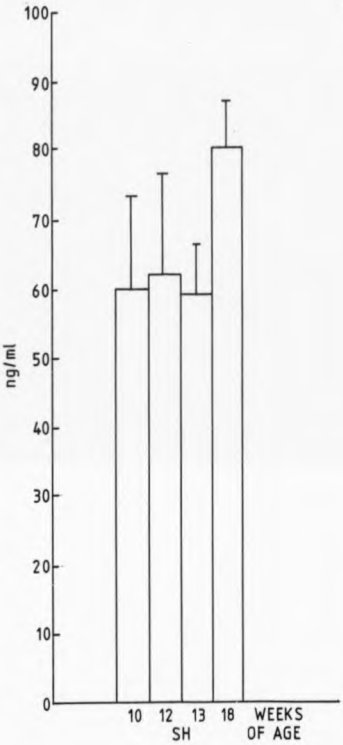
DOMINANT GROUP	F-ratio	T-value	df	Probability
Exchange + Group Housed	1.8931	3.1501	6	$p<0.025$
Exchange + Free range T.H.	2.2479	0.3363	8	N.S.
Group Housed + Free range T.H.	4.255	2.536	8	$p<0.025$

Free range territory holders and exchange control dominants did not differ significantly from each other but both these groups had significantly higher levels when compared with group housed dominants irrespective of time ($p<0.025$). From the histograms it can be seen that for this group, levels remained relatively steady over the eight week period but the other two groups showed marked fluctuations during this time, although these levels did not differ significantly from one another. In the free range territory holders, levels were at their highest one week after removal of the metal barriers, and in the exchange control dominants, levels rose continually after redistribution of the subordinates at week 12. When the data for these three groups were compared with the data for the single caged mice, (Fig. 4:2 and 4:3), no significant differences were found

Peak corticosterone levels in dominant mice Fig 4:2



Peak corticosterone levels Fig 4:3
Single housed mice



between any of the groups (ANOVA c, Table 4:A).

Levels over time for the three groups of subordinates are shown in Figure 4:4. No differences were found between the groups (ANOVA d) but within the groups, levels over time differed significantly ($p<0.0005$). A further breakdown of this result using Student's t-tests showed the following when subordinate groups were combined:-

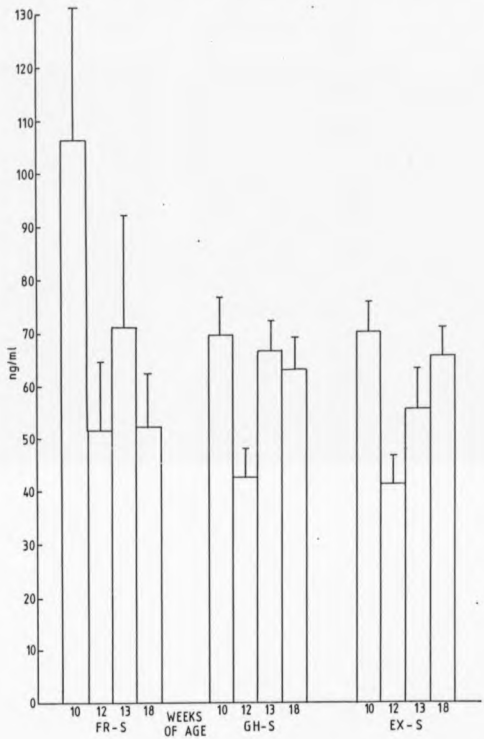
WEEKS	F-ratio	T-value	df	Probability
10 v. 12	1.3042	4.7711	76	$p<0.0005$
12 v. 13	1.6856	2.9382	76	$p<0.005$
13 v. 18	1.9895	0.004	76	N.S.

Significant changes in corticosterone levels occurred between weeks 10 and 12 ($p<0.0005$) and also between weeks 12 and 13 when they rose in all groups ($p<0.005$). Although levels continued to rise up to week 18 in exchange control subordinates, they were by then reduced in the other two groups, particularly the free range subordinates although this result was not significant.

Results for the exchange control animals showed no differences between the dominants and subordinates (Fig. 4:2 and 4:4) nor was the interaction with time significant but levels differed over time ($p<0.012$). Further analysis using Student's t-tests gave the following results when results for exchange dominants and subordinates were combined:-

WEEKS	F-ratio	T-value	df	Probability
10 v. 12	1.4771	3.8	38	$p<0.005$
12 v. 13	1.0731	1.6441	38	N.S.
13 v. 18	1.6471	1.7382	38	$p<0.05$

Peak corticosterone levels in subordinate mice Fig 4:4



Levels dropped in both groups between weeks 10 and 12 ($p<0.005$) and although they rose again at the time of redistribution and continued to rise up to week 18, this increase was only significant between weeks 13 and 18.

There were no differences when group housed dominants and subordinates were compared.

(b) Animal Weights

Figure 4:5 shows the weights for the three social groups of free range mice over ten weeks. Table 4:B shows the two-way ANOVA results for the five weighings carried out on the eight categories of animals. The weights of all groups increased over time ($p<0.0005$). There were no differences between the groups over that time.

The same was true for the three categories of dominant mice although when these data were analysed together with data for single caged mice, a between group difference was found (Fig. 4:6 $p<0.05$ ANOVA c, Table 4:B).

Comparisons of groups using Student's t-tests gave these results:-

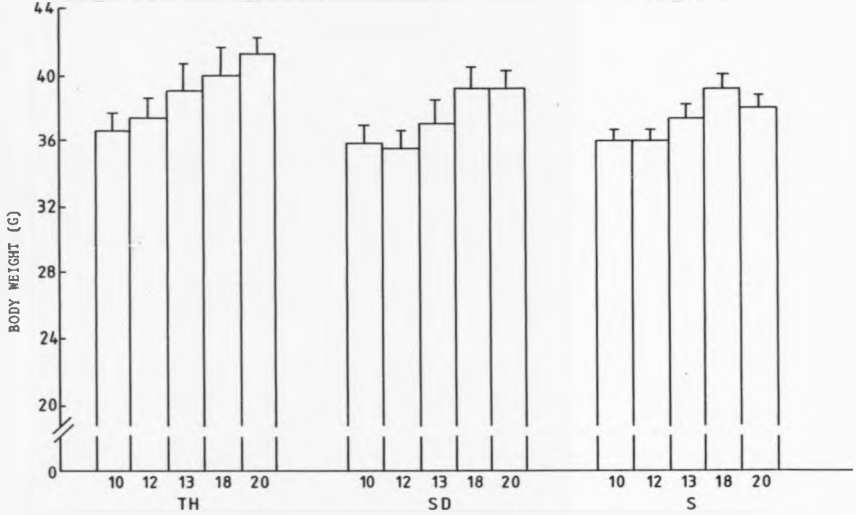
Groups	F-ratio	T-value	df	Probability
Free range TH and single caged	1.2782	2.5487	16	$p<0.05$
Group housed doms + single caged	2.0719	2.1573	14	$p<0.05$
Ex. cont. doms. + single caged	1.2837	2.4261	14	$p<0.05$

The weights of the singly caged mice were found to be significantly lower ($p<0.05$) in comparison with the other three groups.

When data for the three groups of subordinate mice were compared, no differences were found (Fig. 4:7, ANOVA d, Table 4:B)

Weights of free range mice over 10 weeks

Fig 4:5



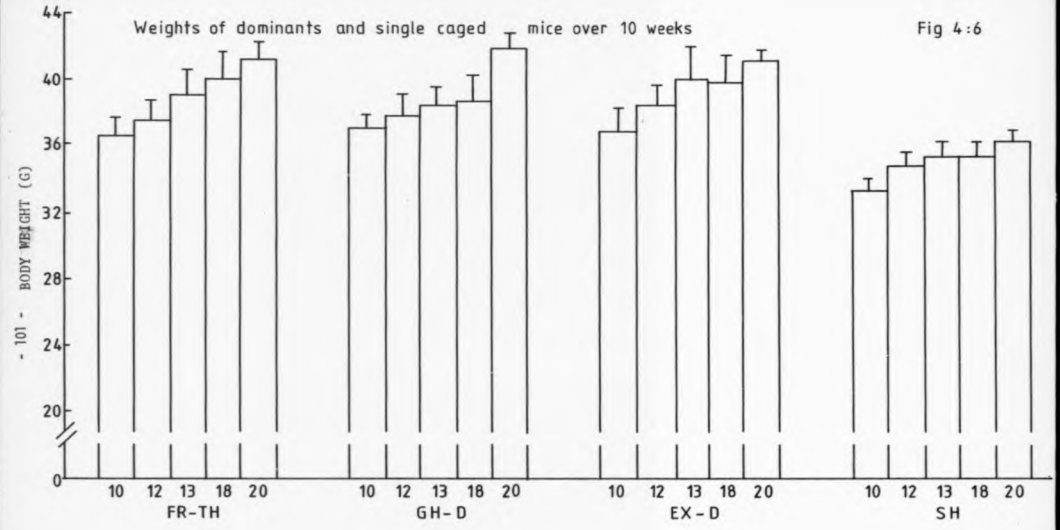

Weights of dominants and single caged mice over 10 weeks
Fig 4:6
- 101 - BODY WEIGHT (G)
44
40
36
32
28
24
20
0
10 12 13 18 20
FR-TH
10 12 13 18 20
GH-D
10 12 13 18 20
EX-D
10 12 13 18 20
SH

TABLE 4B

Results of Two-way Analyses of Variance of Animal Weights (FR3)

	Source	df	F-ratio	Probability
a) Free range territory holders, subdominants and subordinates	Trt. Groups	2+17	0.7786	0.4747
	Time	4+68	40.1372	0.0005
	Trt. x time interaction	8+68	1.4675	0.1855
b) Dominants: free range, group housed, exchange controls	Trt. Groups	2+11	0.08	0.9237
	Time	4+44	18.1769	0.0005
	Trt. x time interaction	8+44	0.516	0.8397
c) Dominants and single caged mice	Trt. Groups	3+22	3.0461	0.05
	Time	4+88	29.1528	0.0005
	Trt. x time interaction	12+88	0.7961	0.6533

TABLE 4B cont...	Source	df	F-ratio	Probability
d) Subordinates: free range, group housed and exchange control	Trt. Groups	2+35	0.2424	0.7862
	Time	4+140	48.5154	0.0005
	Trt. x time interaction	8+140	1.7928	0.0842
e) Exchange control: dominants and subordinates	Trt. Groups	1+17	2.7968	0.1152
	Time	4+68	26.2908	0.0005
	Trt. x time interaction	4+68	2.5496	0.0483
f) Group housed: dominants and subordinates	Trt. Groups	1+17	0.6338	0.4369
	Time	4+68	1.746	0.15
	Trt. x time interaction	4+68	0.3975	0.8098

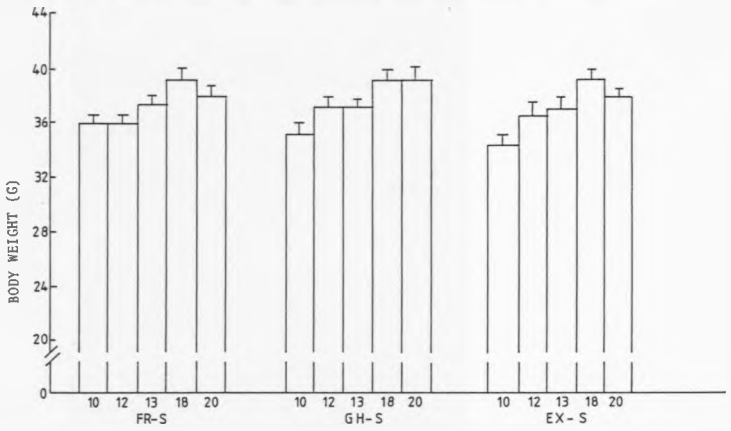
although weights for free range and exchange control subordinates dropped between weeks 18 and 20, but remained level for group housed subordinates, the interaction just failed to reach statistical significance ($p<0.0842$).

Data for group housed dominants and subordinates did not differ significantly but in the exchange control mice, the interaction with time was significant ($p<0.0483$, Fig. 4:6 and 4:7, ANOVA e). Comparisons of the means for each group using Tukey's Multiple Range Test at the probability level of 0.05 showed subordinate weight at 18 weeks differed from weights at 10, 12, 13 and 20 weeks within this group and was also significant when compared with the weighings at 10 and 12 weeks of the dominant animals. Weights at 13, 18 and 20 weeks in this latter groups differed from all other means but not from one another.

(c) Adrenal Weights

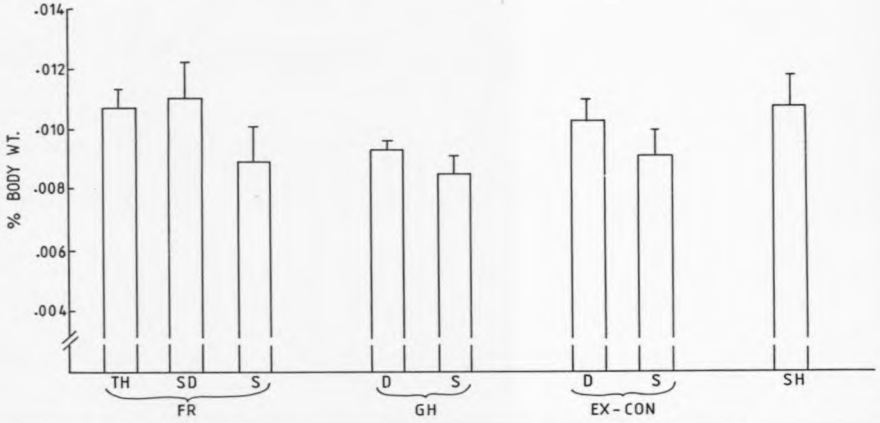
The means and standard errors of left adrenal gland weights, recorded as percentages of total body weight, are shown in Figure 4:8 for every group. Data analysis is shown over:

Weights of caged and free range subordinate mice over 10weeks Fig 4:7



Weights of left adrenals as percentages of total body weight

Fig 4 : 8



1. One-way ANOVA	F-ratio	df	Probability
a) Free range territory holders, subdominants and subordinates	1.098	2+17	0.361
b) Dominants; group housed, exchange control and free range T.H.	0.9	2+11	0.444
c) Dominants in (b) and single caged mice	0.189	3+22	0.903
d) Subordinates: group housed, exchange control and free range	0.076	2+36	0.927

2. Student's t-tests	F-ratio	T-value	df	Probability
a) Group housed: dominants and subordinates	19.3802	0.7911	18	N.S.
b) Exchange control: dominants and subordinates	2.5939	0.8545	18	N.S.

There were no significant results among any of the groups.

Experiment FR8

The aim of these tests was again to examine corticosterone levels in different groups of mice but on this occasion, blood samples were taken to ascertain trough levels. These levels are at

their lowest point in the circadian cycle towards the end of the dark phase, just prior to white lights on (Nichols 1980), and attain values of 61.17 ± 16.8ng/ml in this strain (Creighton 1985). In order to facilitate blood sampling, the lighting regime of the animal rooms was altered for the purposes of this experiment (white lights on 13.00, red lights on 3.00 hours.) This was done 2-3 days prior to the birth of the animals.

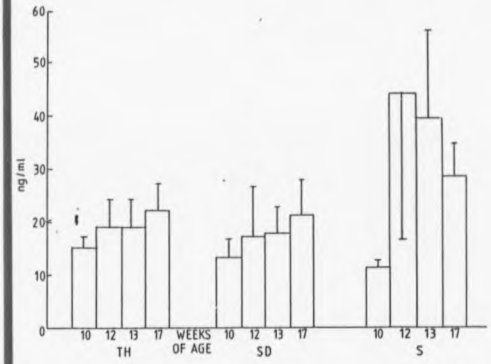
Blood sampling followed the same procedure as in the previous experiment but no exchange control groups were included. The sampling regime was the same as described previously but in this experiment, animals were not weighed. After the fourth sampling at eighteen weeks, the mice in the free range were used in a short follow-up experiment (described in Chapter 5). As a consequence of this, no post mortem data is included. A summary of the mice used in this experiment is given below:-

Condition 1	Free Range	Territory Holders	(5)
		Subdominants	(4)
		Subordinates	(11)
Condition 2	Group Caged	Dominants	(4)
		Subordinates	(16)
Condition 3	Single Caged		(20)

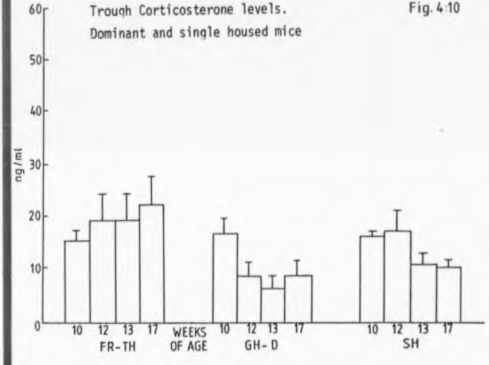
Results

The means and standard errors for each groups' results are shown in figures 4:9, 4:10, 4:11 and 4:12. Data was analysed by two-way ANOVA and details are given in Table 4:C. There were no significant differences between or within any of the groups, although some data

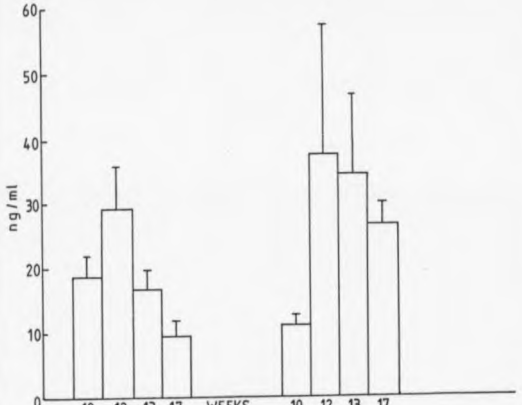
Trough Corticosterone levels. Free range mice Fig. 4:9



Trough Corticosterone levels. Fig. 4:10
Dominant and single housed mice



Trough Corticosterone levels. Subordinate mice Fig. 4:11



Trough Corticosterone levels Fig 4:12
Group housed mice

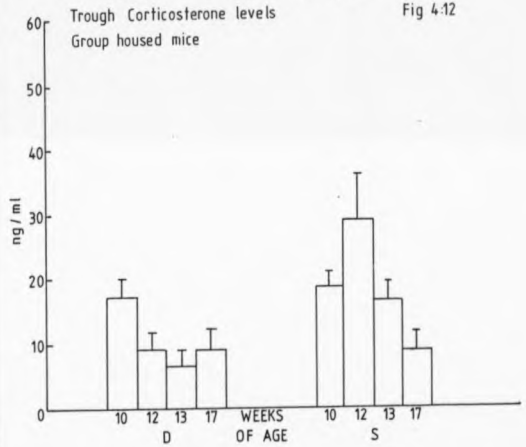


TABLE 4C

Results of Two-way Analysis of Variance. Corticosterone Levels (FR8)

	Source	df	F-ratio	Probability
a) Free range territory holders, subdominants and subordinates	Trt. Groups	2+17	0.7180	0.502
	Time	3+51	0.3322	0.802
	Trt. x time interaction	6+51	0.1681	0.984
b) Dominants, free range, group housed and single caged mice	Trt. Groups	2+26	2.4822	0.103
	Time	3+78	0.5	0.683
	Trt. x time interaction	6+78	0.992	0.436
c) Subordinates: group housed and free range	Trt. Groups	1+25	3.1567	0.0878
	Time	3+75	1.7146	0.1712
	Trt. x time interaction	3+75	0.8206	0.4865

TABLE 4C cont...	Source	df	F-ratio	Probability
d) Group housed: dominants and subordinates	Trt. Groups	1+18	2.3413	0.1433
	Time	3+54	1.2754	0.292
	Trt. x time interaction	3+54	1.284	0.3457

approach the 5% significance level.

Experiment FR5

Using animals from the three housing conditions, the tests carried out in this experiment examined a number of factors: animal weight changes over seven weeks, blood urea levels, pain threshold levels, and adrenal gland weights following post mortems.

1. Animal weights

Animals were weighed three times over a seven-week period. The first measurement was done when the mice were ten weeks of age and housed in groups of five per cage, a total of sixty mice. The second weighing was done at thirteen weeks of age, three weeks after the mice were placed in their experimental conditions - the free range room, single and group cages. The barriers in the room had been removed one week before. A final weighing of all animals was done at seventeen weeks.

Results

Means and standard errors for the weights of each group are shown in Figure 4:13. Data was analysed by two-way ANOVA and the results are given in Table 4:D. Every analysis showed within-group changes in weight over time that were significant, as was seen in the previous weight experiment, though not all groups showed an increase at every weighing. Erratic weight change was evident for the free range subordinates and the single caged mice.

Analysis of data for the free range social groups gave a significant interaction ($p<0.0079$, ANOVA a, Table 4:D). Comparison of the means was done by Tukey test with a probability level of 0.05. Means of the three weighings for territory holders and subdominants

Body weights over seven weeks

Fig 4 :13

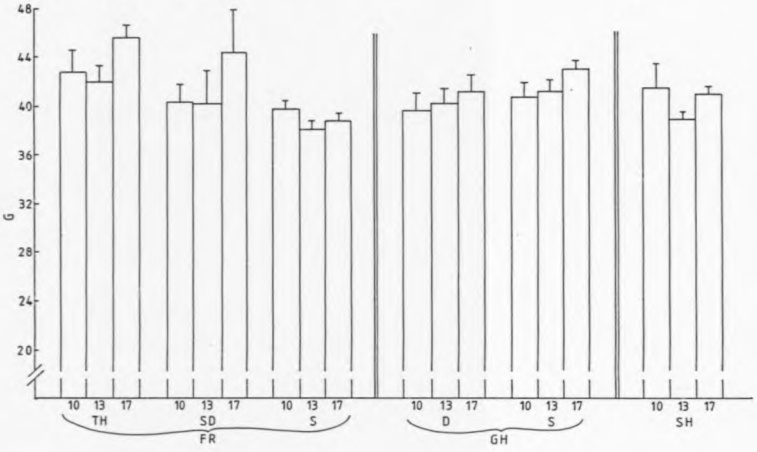


TABLE 4D

Results of Two-way Analysis of Variance - Animal Weights (FR5)

	Source	df	F-ratio	Probability
a) Free range territory holders, subdominants and subordinates	Trt. Groups	2+17	3.2183	0.0653
	Time	2+34	14.5588	0.0005
	Trt. x time interaction	4+34	4.1227	0.0079
b) Free range territory holders, group housed dominants and single caged mice	Trt. Groups	2+23	5.1369	0.0143
	Time	2+46	20.3503	0.0005
	Trt. x time interaction	4+46	1.3433	0.2683
c) Subordinates: group housed and free-range	Trt. Groups	1+28	10.0211	0.0037
	Time	2+56	12.2278	0.0005
	Trt. x time interaction	2+56	21.9068	0.0005

TABLE 4D cont...	Source	df	F-ratio	Probability
d) Group housed: dominants and subordinates	Trt. Groups	1+18	0.3710	0.5501
	Time	2+36	34.4348	0.0005
	Trt. x time interaction	2+36	1.0022	0.3277

differed significantly from the three means for the subordinate mice and also within and between these two groups for weeks 10 and 13 compared with week 17.

Data for free range territory holders, group caged dominants and single caged mice showed a significant difference between the groups (Anova b, Table 4:D). Comparison of the means between groups using Student's t-tests gave the following results:-

GROUPS	F-ratio	T-value	df	Probability
Free range TH and group housed dominants	1.0054	1.5579	2+3	N.S.
Free range TH and single caged mice	1.73	3.4449	2+18	$p<0.005$
Group housed dominants and single caged mice	1.72	1.1323	3+18	N.S.

Only free range territory holders and single caged mice differed significantly from one another with weights of the free range mice increasing irrespective of time. In single caged mice, weights were found to drop and then recover to their original levels.

A pronounced difference was found between the group caged and free range subordinates. The ANOVA showed significant differences both between and within the groups as well as a significant interaction ($p<0.0037$, $p<0.0005$, $p<0.0005$, Anova c, Table 4:D). Mean weights of animals were compared using a Tukey test which showed that the group housed subordinates were significantly heavier in weeks 13 and 17 when compared with the weights of free range subordinates for each time that weighing was done ($p<0.05$).

There were no differences between group caged dominant and subordinate animals.

2. Blood Urea Levels

At seventeen weeks, blood samples were taken from animals in all groups as part of an experiment to determine testosterone levels, the details and results of which are given in Chapter 5. At the time when samples were obtained, blood from each animal was tested for levels of urea; details of the method are given above. Figure 4:14 shows the frequency distribution for the urea scores.

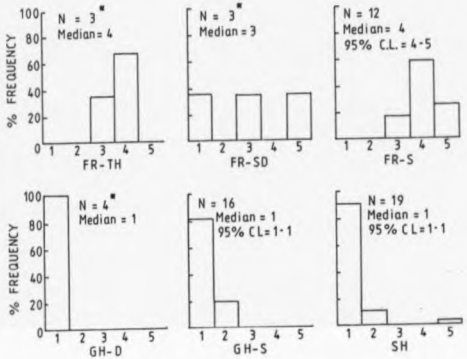
Comparison of data for the groups using the Kruskal-Wallis and Mann Whitney - U tests gave the following results:-

a)	Free range territory holders, subdominants and subordinates	H=3.214	N.S.
b)	Free range T.H., group housed dominants and single caged mice	H=6.611	$p<0.05$
c)	Group housed dominants and subordinates	U=26	N.S.
d)	Free range and group housed subordinates	U=0	$p<0.005$
e)	Free range territory holders and group housed dominants	U=0	$p<0.05$

Levels were raised above the lowest value (3.3nmol/l) in all groups of free range mice and no difference was found between them.

Percentage frequency distribution for urea scores

Fig. 4:14



nmol/l	3.3 ± 2.1	5.4 ± 2.1	7.5 ± 2.1	10.35 ± 3.3	14.2 ± 3.3	17.9 ± 3.7	21.6 ± 3.7
Point score	1	2	3	4	5	6	7

Values of Blood Urea Levels and Associated Point Scores

In contrast, low levels were found in group housed dominants and subordinates as well as in the single caged mice. The raised levels seen in free range mice resulted in the significant differences found when these animals were compared with the ones in the caged conditions.

3. Pain Threshold Levels

At week 15, animals from the three housing conditions were tested for latencies to first and second paw licks in three successive tests on the same day, which employed the hot-plate method described above. The means and standard errors for these tests are shown in Figures 4:15, 4:16, 4:17 and 4:18, and analysis of the data is given in Table 4:E.

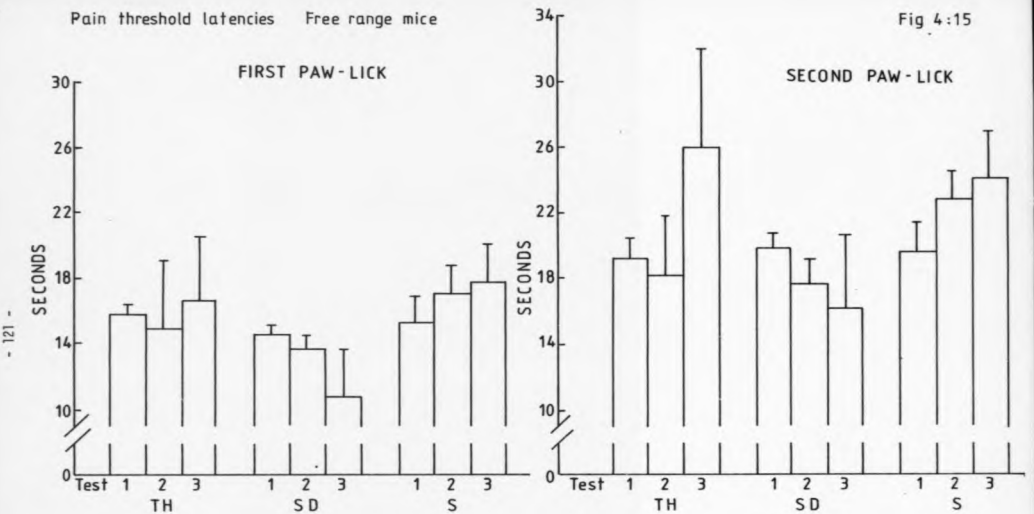
When data are compared, only free range and group housed subordinates showed any difference between the groups ($p<0.0445$, $p<0.0159$, ANOVA d, Table 4:E, Fig. 4:18) for both latencies. Caged subordinates appeared to take significantly more time to respond to the heat stimulus of the hot plate in the first and second tests. No other group gave results which were significant, although one result for territory holders, caged dominants and single housed mice showed an interaction of $p=0.07$.

4. Adrenal weights

Animals from the free range and group cages were sacrificed at twenty weeks and the left adrenal glands were removed and weighed. Mean weights and standard errors as percentages of total body weight are shown in Figure 4:19 and results of analysis on arcsine transformed data are given below. A number of single caged mice developed attack by cage mite during the last two weeks of the experiment and therefore this group was excluded from post mortem

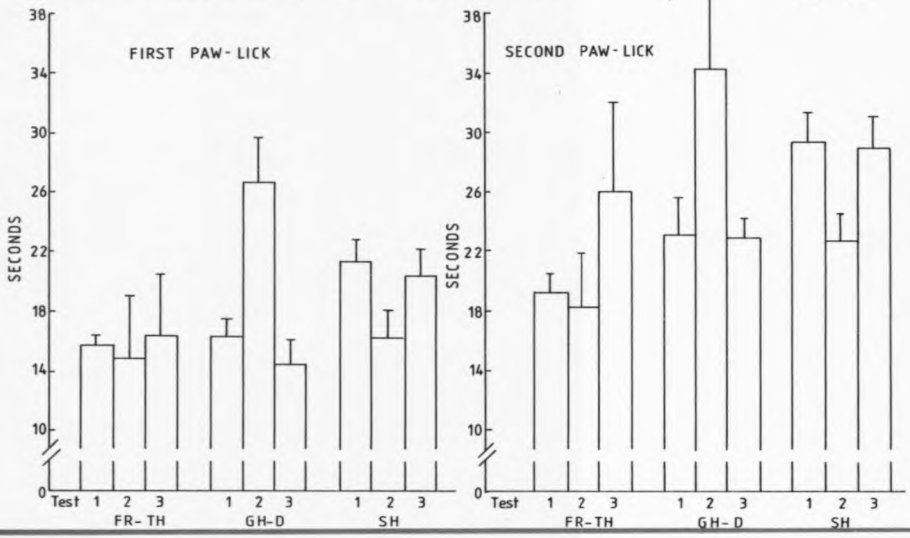
Pain threshold latencies Free range mice

Fig 4:15



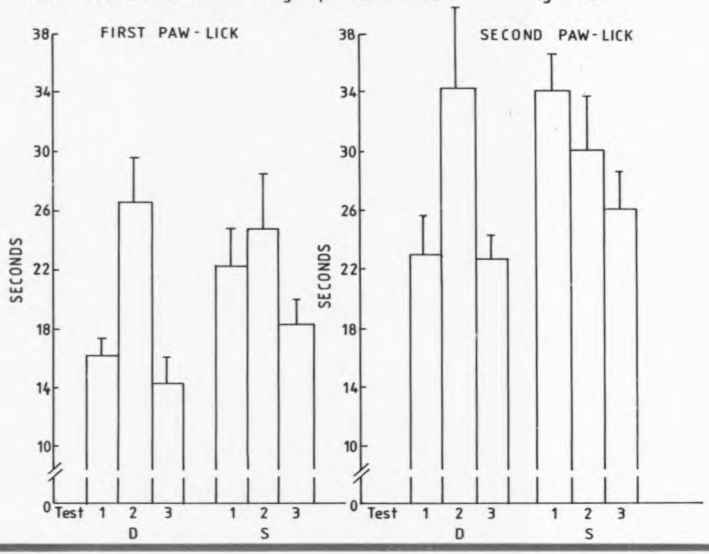
Pain threshold latencies in dominant and single caged mice

Fig 4 : 16



Pain threshold latencies in group housed mice

Fig 4:17



Pain threshold latencies in subordinate mice Fig 4:18

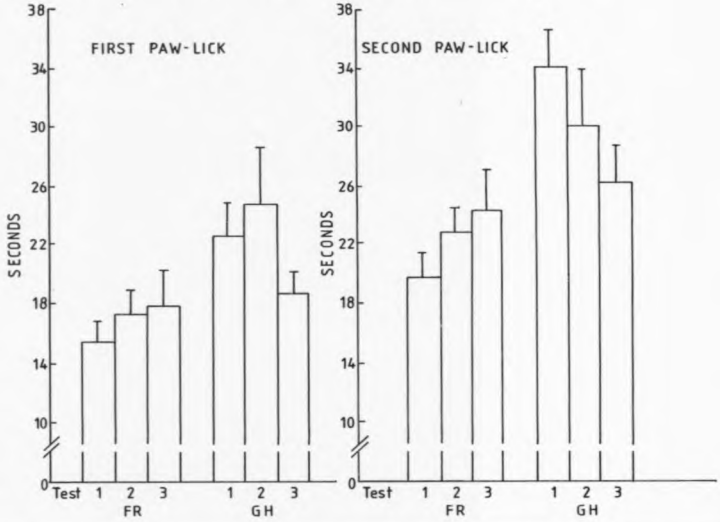


TABLE 4E

Results of Two-way Analysis of Variance - Pain Thresholds

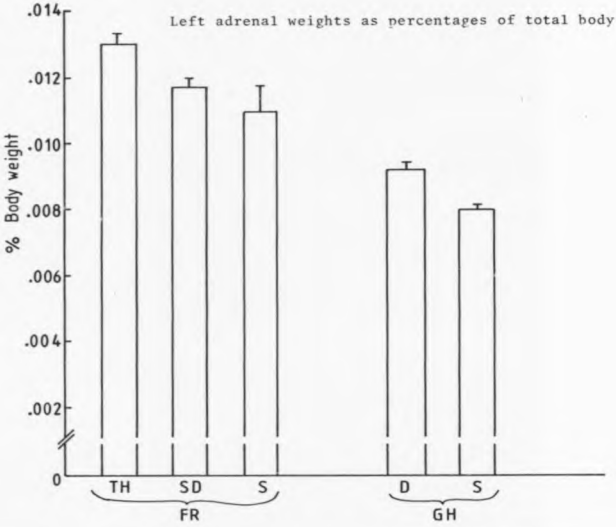
	Source	df	F-ratio	Probability
Latencies to First Paw Lick				
a) Free range territory holders, subdominants and subordinates	Trt. Groups	2+15	0.5535	0.5862
	Tests	2+30	0.0105	0.9896
	Trt. x tests interaction	4+30	0.7096	0.5918
b) Free range territory holders, group housed dominants and single caged mice	Trt. Groups	2+22	0.6766	0.5186
	Tests	2+44	0.3962	0.6934
	Trt. x tests interaction	4+44	2.3143	0.0723
c) Group housed: dominants and subordinates	Trt. Groups	1+15	0.5077	0.4871
	Tests	2+30	2.3971	0.1084
	Trt. x tests interaction	2+30	0.4568	0.6376

TABLE 4E cont...	Source	df	F-ratio	Probability
d) Subordinates: group housed and free range	Trt. Groups	1+24	4.4946	0.0445
	Tests	2+48	0.8817	0.4207
	Trt. x tests interaction	2+48	1.4870	0.2363

TABLE 4E cont...	Source	df	F-ratio	Probability
Latencies to Second Paw Lick				
e) Free range territory holders, subdominants and subordinates	Trt. Groups	2+15	0.5604	0.5825
	Tests	2+30	1.2876	0.2907
	Trt. x tests interaction	4+30	1.9369	0.13
f) Free range territory holders, group housed dominants and single caged mice	Trt. Groups	2+22	1.5956	0.2254
	Tests	2+44	0.2105	0.811
	Trt. x tests interaction	4+44	2.3478	0.069
g) Group housed: dominants and subordinates	Trt. Groups	1+24	4.4946	0.0445
	Tests	2+48	0.8817	0.4207
	Trt. x tests interaction	2+48	1.4870	0.2363

TABLE 4E cont...	Source	df	F-ratio	Probability
h) Group housed and free range subordinates	Trt. Groups	1+24	6.7334	0.0159
	Tests	2+48	0.1398	0.8699
	Trt. x tests interaction	2+48	2.2387	0.1176

Left adrenal weights as percentages of total body weight Fig 4:19



examination.

1. One-way ANOVA	F-ratio	df	Probability
Free range T.H., subdominants and subordinates	0.358	2+13	0.706

2. Students' t-tests	F-ratio	T-value	df	Probability
Free-range T.H. and group housed dominants	7.48	1.2225	5	N.S.
Group housed dominants and subordinates	7.24	0.5835	18	N.S.
Group housed and free range subordinates	3.6325	0.2703	24	N.S.

There were no significant differences between any of the groups.

Discussion

The aim of these experiments was to test the prediction that social stress would be evident in subordinate mice, particularly those housed in the free range room. A number of parameters were examined: peak and trough levels of corticosterone over a number of weeks as a measure of stress over time, body weight changes and adrenal weights at post mortem. Blood urea levels and pain thresholds were also measured and observations were made on general physical condition and the appearance of kidneys at autopsy.

The measurement of plasma hormone levels provided an instantaneous assessment of adrenocortical activity and samples were obtained from both peak and trough times to counter the possibility of stress being evident at one time of day but not the other. From the results on peak levels, no differences were found between the social groups of free range mice although within each group, levels fluctuated. For the territory holders and subordinates, levels achieved their highest at week thirteen, the time when the barriers were removed and mice encountered each other for the first time, which resulted in social conflict together with the risks of loss of status. After this time, all three social groups showed a decline in levels.

Unlike the free range mice, the exchange control animals in cages showed evidence of chronic stress with failure to cope with their conditions as levels continued to rise in both dominants and subordinates over five weeks despite an initial drop. This could have been due to continual high levels of aggression. However, comparison of levels among the three groups of subordinates (free range, exchange control and group caged control) showed no differences although the free range mice demonstrated the greatest fluctuations over time. At ten weeks, mice destined to become free range

subordinates were found to have high corticosterone output compared with other groups even though they were still housed under caged conditions. This finding is hard to explain but it is possible that poor blood sampling technique may have been responsible, blood being obtained near or on the three minute limit permitted. Fighting in the cages just prior to sampling could also have been a cause of these raised levels.

Single caged mice have previously been compared with dominants in terms of their physiology and behaviour (Brain 1975). The results in this work showed that peak levels of corticosterone for these animals were comparable with those of the three goups of dominants and did not differ statistically from them. However, levels were raised in this group at eighteen weeks following eight weeks in "isolation" when levels remained constant, a finding in line with that of Goldsmith et al. (1976) who also was working with TO strain mice. Despite the argument put forward by Brain (op cit.) and Brain and Benton, (1977), and supported by the corticosterone results, that single housing is non-stressful and similar to territory occupancy, social isolation is plainly not a natural condition and may eventually result in adrenocortical activation, as seen at week eighteen.

In a second experiment, trough levels of corticosterone were investigated but in these tests, no exchange control animals were used. Results for peak levels had not been found to differ significantly between the two group caged conditions. Again, no statistical differences were found between any of the groups tested although levels for free range subordinates were found to be high in the early part of the experiment, dropping down later on. When the barriers were removed, the corticosterone response observed in the territory holders at peak sampling time was not repeated nor were

levels altered in subdominants. The corticosterone trough occurs during the period of inactivity, when white lights are on and the fact that at this time, most animals were resting may have influenced the results. Changes in levels for the territory holders overall resembled the pattern found at peak time and were generally higher than in caged dominants and singly caged mice.

The conclusion drawn from the corticosterone measurements is that any stress present in the free range room was of an acute rather than chronic nature. The findings compare well with the work by Henry and Ely (1976) and Ely and Henry (1978) in mice using the Reimer-Petras style of population cage where social conditions were stable over time, and with Keverne et al. (1982) using talapoin monkeys. These studies show that the process of socialisation among males results in initial high titres of corticosteroids but after stabilisation of a colony or group, levels decrease as dominance status and territory are established and subordinates learn to cope with their social position and the aggression directed towards them. The results are however in conflict with those of Louch and Higginbotham (1967) and also Henry and Stephens (1977) using the Henry-Stephens population cage where the social structure over time was more fluid and less well defined. Whether caged dominants and territory holders responded to social stimulation with raised adrenal-medullary output is certainly possible though unknown in the context of this study. However, from the corticosterone data for the territory holders and exchange control dominants, it appears that encounters with strange mice are stressful for mice of high social position resulting in endocrine changes, an observation that was not matched by the data for control caged dominants that never met other mice.

Among subordinates, particularly from the free range, successful

coping behaviour would appear to be the mechanism operating to control overall activity of the pituitary-adrenal system. It has been shown that subordinate animals with elevated levels of ACTH can more readily change their behaviour and avoid attacks by dominants either by escaping or by displaying submission responses (Brain and Poole 1974). In addition, Bohus (1975) has shown that if coping behaviour is permitted expression then this may reduce or suppress the pituitary-adrenal response to an otherwise stressful situation, for instance, the plasma ACTH response in rats yolked together and exposed to shock is lower if animals have the opportunity to fight each other suggesting alleviation of the defeat reaction (Connor et al. 1971). Eating, drinking, escape and avoidance are also behaviours that inhibit pituitary-adrenal activation in a potentially stressful context (Vernikos-Danellis and Heybach 1980). Certainly the opportunities for escape and avoidance were greater in the free range subordinates compared with those from the exchange control groups given the extra space available to them, an advantage denied to the caged mice.

Levine and Coover (1976) have demonstrated that the rhythmicity of plasma corticoids can be altered to coincide with the presentation of stimuli such as food or drink, at a given time. Repeated presentation of a stimulus eventually leads to a waning of pituitary-adrenal activity and the spontaneous rise in glucocorticoids eventually disappears. When this point is reached, the response is said to be habituated (Levine et al. 1978). Conversely, in other circumstances, the pituitary-adrenal peak may entrain and be enhanced. Most organisms live in environments that involve repeated aversive stimulation yet they do not respond continually to these stimuli but appear instead to cope (Levine et al. op cit.). Coping differs from habituation in that the stimuli

that elicit the coping response continue to threaten but the individual no longer responds either behaviourally or physiologically to them; this is in contrast to habituation where the stimuli themselves are relatively neutral. The decrease in corticosterone levels in the free range subordinates reflects the coping ability of these animals despite both the continual threat of attack and periodic bouts of aggression from territory holders.

Levine et al. (1978) and others have argued that it is not just the stimulus or the physical environment per se that determines physiological response but the individual's evaluation of the stimuli; indeed as Mason (1975a) has also shown, response is largely dependent upon how an individual perceives the situation. This position is also supported by Henry (1985) who has put forward the view that adequate social experience is also a vital component in the development of self perception as part of not only behavioural but also physiological response. If mastery of the circumstances occurs together with enhanced learning of new patterns of behaviour (de Wied et al. 1972), the resulting physiological response to stress will be diminished with an eventual decrease in pituitary-adrenal activity and it is concluded that the data for the free range subordinates provides evidence to support this view. However, these results go against the findings of Bronson and Eleftheriou (1965b). Mice exposed either physically and visually or just visually to a fighter who had defeated them, showed intense adrenal activity compared with controls that never experienced fighting. These workers argued that the psychological component of fear due to the presence of the fighter was sufficient to stimulate and maintain this physiological response provided that the subjects had previously experienced defeat however, there is no evidence that these animals learned to habituate to their situation. The results conflict with the present data despite the

similarity of conditions. Both caged and free range subordinates not only suffered physical defeat but also endured constant exposure to aggressors and yet, in both housing conditions, adrenal activity declined over time.

Brain (1971) has suggested that the effects of ACTH and the glucocorticoids on fighting behaviour may be partially mediated by their effects on learning and the retention of avoidance responses. Dominant animals in other studies, have been characterised by low activation of the pituitary-adrenal system and do not apparently show much evidence of avoidance reactions as they will readily approach any strange conspecific as a prelude to fighting. Conversely, subordinate mice, characterised by high titres of ACTH and glucocorticoids in their plasma, especially as a result of being subjected to defeat, may learn to avoid dominant individuals rapidly and to retain this response over a long period of time without reinforcement. Indeed, it has been shown that only with great difficulty can a thoroughly subordinated mouse be trained to act aggressively thereafter (Ginsburg and Allee 1942). Behaviours such as avoidance of attack and submission, it has been argued, cannot come about without prior experience of defeat (Nock and Leshner 1976) The knowledge of when to submit and the context and cues that demand this are intimately linked with glucocorticoid output and Bohus (1973) has stated that these steroids increase the discriminative capacity of the organism. These ideas also fit in well with observations on subordinate mice from the free range. It was notable that patterns of behaviour for these mice did not become fixed and rigid. These animals, as well as submitting to territory holders and subdominants, were seen on occasion to attack intruders that entered the territory area they inhabited. It would appear that rather than having learned never to attack another mouse, subordinates learned instead to avoid

specific individuals. Indeed it is known that defeated rats will successfully attack and defeat intruders that enter their home cages (Koolhaas et al. 1980).

In contrast to the immediacy of corticosterone measurement, adrenal weight provides an historical perspective of the effects, if any, of prolonged adrenal activity. In the experiments where this parameter was measured, no statistical differences were found between the groups. It was noticeable though that in the first set of organ weights (exp. FR3), free range subdominants and single caged mice showed the highest recorded. Also, in both sets of data, dominants of all categories were found to have higher adrenal weights than those of subordinates though these values did not differ statistically. This may have been due to the small subject numbers in the groups. The data provide evidence to support the earlier conclusion that chronic stress was not a feature of mice housed in any of the three housing conditions. As a consequence, the results do not support the findings of Christian (1956) and Davis and Christian (1957) for mice and Clarke (1953) for voles nor those of other workers discussed earlier. Despite both inter- and intra-group fighting among the free range animals, adrenal hypertrophy was not evident yet Christian (1955) found increases in adrenal weights in mice housed in densities of greater than one per cage although maximum increase was only observed with sixteen and above in a cage. It is possible that the strain of mice he used (NMRI strain) possessed adrenals that were more responsive to stress than those of the TO strain used here. In a separate study, Archer (1970) found an increase in adrenal weight in mice that were made to fight intensely in pairs for short periods, during which time there was no opportunity to escape. By contrast, the free range conditions provided sufficient space for animals to flee and avoid attack although fighting did still take place, as

among caged controls also, and there was also a certain advantage in having safety in numbers so that aggression from a dominant animal was distributed among a number of individuals, an advantage that Archer's defeated mice did not possess. Certain individuals in the free range on occasions appeared to be singled out for attack but generally, a mouse in these circumstances could lose itself in a huddle with others.

In support of the findings here, two studies measuring the effects of social interaction (Ely et al. 1974, Henry and Ely 1976) showed that the adrenal weights of all males remained normal despite changes in catecholamine activity and a period of raised corticosterone levels. All values returned to normal after mice had been interacting for three months which suggests that gland weight is only reflective of neuroendocrine disturbances over long periods.

Although it is a popular methodology, gland weight is considered to be a fairly crude form of measure by certain workers (Benton et al. 1978, Brain and Benton 1979) and it has been argued that it is impossible to be sure to what extent the weight of endocrine glands reflects the influence of different housing or differential experience. The use of adrenal weight as an index of stress also suffers from the problem that this gland's activity is further influenced by gonadal steroids (Kitay 1968) and by medullary activity (Brain and Benton 1979). As a consequence, although the adrenal weights recorded in these experiments support the corticosterone findings, these data are seen as being important only in relation to other measures of stress.

No attempt was made in this study to relate the degree of wounding in subordinates to adrenal changes and in the literature, there is some confusion about this. Southwick and Bland (1959) found adrenal enlargement only in their wounded subordinate mice while

Christian (1959) could find no correlation between severity of wounding and adrenal weight. More recently however, Henry et al. (1982) have shown that subordinates with tail scarring and nicks also possess adrenal hypertrophy.

In two experiments as a further indicator of stress, body weights of all mice were recorded. Over ten and seven weeks, it was noticeable that most groups shared increases in weight although in comparison to the different groups of dominants, single housed mice showed very little change in weight in the first experiment (FR3) and in the second (FR5), lost weight before regaining it. Weights of dominants and territory holders did not differ nor did those for the three social categories of free range mice, the three categories of subordinate mice or the group caged dominants and subordinates in the first set of weighings that were done (FR3). Among the exchange control animals, the weights of subordinates were reduced over time resulting in a significant difference when these animals were compared with this category of dominants.

In the second set of weight data (FR5), significant weight loss was observed among free range subordinates when compared with the caged mice but when compared with the free range territory holders and subdominants, this loss was not significant. The weights of all group housed mice increased over time but in contrast to the findings of Davis and Christian (1957), dominant mice were not always the heaviest animals in a group. Loss of weight is known to be associated with the social stress of subordination in tree shrews (von Holst 1985) although whether it is a consequence or a cause of low status is hard to say. The group housed subordinates were heavier than those of the free range and although the statistically significant interaction between these two groups shows weight loss by the free range mice was directly opposite to the gains made by those in group

cages over time, this result may reflect the duration of the experiment and given longer, the free range subordinates could have regained the earlier weight loss. Mice living in the room tended to distribute food pellets from the baskets all over the floor so that even if subordinates were forced to huddle together on the tops of bricks, food was always available nearby without the necessity of making forays to a central source. It is thought therefore that any low weight among this group was unlikely to be due to inhibited feeding and some unknown physiological difference may be the cause.

Reasons why the single housed mice showed both the smallest increases in weight and at one point, actual loss, are hard to determine. There appears to be no evidence in the literature that this form of housing for mice depresses weight, indeed certain organ weights, such as preputials, are found to be increased (Benton et al. 1979). As these mice were lighter in weight that the two categories of high status males, caution is taken in stating that singly housed mice are equivalent to territory holders (Brain 1972a, 1975).

In general the data shows an absence of severe weight loss and instead, the more normal pattern was one of increase. Although there were a number of statistically significant differences and interactions, it is felt that this alone does not provide sufficient evidence of chronic stress particularly as it is unsupported by the data for adrenal activity.

Data for urea levels was found to contrast with the measurements of stress discussed above. The high levels in the free range mice were not found in any of the mice from the two other housing conditions. Results for the free range subordinate mice are similar to those of population cage mice (Henry et al. 1982) and for tree shrews (von Holst 1985). In the study by Henry, a correlation was found between the degree of scarring and raised blood urea. Their

dominant animal was generally unmarked with a low urea score, in contrast to the territory holders. In the population cages, although on occasions more than one dominant defended territories, it appears that these animals were rarely attacked and had little contact with one another, separated by the long tubes that attached to individual cages. By contrast, in the present experiments, although the general quality of the coats of the free range territory holders tended to be superior to those of other males, these animals nevertheless sustained wounding on the tail and occasionally, on the rump also due to encounters with animals from other territories. The room permitted a freedom of movement thereby increasing the number of chance encounters between animals which the boxes and tubes of the population cage presumably did not.

Both in this study and in the one of Henry et al. (1982), it was found that subordinate mice at post mortem either voided a large amount of urine or had grossly distended bladders (see photographs) indicating a behavioural inhibition to urinate. Henry (op cit.) has argued that these observations suggest rapidly developing renal damage with reflux nephropathy as evidenced by elevated blood urea. In the study here, it was noticeable that of a total of seventeen free range subordinates examined at autopsy in experiment FR5, twelve had full bladders of which five were very distended. Five urinated a considerable volume at death and nine mice had kidneys which were notably grey in colour and contained small pits. This was in contrast with the shiny, deep red kidneys of all other mice. Henry has argued that these physiological events are a consequence of intense social stress and urine deposition is suppressed in subordinates housed in the presence of dominants (Desjardins et al. 1973). The latter point about dominants may be relevant to the free range mice but in caged subordinates, very little urine was ever found in their bladders at

post mortem. The findings suggest that the uremia may be indicative of kidney damage. Because of the odour properties of mouse urine and the influence these have on behaviour, the observed urine retention may be a species-specific phenomenon.

Male odours are a major influential factor in agonistic encounters (Mackintosh and Grant 1966, Mugford and Nowell 1970) and this may play a decisive role in the way urine is deposited. If subordinate mice possess a urine characteristic that induces aggression, then these animals may withold their urine in order to avoid potential attack from territory holders and subdominants. Support for this argument comes from observations of the very few occasions when a territory holder was deposed and gained subordinate status. Although no formal measurements were made, two to three weeks following their loss of status, these animals were still found to produce the urine marking pattern of intense overall spotting characteristic of a high status male rather than a pattern of large pools associated with subordination. In addition, it was noticeable that these mice were singled out for attack by the new territory holders which would suggest that these deposed mice had not learned to withold their urine so producing a different pattern and perhaps reducing the level of aggression directed towards them.

The final results to be discussed are those from the hot plate tests. These data were not found to support the original prediction that subordinate mice, particularly those in the free range, would have raised pain thresholds as a consequence of social stress when compared with other groups. However, the findings for the free range mice are in line with those for corticosterone levels and adrenal weights which showed evidence of an acute response to stress only. The one statistical difference was between caged and free range subordinates and it was mice from the group cages that demonstrated

the longest latencies to paw lick. There were no other differences found. Miczek et al. (1982) working with B6AF$_1$ mice, have argued that the expression of defeat behaviour is of prime importance in the onset of stress induced analgesia whereas Rodgers and Hendrie (1983) have suggested that lack of control over the behaviour of the attacker may be the major factor. In a recent study (Rodgers and Randall 1986), which sought to dissociate nociceptive consequences of defeat per se from those of defeat plus further attack, it was found that defeat alone induced a short-lasting, non-opioid analgesia whilst exposure to extended attacks activated a much longer lasting opioid analgesia. This point is important for the results for caged subordinates in the present study which were subjected to almost continual attack and defeat by dominants. The free range subordinates, although always confined with aggressive animals also, did not have the same constant degree of proximity to them in the same way and incidences of defeat followed by further attack may have been less frequent.

From the results of these tests, two further points are worth making: firstly, levels of corticosterone were not measured in these groups of mice during this experiment and had they been, the results may not have been the same as those found in other experiments, particularly as groups of mice tend to differ. Secondly, it is possible that as none of the animals in the pain threshold tests underwent any controlled stress directly prior to testing, mice removed from a relatively peaceful environment therefore showed little or no delayed response to inflicted heat. It is therefore concluded that these tests only examined pain threshold levels rather than stress-induced analgesia. It is postulated that the second or third test might have been stress-related if corticosterone affects SIA very late on. Certainly in tests on DBA/2 strain mice, social

conflict analgesia has a duration of forty to sixty minutes which is blocked and reversed by naloxone (Rodgers and Randall 1985a,b) so a form of social stress (eg. fighting behaviour) prior to testing in the study here, could have produced a more marked response.

To sum up from the results of experiments discussed in this chapter, the overall conclusion that can be drawn is that chronic social stress was not evident among subordinate mice as was originally predicted. This is supported by the data from measurements of corticosterone, body and adrenal weights and pain threshold tests. However, the data for urea levels and observations on bladder distention and the state of kidneys at post mortem together with general physical condition, indicate that some degree of stress among free range mice must have been influential at some point. It is proposed that acute stress in the early weeks of free range experiments, particularly at the time when barriers were removed, was sufficient to influence behavioural patterns of urination which subsequently led to raised urea levels and kidney damage though this was not sufficiently prolonged to maintain high corticosterone levels over time and influence adrenal and body weights long term. By the time the hot plate tests were carried out (during the latter part of experiment FR5), the level of stress was insufficient to produce any substantial results.

It is recognised that this final conclusion is not consistent with previous findings. Chronic tubulointerstitial nephritis is generally a condition that occurs over a long-term period in stressed mice and is known to be associated with raised adrenocortical output (Henry and Stephens in press). The results from the present study suggest a short term effect on renal physiology together with a lack of prolonged involvement of the pituitary-adrenal axis. However, it must be said that no generalisations are being made to work outside

of these experiments and conclusions have been based solely on the evidence derived from the results reported here.

Free range subordinate mice with gross bladder distention.

CHAPTER 5

TERRITORIAL AND DOMINANCE STATUS AND GONADAL ACTIVITY

Introduction

The experiments described in this chapter investigated levels of testosterone in mice of different social status, housed in cages and the free range room. Previous studies have demonstrated a relationship between circulating testosterone levels and a number of factors such as social status, aggression levels and sexual behaviour in a variety of species. The aim of this study was to investigate whether the same correlations could be seen to hold true for the male mice housed in the conditions mentioned above.

To test this, a number of predictions were made (briefly described in chapter one). The primary expectation was that levels of testosterone would be directly linked to an animal's social position so it was anticipated that as high status males, free range territory holders and group caged dominants, as well as singly housed males, would show corresponding high circulating plasma levels of testosterone. In contrast, it was predicted that the low status males, subdominants and subordinates, would have depressed testosterone output in association with the social stress frequently experienced by these animals which has been described in a number of other studies. For each of these social groups, as a further indicator of gonadal activity, measurements of sex accessory gland weights, preputials and testes, were also made based on the prediction that gland weight would also correlate positively with social status.

The second area of investigation examined the known relationship

between testosterone and aggression, and here it was predicted that males with the highest testosterone levels would also be the most aggressive as determined by observation. As a consequence of the results obtained from a number of initial experiments which yielded unexpected data from testosterone levels in the free range territory holders, a further series of experiments were carried out using animals housed only in cages, which investigated the influence of short, inconclusive fights on androgen output. This was based on the expectation that such fights would serve to depress testosterone output in dominant males simulating the experiences of territory holders. In the cages, dominant males only encountered animals they could defeat and knew they had beaten. The same held true for territory holders in the free range with regard to the subordinates with which they lived. However, outside a territory area were other animals that posed a threat. The hypothesis was therefore that testosterone levels differed between territory holders and group housed dominants because territory holders were not always outright winners of fights. Because of their situation, certain fights were not lost but were not won either.

In the light of these predictions, the introduction to this chapter is based upon a number of topics. The first concerns details of the pituitary gonadal system together with mention of the hormones and brain peptides that influence testosterone secretion. This is followed by an evaluation of previous studies which have examined the relationship between testosterone and social status. The links between androgen output and aggression are then explored: the literature covering this area of research is very extensive and since this topic formed only a single part of the work here, a concise review only is given. In view of the fact that testosterone levels were measured in both high and low status males, a discussion on the

effects of acute and chronic stress on testosterone output is also included. This association between social stress and androgens is linked to and leads into the final subject for discussion: the influence of the social environment on androgen activity particularly in relation to early studies on crowding in rodents.

Peripheral levels of testosterone are under the control of the hypothalamo-pituitary-testicular axis which is composed of the hypothalamus and its neural connections to the rest of the brain, the gonadotrophin-producing cells of the anterior pituitary and the testes. Besides the testes, the adrenal cortex is also a source of testosterone but to only a limited extent and it appears that there are considerable species differences in testosterone production from this organ (Kime et al. 1980). The testes are functionally divided into interstitial tissue containing Leydig cells, the sites of testosterone production, and seminiferous tubules which are in turn, composed of Sertoli cells and their accompanying germinal cells and are responsible for spermatogenesis. Hypothalamic gonadotrophin-releasing hormone (GnRH) mediates pituitary release of the glycoproteins, leutinising hormone (LH) and follicle-stimulating hormone (FSH). When LH binds to specific membrane receptors on testicular Leydig cells, the biochemical reactions that follow result in secretion of testosterone and oestradiol together with a number of other sex steroids including androstenedione, androstenediols and 5α-dihydrotestosterone (DHT). The sex steroids in turn act on the brain and anterior pituitary exerting control over gonadotrophin release via negative feedback mechanisms.

Testosterone is the major androgen produced in the testes with the primary site of synthesis being the Leydig cells, as stated above. Here, cholesterol is synthesised from glucose and fatty acids, or it may be accumulated from the blood. In the same way as in the

adrenal, side chains are cleaved from the molecule to yield pregnenolone. This 21-carbon steroid is the precursor for all steroids. In the testes, it is converted to testosterone via a number of LH-activated microsomal enzyme steps (Bardin and Paulsen 1982) which includes other products such as androstenedione and androstenediols. There is evidence to show that testosterone is converted to DHT and androstenediols in a number of other tissues including skin, brain, salivary glands, lung, heart and pectoral muscle (Mainwaring 1977). DHT is largely responsible for maintenance of accessory sexual glands (Gower 1984) although rates of formation in these tissues vary enormously with the species (Wilson and Gloynar 1970 cited in Turner and Bagnara 1971).

Testosterone itself may be seen as both a hormone, in that it acts in its own right, without metabolism, and as a pre-hormone when it serves as the precursor of 5α-DHT and other active steroid metabolites (Mainwaring 1979 cited in Bardin and Paulsen 1982). To assume that a given androgen target cell responds to only one particular testosterone metabolite may be incorrect (Baulieu et al. 1969 cited in Kime et al. 1980). It is likely that in all mammals, testosterone is bound to proteins in plasma, in particular, sex-hormone binding globulin (SHBG). It is these proteins, when bound to testosterone that prevent excessive androgen activity, so that even if overall testosterone concentrations are high, it is only the small proportion of free or unbound steroid in plasma that is biologically active; the remainder is biologically inactive but in equilibrium with the free fraction (Nieschlag et al. 1973 cited in Kime et al. 1980).

Although it might be expected that under resting conditions, plasma testosterone levels are relatively stable, without large fluctuations and closely controlled by the feedback mechanism of the

pituitary-gonadal axis (Turner and Bagnara 1971), it appears that for a number of species, an irregular pattern of testosterone secretion is discernible on a daily basis. Low plasma testosterone concentrations were found to alternate with very high ones in rats (Bartke et al. 1973) and similar fluctuations have been seen in mice (Bartke and Dalterio 1975) with values ranging from 0.3ng/ml to 44.4ng/ml within a number of strains. Work with rats has shown that LH is released in well-defined pulses (Ellis and Desjardins 1982). Plasma LH rises occur within five to ten minutes followed by a gradual decline over the next fifty to seventy minutes. Episodes of testosterone secretion spanned three to six hours and were marked by a slow graded rise and fall. In this study, the testosterone pulses were preceded by a train of closely coupled LH peaks. The number, amplitude and timing of hormone episodes varied on different days although in a separate study on male rats, Keating and Tcholakian (1979) found a distinct diurnal rhythm for testosterone consisting of a bimodal pattern with the highest mean testosterone concentrations occurring in the dark period. The same pattern was found to exist when the investigation period was extended from 24 to 48 hours. These results seem to conflict with those of Bartke et al. (1973) and Bartke and Dalterio (1975) for mice as do the studies of Mock et al. (1978) who demonstrated a trimodal rhythm of plasma testosterone concentration in 24 hours in the rat. This result is also at variance with those of Koolhaas et al. (1980) also working with rats, where the data demonstrate a rather random distribution of peak values of plasma testosterone concentrations although a higher incidence of peaks is seen in the middle of the light period and at the beginning and end of the dark period. The function of this pulsatile nature of plasma testosterone is unknown. Taken together, it appears that there is still a controversy in the literature, at

least on rodents, about the number of peaks as well as their temporal distribution.

It is however, accepted that an appropriate hormonal environment needs to exist for the process of spermatogenesis. Androgens are essential for this latter event though it has been shown that pharmacological doses of testosterone alone can maintain spermatogenesis in hypophysectomised animals (Harris et al. 1977) while more physiological doses do not (Simpson and Evans 1946 cited in Cunningham and Huckins 1979). Despite this, normal rats given 100µg TP/100gm of body weight per day showed a thirty-fold drop in levels of intratesticular testosterone yet FSH levels were only partially suppressed and complete sperm production was evident together with only slight reductions in testicular weight (Cunningham and Huckins 1979). This study suggests that the full process of spermatogenesis is apparently not dependent on the presence of high levels of intratesticular testosterone provided FSH is present, which is known to regulate Sertoli cell function, and maybe as a result, secondarily influences germ cell maturation.

The feedback system that operates within the pituitary-gonadal axis is not insensitive to the influence of factors such as prolactin. In golden hamsters, Bartke et al. (1978) have demonstrated the important role of prolactin in regulating the seasonal cycles of regression and recrudescence of the testes in this species and in a separate study on dwarf mice, PRL was shown to be responsible for increases and decreases in the concentration of cholesterol esters in the testes (Bartke 1969, 1971). However, the data from other studies appears to be in conflict with these findings.

In mice and rats with experimentally-induced chronic hyperprolactinemia (Bartke et al. 1977), despite dramatically elevated PRL levels, over a five month period LH levels rose only

slightly, testosterone levels remained indistinguishable from those of controls and testicular weights also showed no differences. These results also differ from the observations of Fang et al. (1974) who demonstrated a lowering of testosterone levels and testes weights together with raised peripheral LH levels in male rats, in response to transplanted PRL-secreting pituitary tumours. It appears that whether PRL is able to suppress the activity of the testes in rodents remains to be conclusively demonstrated. However, from social studies of primate groups, work on Talapoin monkeys (Keverne et al. 1984) shows results of high PRL and low LH profiles among subordinate male individuals.

Moving on to the topic of testosterone secretion and social status, a number of studies have correlated these two factors. Firstly, the work carried out on human males has provided interesting results linking dominance behaviour and testosterone levels. Mazur and Lamb (1980) in an intriguing study, demonstrated elevated levels of testosterone in young men who scored decisive victories in tennis matches while testosterone declined in the losers. In addition, they found that in a hard-won match where victory was only determined after an eleventh tie-break, testosterone levels were unaffected. Furthermore, they also showed that gain which had required no effort, such as winning a $100 lottery, also had no effect on testosterone levels. In a subsequent study, Elias (1981) discovered greater elevations of testosterone in the winners of college wrestling matches as compared with losers. It is notable that in these studies reported here, human males displayed higher levels of testosterone after social interaction in which they emerged victorious and dominant over competitors, or attained significant elevations of social status. In addition to this work, there are numerous confirmatory animal studies.

High androgen secretion has been correlated with high social status in a number of species: mice (Bronson and Marsden 1973, McKinney and Desjardins 1973), rats (Bermond 1982), monkeys (Eberhart et al. 1980) and tree shrews (Fischer et al. 1985) for example. In a study which looked at the effects of castration and androgen on the social dominance of male BALB/cJ mice, Lee and Naranjo (1974) showed that nine out of ten males lost their social position following castration, an effect that was not observed in sham-operated controls. Differing doses of testosterone propionate given daily to castrated dominants did not restore their pre-operative status, which may have been partly due to the irregular levels of steroid resulting from the treatment method. The authors concluded that testosterone does play a vital role in the maintenance of high status. In another experiment using mice, Raab and Haedenkamp (1981) examined the influence of social conflict on testosterone output. Previously isolated males were housed in pairs as residents and intruders, and status was determined by the degree of wounding that was observed. Testosterone levels after ten days showed a marked decrease in subordinates whereas levels for dominant mice were little changed when compared with isolated controls.

Recent work on rats (Albert et al. 1986) has demonstrated that the removal of endogenous testosterone results not only in the loss of dominance status but also in a decrease in social aggression, an effect that was not seen in castrated rats given testosterone-filled silastic tubes. In contrast to the results obtained for mice by Lee and Naranjo (1974), when castrated rats with empty silastic tubes were given testosterone, the degree of aggressiveness was seen to return to levels similar to those emitted by sham castrated control animals. Loss of dominance following the removal of testosterone with the accompanying decrease in aggressiveness was not a consequence of

interactions with a subordinate male. The decline in the high status male's aggressiveness occurred following castration whether a subordinate was present or not.

The behaviour and physiology of dominant animals differs from that of subordinates, but the data from the animal studies also contains a number of conflicting results. Work using mice has shown that testosterone levels in dominant, inbred males were not always elevated compared with subordinates (Barclay and Goldman 1977a, Dessi-Fulgheri et al. 1976a, Selmanoff et al. 1977). However, Buhl et al. (1978) found that dominant male collared lemmings had higher plasma testosterone levels and heavier ventral prostates than did both active and passive subordinates and in a recent study of aggressive dominance using brown lemmings, Huck et al. (1986) found that dominants presented with both the highest testes and seminal vesicle weights together with the highest plasma testosterone levels.

In addition, a number of primate studies, which have looked at the testosterone-social status relationship, have produced a variety of results: in rhesus monkeys, Rose et al. (1971, 1972) found plasma testosterone levels to be positively correlated with an animal's social position as did Eberhart et al. (1980), working with male Talapoin monkeys in mixed sex groups, although prior testosterone levels were not predictive of rank. Similarly, Mendoza et al. (1979) found that when dominance hierarchies were being established in various groups of male squirrel monkeys, the dominant animal showed marked increases in testosterone levels while subordinates showed equally marked decreases. Similar to the findings of Eberhart et al. (op.cit.), the testosterone levels in Mendoza's study prior to interaction, were not predictive of subsequent dominance status. In an experiment which lasted nine months, Rose et al. (1971) found that their thirty-four adult male rhesus monkeys formed linear hierarchies

which were stable for most of the time period. The highest ranking males again had the highest testosterone titres and the data suggest that among these animals, androgen concentrations were a function of dominance and aggressive behaviour and that dominance changes alter testosterone concentrations.

Tree shrews, which have been regarded as primitive primates, when housed in pairs of males under laboratory conditions, very quickly form dominant-subordinate relations (von Holst 1985). Within ten days, subordinate males show a decrease in serum testosterone concentrations of between 30-60%. In contrast, levels for dominants show a marked increase, a trend which was also evident in the weight data of testes, epididymes and sex accessory glands.

As mentioned earlier, a secondary indicator of androgen activity is sex accessory gland weight. Indeed, prior to techniques such as radioimmunoassays for the measurement of hormone levels, gland weight provided valuable information in this area. Besides the work of von Holst, cited above, a number of studies on mice have employed this technique (Christian 1955, Brain 1972a, Bronson 1973, Bronson et al. 1973, Barclay and Goldman 1977a,b). Benton et al. (1978), in a study on male TO mice housed differentially: either singly, in pairs or in groups of twelve, for three weeks, showed that dominants from pairs of males had the heaviest preputial glands compared with all other groups, but their seminal vesicle weights did not differ from other groups although weights for this gland were significantly lower among the subordinate mice. These results are consistent with a number of other reports (Brain 1972a, Chapman et al. 1969, Evans and Mackintosh 1976). Bronson and Marsden (1973) showed that preputial glands in dominant males were 84-86% heavier than singly caged controls and indicated that this gland actually grows in size during attainment and/or maintenance of a dominant social position.

In partial contrast to these last findings, McKinney and Desjardins (1973) reported that the weights of sex accessory glands were greater in isolated males as compared with grouped male mice. They also found that dominants and isolated males were indistinguishable from each other in terms of testosterone production yet both differed significantly from subordinates. These results are supported by Brain and Nowell (1970) who showed that testes and ventral prostate weights were significantly heavier in singly caged mice than in males housed in groups numbering two to sixteen per cage. These authors suggest that pituitary-gonadal function is stimulated by single housing (Brain and Nowell 1971). The subject of single housed males has already been discussed elsewhere (chapters one and four). However, males housed under these conditions were used in the work here for comparison with caged dominants and free range territory holders with the expectation that these three groups would produce similar results (McKinney and Desjardins 1973). Goldsmith et al. (1976) have also shown that singly caged males are able to take on the behavioural and endocrine characteristics of dominant territory holders although it appears that these effects are somewhat dependent upon the age when this form of housing is started. The study by Benton et al. (1978) mentioned above, and Benton and Brain (1979), also showed that isolated males were similar to dominants, having similar seminal vesicle weights. Other workers (Brain 1972a, 1975, Welch and Welch 1971) have also made reference to these similarities and suggest that the behavioural responses are similar to those in a territory-holding mouse. This territorial view has regarded single housing as unstressful because the animals do not fight.

Moving on to the relationship between androgen output and agonistic behaviour, reviews of work carried out on rodents are to be

found in Uhrich (1938), Scott (1966), Valzelli (1973), Brain (1979), Leshner (1975), Gandelman (1980, 1981), Blanchard and Blanchard (1981) and Brain (1983). Interest in the pituitary-gonadal effects on aggression appears to have emerged from early observations that in most species, the male is more aggressive than the female (Uhrich 1938, Seward 1945) and it was held that a straight-forward link existed between androgens and aggressive behaviour. Castrating male mice reduces their aggressiveness and replacement therapy with moderate doses of testosterone is effective in restoring the aggressiveness of castrates to normal levels (Beeman 1947, Bevan et al. 1957). In addition, treatment with testosterone propionate (TP) early in life accelerates the development of aggressiveness in mice. Levy and King (1953) demonstrated that testosterone-treated male mice first show aggression at eighteen days rather than thirty-four days of age. Also, injecting non-aggressive mice with testosterone increases their aggressiveness (Banarjee 1971).

A number of studies have produced results suggesting that the ability of males to fight as adults is dependent upon exposure to testosterone during the neonatal period of development (Bronson and Desjardins 1970, Whitsett et al. 1972, for example). Edwards (1969) has suggested that endogenous testosterone stimulates organisation or causes differentiation of a neural substrate for fighting. The implication then is that early exposure to testosterone produces a permanent alteration of central neural tissue, rendering it responsive to the aggression-activating properties of testosterone, behavioural activation normally occurring in the male at puberty. This has been named the organisation-activation model of gonadal hormones.

On the basis of this model, it can be predicted that animals which have not been exposed to testosterone as neonates, will not

display aggression when administered the hormone during adult life. Recent data however, are not in accord with this prediction and have challenged this model. Work by Svare et al. (1974) shows that doses of testosterone propionate given to ovariectomised adult females, non-neonatally androgen-exposed mice over a period of six weeks resulted in attacks by these animals towards male intruders rendered non-aggressive by extirpation of the olfactory bulbs. Similar findings have been presented by Barclay and Goldman (1977c) and Simon et al. (1984) and it would seem therefore that chronic administration of testosterone in the absence of neonatal exposure to the hormone is sufficient to activate fighting.

From recent work employing both intact animals and castrates given hormone replacement therapy, it appears that although hormones must be present in many cases for the initiation of agonistic behaviour, the behaviour can be emancipated, at least for a time, from the constraints of hormones by appropriate environmental manipulation (Gandelman 1980, 1981). Certainly when aggression takes place among social groups of animals, the actual form of fighting itself is known to differ depending on whether dominant animals fight subordinates or intruders. In the former context, in laboratory cages actual fights and severe injuries are rare compared with the latter and this in itself may result in different endocrine responses (Dijkstra et al. 1984). Indeed questions concerning the effects of fighting on androgen response have stimulated a number of studies. There has been a tendency to adhere to the idea that the rise in levels of aggressiveness that results when rodents, particularly mice, are singly caged, is simply a result of the increased androgen titres that are observed. This would seem to be a rather simplistic interpretation. Work on mice (Clark and Nowell 1978) has demonstrated that a causal relationship may not, in fact, exist.

These workers have shown that isolation of animals resulted in an immediate and continuous rise in levels of aggressiveness when male mice were paired with conspecifics but testosterone levels showed a significant rise only after seven weeks in isolation. They concluded that testosterone is essential for the maintenance of aggressive behaviour but that actual levels are more dependent upon such variables as previous experience of victory rather than reactivity to the presence of a conspecific.

Using rats, Schuurman (1980) has conducted a very extensive series of studies examining the testosterone response to competition. He found that competition had no effect on the testosterone levels of winners but very marked depressive effects on those of the losers. From long-term work on rhesus monkeys, Rose et al. (1971, 1972) also found that defeat experiences depress testosterone secretion markedly. Thus, although there has been controversy over the effects of competition on pituitary-gonadal function, those effects now seem somewhat clearer. Defeat leads to a marked and sustained depression of gonadotrophin secretion and to a decrease in circulating testosterone levels. Victory also leads to a depression in gonadotrophin levels, though less marked than that following defeat (Bronson et al. 1973) but to no change in testosterone levels (Schuurman 1980, Koolhaas 1980). This necessarily leads on to questions concerning the functions of those hormonal changes occurring after agonistic experiences; can they be seen as simply physiological responses to the experience or do they play some important role in determining the individual's future agonistic behaviour patterns? Nock and Leshner (1976), in a study examining patterns of agonistic response in both winners and losers, using castrated mice given hormone replacement therapy, showed that preventing a testosterone response to competition had no effect on

continuing or future agonistic responses. These workers have argued that there appears to be no behavioural significance to the gonadal hormone responses to agonistic experiences. One of the problems of this study was that the testosterone injection regimen used may have resulted in supra-fluctuations in circulating testosterone levels which could have affected results. However, in the following year, in a study employing a more sophisticated method for controlling testosterone levels, by using silastic implants which lead to steadily released and constant circulating levels, Maruniak et al. (1977) obtained the same results as Nock and Leshner. These two sets of data go against a hypothesis that the gonadal hormone response to agonistic experiences has behavioural significance. Further, Lesher (1983) has argued that if these hormonal responses do have any significance, it is either relevant to some non-agonistic behaviour or it is purely physiological, at least in mice.

These arguments however, ignore the possibility that other factors play an important role. For instance, the context within which the interaction of testosterone and aggression takes place may be of significance, be it physical or social. Koolhaas et al. (1980) have shown that after castration, attack latencies in rats increased slowly in the home cage whereas a very rapid increase was noted when animals were tested in unfamiliar cages. These authors have pointed to the complexity of the testosterone-aggression relationship and have argued that the relevance of testosterone for this behaviour depends upon the situation in which it occurs. Schuurman (1980) also found that castrated rats continued defending their home cages against male intruders for weeks whereas in an unfamiliar cage setting, these animals stopped attacking opponents almost immediately which indicated that the former type of aggression is much less testosterone dependent than is maintenance of the latter one.

Results from this study are further emphasised by the findings of Barfield et al. (1972). Testosterone propionate replacement therapy given to castrated rats with pre-operative fighting experience showed that androgen clearly promoted the exhibition of agonistic behaviour compared with untreated castrates although this latter group still fought if sufficiently provoked - the response theshold was elevated but the ability to fully respond was still present. Again, the nature of this effect appears to be much influenced by the test situation employed: dominance relations being less predictable in neutral cages compared with home cage settings for the resident castrates given testosterone propionate. Indeed, both treated and untreated castrates were commonly found to be dominant to intact males especially in their home cages, despite the decline in agonistic behaviour that occurred among untreated castrates.

Besides the physical setting of the cage, depending on the social environment, the link between testosterone and the propensity of male rats and mice to fight may be altered according to other circumstances. Male mice housed with females appear to fight as much as males housed singly whereas aggression levels in all-male groups may be lower (Crawley et al. 1975). Certainly more male mice that have cohabited and mated with intact females subsequently display aggression after castration than do males that have lived with non-cycling (ovariectomised) females (Palmer et al. 1984). Regardless of the presence or absence of females, Gandelman (1981) has shown that fighting experience prior to castration in mice, permits the expression of that behaviour in the absence of gonadal hormones. In rats, Dessi-Fulgheri et al. (1976b) established that males maintained in individual cages or separated by wire-mesh screens from males or females had lower testosterone and estradiol

titres than did males housed in groups of four with four females. This study was discussed in terms of "isolation" and "deprivation" and it is possible that the sexual activity among the mixed sex groups could have been an influential variable besides any inter-male fighting that occurred, in stimulating gonadal output. In an earlier paper, Dessi-Fulgheri et al. (1975) reported that the long-term isolation of male rats resulted in circulating testosterone levels that were higher than those recorded in group housed animals. This was thought to be due to increased "irritable aggression" in response to a plastic bar.

In conclusion, it would seem from the studies reported above that the effects of testosterone on aggression and the converse of this are gradually being clarified. However consideration of the social and physical contexts within which these events are situated, also require due attention and study.

The social environment with respect to the role it plays in connection to subordinate status and gonadal output is now discussed. Besides acting as a stimulus to pituitary adrenal activity, another of the observed effects of social stress is the reduction in output in plasma testosterone. Activation of the pituitary adrenal system is a consistent physiological correlate of stress and it may be that ACTH and/or corticosteroids mediate the suppression of testosterone, either directly or via inhibition of gonadotrophin secretion (Selye 1939, Christian and Davis 1964). However, interaction of the pituitary adrenal and pituitary-gonadal systems with the neuro-endocrine mechanisms responsible for testosterone suppression are poorly understood. Part of the suppressive effect may also involve an increase in the clearance of testosterone from the circulation and/or a decrease in secretion (Gray et al. 1978). This possibility is supported by data from experiments where reduced

gonadotrophin levels have been linked to chronic stress (Bronson 1973, Oyama et al. 1976 cited in Gray et al. 1978). This effect has already been discussed in terms of the consequences of defeat in rodents and monkeys (Schuurman 1980, Koolhaas 1980, Rose et al. 1971, 1972) although reductions in testosterone due to stress are known not to necessarily be paralleled by changes in LH output (McGrady 1984, Sapolsky 1986, Armario 1986) although other results do seem to conflict. In bulls, it has been shown that ACTH-induced increases in serum concentrations of adrenocorticosterioids suppressed the episodic secretory pattern of LH and testosterone and also suppressed the basal secretary rate of testosterone (Johnson et al. 1982). However a decline in testosterone output is not necessarily a direct consequence of ACTH output. In human males, a decrease in testosterone levels was found after ACTH administration (Scharson et al. 1987), an effect not observed after a metyrapone-induced ACTH increase. This result indicates that the reduction in androgen levels was related to cortisol rather than to ACTH itself. Other data from humans does show that stressful events such as intense exercise or fear-provoking situations are associated with a reduction in testosterone levels (Davidson et al. 1978).

Although it appears that the effects of psychological stress in both humans and rodents serves to decrease testosterone output (McGrady 1984), the relationship between these two is less than clear. In addition, whether the stressor is of an acute or chronic nature has been found to produce very different gonadal responses. There is a large literature containing evidence to show that a reduction in testosterone titres is an observed consequence of social stress (for example, in mice, Bronson (1973) and in monkeys, Eberhart et al. (1980), Sapolsky (1985), where subordinate animals have both lower circulating androgen levels and a lower rate of testosterone

synthesis (McKinney and Desjardins 1973) together with lower sex accessory gland weights compared with dominants and single housed animals. Indeed, among these low status animals reproductive function may also be inhibited (Selye 1939). For male tree shrews housed in pairs (Fischer et al. 1985), chronic subordination for fifty days led to severe gonadal regression with reduced testes and prostate weights. Within the epididymis, besides loss of organ weight, there was an enormous increase in the number of immature germ cells indicating serious impairment to fertility.

Other studies by Rose et al. (1975) have shown that rhesus monkeys exhibited marked depression of testosterone due to chronic increases in socially induced stress. In order to explain this, these authors have suggested that these falls in androgen output may be adaptive in that such reductions following defeat or loss of social status decrease the probability of aggressive action on the part of the subject, thereby precluding the likelihood of instigating additional combat and repeated defeats. Interestingly in the wild baboon, Sapolsky (1982) has shown that rapid capture and immobilisation stress resulted in suppressed testosterone concentrations in low ranking males. Middle ranking males were less affected whereas higher ranking animals had elevated testosterone output for the first post-stress hour. Conclusions drawn from this and a subsequent study (Sapolsky 1983) suggest that both acute and sustained stress can suppress gonadal function in subordinates, at least in primates living under natural conditions.

The findings of Sapolsky (1982) and others are illuminating in that they demonstrate how reaction to a stressor, rather than being straight-forward and stereotypic, is also largely dependent upon additional factors such as status so that the way an animal perceives the environment or its own social standing in relation to

conspecifics has a pronounced effect on its behaviour and endocrine function. Despite this, many studies have necessarily ignored complicating variables and have looked directly at the response of gonadal activity to various stressors. In general, the results from a number of workers suggest that the observed response to acute forms of stress is a rise in testosterone output. This has been demonstrated in rats (Armario et al. 1986), rabbits (Faulborn et al. 1979), pigs (Liptrap and Raeside 1975, 1978) and baboons (Sapolsky 1982). In rabbits, Pitzel et al. (1984) have shown that intravenous injections of $ACTH_{1-24}$ produce a biphasic effect on plasma testosterone levels. After an initial rise, twenty minutes post injection, there followed a significant suppression of testosterone by 120 minutes post injection. Although LH and testosterone have been observed to rise transiently in the early stages of stress in a separate study on mice (Frankel and Ryan 1981), Sapolsky (1985) has argued that the suppression of testosterone that may follow is due in part to stress-induced secretion of opioids acting at either the pituitary or hypothalamus to decrease LH secretion, and of cortisol (in monkeys) acting upon the testes to desensitise them to LH. However, considerable individual variation in this phenomenon was observed which was related to an individual's social status. Male rhesus monkeys allowed individual access to receptive females showed a two-to-three fold increase in testosterone which dropped dramatically when these males were subsequently subordinated following the stress of defeat by a large all-male group for a two hour period (Rose et al. 1972). These findings compare well with those of Sapolsky in that testosterone levels were seen to show a marked responsiveness to negative aspects of the social environment. However, interestingly, when two of the subordinated males were reintroduced to the females, nine and fifteen weeks after their

defeat experience, levels of testosterone again rose rapidly to the previously elevated concentrations. More recent contrasting findings in Talapoin monkeys has shown that following chronic social stress, testosterone levels for subordinate males did not increase when these animals were permitted access to receptive females in the absence of other males thereby removing the threat of aggression. The differences between these two studies may be due to whether the animals were acutely or chronically stressed. Male Talapoin monkeys that lose status and become subordinated have lower testosterone levels than those of dominant males (Eberhart et al. 1980) and the administration of sufficient testosterone to produce supranormal "physiological" levels in subordinates does not improve their status in the hierarchy (Dixson and Herbert 1977). From long-term studies on this species, the conclusion has been drawn that it is the social hierarchy which is primarily influential in determining behaviour associated with different social status and which can override the effects of the gonadal hormones (Keverne et al. 1984).

In conclusion to this section, brief mention is made of the effects of stress on gonadal function in response to housing conditions, in particular increased density and crowding. High density has been shown to adversely affect the weights of reproductive organs, particularly in low status animals. The behavioural and endocrine correlates of crowding in rodents have been reviewed many times (Archer 1970, 1979, Calhoun 1962, 1963, Christian 1963, 1971, Brain 1971). The work of Christian (1955) demonstrated that chronically crowding adult male mice resulted in reduced androgen output as measured by sex accessory gland weights. More recently, in a developmental study to investigate the effects of dense housing on the growth of sexual organs and on testosterone levels, Jean-Faucher et al. (1981) showed that by ninety days of age,

male mice in mixed-sex groups of thirty per cage had lowered body, testes and seminal vesicle weights yet testosterone levels were raised, when compared with males from mixed-sex groups of six after puberty between 60 and 90 days. However the large groups had lower testosterone levels between weaning and 50 days although across all groups, the time of first matings was the same. These authors acknowledge that this study does not distinguish between the effects of animal numbers and animal density upon reproductive functions since these two parameters were both increased in the large population group, but they have questioned whether group size is more important than the amount of space available. It would appear though that the exact mechanisms mediating the stress of grouping effects on the pituitary-gonadal axis are not fully understood.

This discussion has centred on a number of subjects including an outline of pituitary-gonadal physiology, the relationships between testosterone levels and social status and also with agonistic behaviour. The effects of social stress, both acute and chronic on testosterone output have also been reviewed together with a brief survey of the effects of dense housing conditions. From the results of a number of these studies, the predictions outlined at the start of this chapter were put forward. The next two sections describe the methods and experiments that were carried out to test these proposals.

Summary of Experiments

Experiments are reported in the following order: FR3, FR5, FR6, FR8, Expt. 11, FR10, FR12.

FR3: Levels of plasma testosterone in caged and free range mice measured at thirteen and nineteen weeks of age.

FR5: Tests carried out on caged and free range mice for levels of testosterone at ten, twelve and seventeen weeks of age.

FR6: Further measures of testosterone levels at twelve, fifteen and seventeen weeks of age.

FR8: Testosterone and trough levels of corticosterone measured at ten, thirteen, fifteen and seventeen weeks of age.

Results for these four experiments showed a marked decline in testosterone levels over time in free range territory holders. Stress, as measured by corticosterone, was not found to be associated with this effect.

Expt. 11: Tests of aggression and testosterone levels in caged, dominant males. Results showed that aggression did not influence circulating levels of testosterone.

FR10: Levels of testosterone in free range mice housed with females over a seven week period. Levels for territory holders were notably raised when females entered post-partum oestrous. Agonistic behaviour recorded in free range males.

FR12: Testosterone levels in free range mice following short exposure to strange females. Levels were found to rise in territory holders only. Agonistic behaviours recorded in free range mice.

Methods

1. Radioimmunoassay for Testosterone

Total plasma testosterone levels were measured using a radioimmunoassay technique after Wheeler and Luther (1983). In terms of the total concentration of circulating testosterone, the percentage of free steroid relative to the amount bound remains the same for each animal regardless of status or other factors.

The following reagents were supplied for the assay from the Department of Chemical Pathology, St. Thomas's Hospital, London.

1. Testosterone standard solution of 10.08ng/ml (Steraloids Ltd., Croydon) in 0.1% phosphate buffered saline (0.1% BSA-PBS).
2. Sheep anti-testosterone antiserum (HP/S/55/1A) (Guildhay Antisera, University of Surrey, Guildford) raised against T-3-(O-carboxymethyl) BSA, 1:25,000 dilution in 0.1% BSA-PBS
3. Iodinated testosterone concentrated in ethanol. The label was prepared by O-linking Carboxymethyl oximino-(2-[^{125}]iodohistamine) using the method of Nars and Hunter (1973), (cited in Wheeler and Luther 1983).
4. Precipitating agent: donkey anti-sheep Ig precipitating antiserum (HP/D/14 - XIIIA, Guildhay, Ltd.) 1:100 dilution with carrier sheep serum at 1:2000 in polyethylene glycol 6000 (PEG).
5. Buffer 1 - 0.1% BSA-PBS, for diluting standards and plasma samples.
6. Buffer 2 - 2.5% BSA-PBS, for diluting iodinated testosterone and for a wash step.
7. Plasma quality control samples of known concentrations.

Glassware

Glassware for extraction and evaporation of solvent was soaked overnight in 4% Lipsol, then rinsed in deionised water, soaked for 2-3 hours in 5% HCl, rinsed in distilled water and oven-dried. Just

prior to use, all glass tubes were washed with 1.0ml of diethyl ether (BDH) and air dried. Disposable Luckhams tubes and caps were used in the assay.

Assay Procedure

On day 1, plasma samples (typically 30µl) and standards diluted in 0.1% BSA-PBS, together with a zero standard of 0.1% BSA-PBS, were extracted by adding 3.0mls of fresh diethyl ether (ANALAR, BDH) and vortexing for 8 minutes. The aqueous phase was then frozen quickly in cardice (dry ice/acetone) and the organic phase decanted and evaporated to dryness in a warm water bath at 37°c for approximately one hour. Samples were redissolved in 300µl 0.1% BSA-PBS and vortexed at 10 minute intervals for 30 minutes. 0.1ml aliquots were then assayed in duplicate by the addition of 0.1ml of sheep antiserum to all tubes except those for total counts and non-specific binding (NSB), and 0.1ml of iodinated labelled testosterone, diluted 1:100 in 2.5% BSA-PBS, to all tubes. After vortexing, tubes were covered and incubated for 16-18 hours at 4°C.

On day 2, 0.5ml of PEG precipitating agent (containing the second antibody) was added to all tubes except total counts. After vortexing and standing at room temperature for 30 minutes, tubes were centrifuged at 3000rpm for 20 minutes at 4°C, followed by partial aspiration. The pellet was then resuspended in 1.0ml of 2.5% BSA-PBS which was added to all tubes except total counts tubes, vortexed and centrifuged for 30 minutes as before. This was followed by full aspiration of the supernatant. Tubes were then capped and counted for one minute each on a Packard Modumatic 6. Counts were converted to ng/ml and read off against the standard curve using a least-squares fit computer programme (Amersham Radioassay Curve Fit Program).

The testosterone antiserum cross-reacted significantly with only

5α-dihydrotestosterone (20%) and less than 0.1% with other steroids. The antiserum bound 85.6% of the testosterone label (S.D.=7.13%); mean recovery (±S.D.) was 97.6(±12.0%) and was not corrected for; non-specific binding (±S.D.) was 1.3(±0.4%) for 31 assays. Intra- and interassay variability for different known concentrations of plasma testosterone is shown in Table 5:A. The zeros (mean 3.3 pg/ml, S.D. 1.0 pg/ml) were below the designated sensitivity of the assay. The standard curve was parallel to plasma dilution curves for human and mouse plasma over a range of sample sizes used (Figure 5:1, 5:2a, 5:2b). A precision profile of the standard curve as %CV is shown in Figure 5:3 with further details in Table 5:B. Values are derived from duplicates for each of the eight points from six standard curves.

2. Post Mortem Data

Procedures for the removal of left testes and preputial glands are detailed in Chapter 2.

3. Blood Sampling

Blood from each animal was obtained followed the procedure laid out in Chapter 2. As far as possible, blood was collected within three to five minutes of disturbing a cage or opening the door to the free range room. This precaution was taken because although evidence is lacking for mice, there is data to suggest that acute forms of stress may produce a rise in testosterone levels prior to their decrease, five to ten minutes after the onset of stress in baboons (Sapolsky, 1982) and within ten to twenty minutes in rabbits (Pitzel et al., 1984). In rats, an acute stress-induced rise in plasma testosterone levels was found to occur five minutes after ether anaesthesia, reaching concentrations that were significantly high, compared with basal levels, by thirty minutes, (Frankel and Ryan, 1981). Sampling was carried out between 11.00 and 12.30 hours.

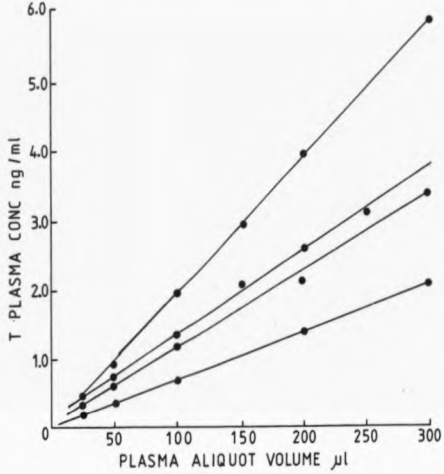
TABLE 5:A WITHIN AND BETWEEN ASSAY VARIATION

Pool Conc ng/ml	No. of Samples	Conc. of Testosterone	S.D. %	CV %	RECOVERY %
0.835	8	0.783	±5.6	±7.2	93.7
0.993	7	0.942	±5.9	±6.2	94.7
0.85	14	0.84	±9.5	±11.3	98.8
4.17	8	3.93	±17.0	±12.2	94.3
6.19	6	7.0	±22.4	±7.7	114.5
6.33	14	6.38	±23.2	±13.5	100.7

Mean recovery = 99.5 ± 7.9% (S.D.)

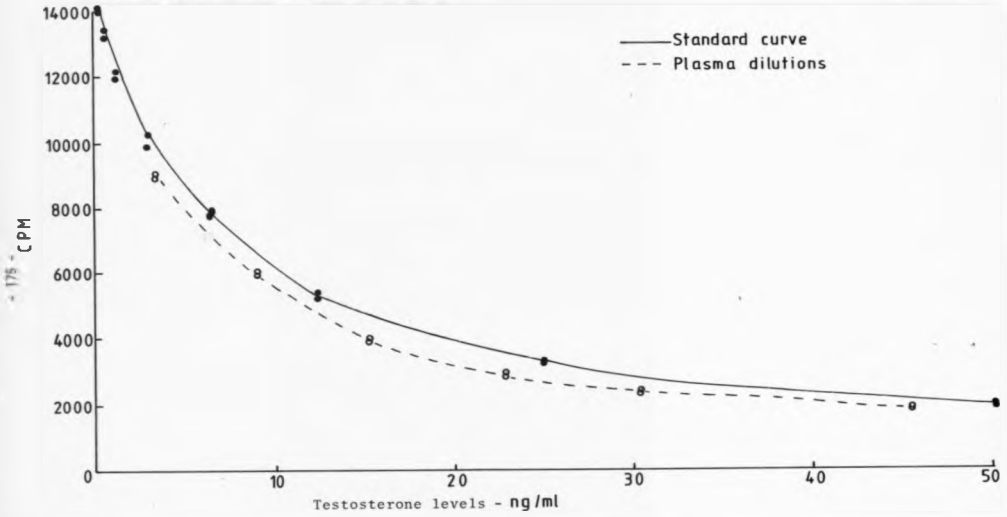
Mean Coefficient of Variation = 9.68 ± 3.02% (S.D.)

Serial dilutions of plasma Fig 5:1



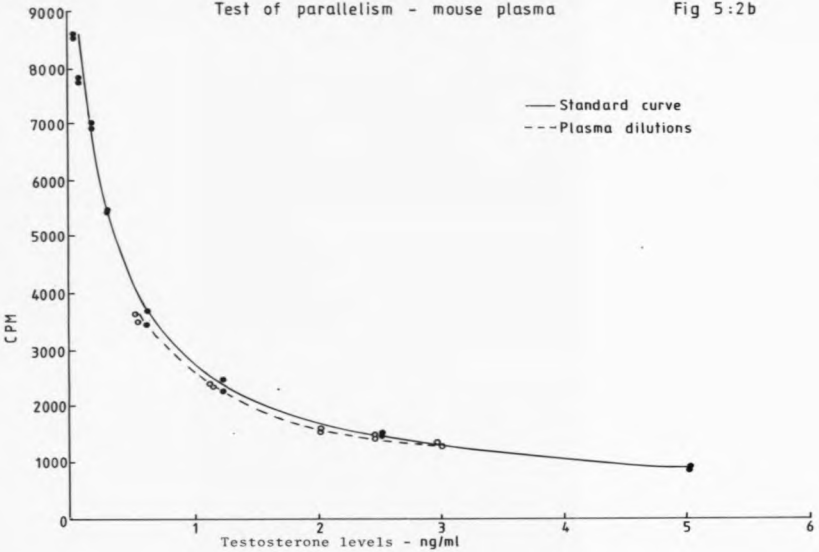
Test of parallellism - Human plasma

Fig 5:2a



Test of parallelism - mouse plasma

Fig 5:2b



Testosterone RIA - Precision profile, coefficient of variation (%)

Fig 5.3

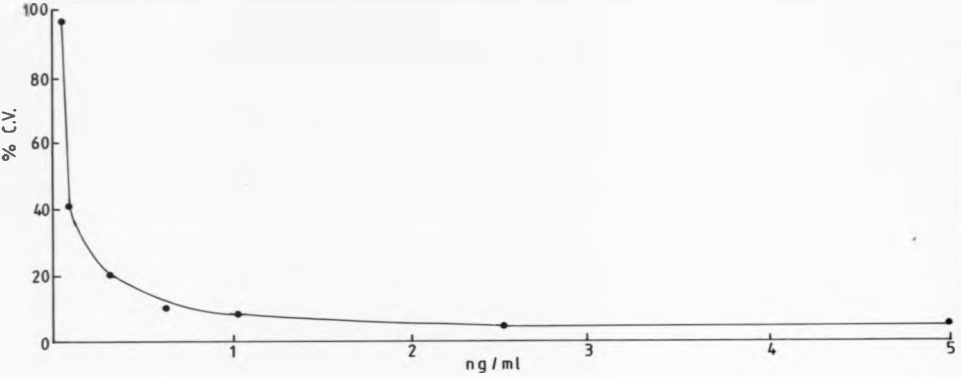


TABLE 5:B PRECISION PROFILE

Conc. ng	N.	Mean	S.D.	CV %
5.04	6	4.90	0.32	6.6
2.52	6	2.54	0.11	4.6
1.26	6	1.26	0.11	8.4
0.63	6	0.63	0.06	9.9
0.315	6	0.31	0.06	20.8
0.157	6	0.15	0.04	28.9
0.078	6	0.08	0.03	41.0
0.039	6	0.04	0.04	97.4

After every occasion when blood sampling was carried out, observations were made two or three times weekly for fifteen to twenty minutes each day under red light, in order to verify that no changes in social status had taken place.

4. Data analysis

Unless otherwise stated data were analysed by parametric statistics: one-way and two-way mixed design, analyses of variance, Student's t-tests and Tukey's Multiple Range Test. All data for organ weights were recorded as a percentage of total body weight and arcsine transformed prior to statistical analyses. Analysis of data based on actual organ weights did not differ in the results from those data which were recorded as percentages and arcsine transformed. As a consequence only this latter form of data analysis is presented in the results. Values depicted as histograms are shown as means and standard errors of means.

Experiments

Experiment FR3 A preliminary study was carried out to examine basal levels of testosterone in both caged and free range mice. Blood sampling was carried out on two occasions. The first was at thirteen weeks of age when the mice had lived in their respective housing conditions for two to three weeks, and after the barriers in the free range room had been removed one week earlier. Six weeks later, at nineteen weeks of age, a second blood sample was taken from all animals.

Plasma levels of testosterone were determined in the following social groups:

Free Range	Territory holders	n=6
	Subdominants	n=7
	Subordinates	n=7
Group Housed	Dominants	n=4
	Subordinates	n=14
Single Housed		n=11

After the second samples were taken, animals were killed by cervical dislocation and the left testes removed and weighed within one hour of death.

Results and Discussion

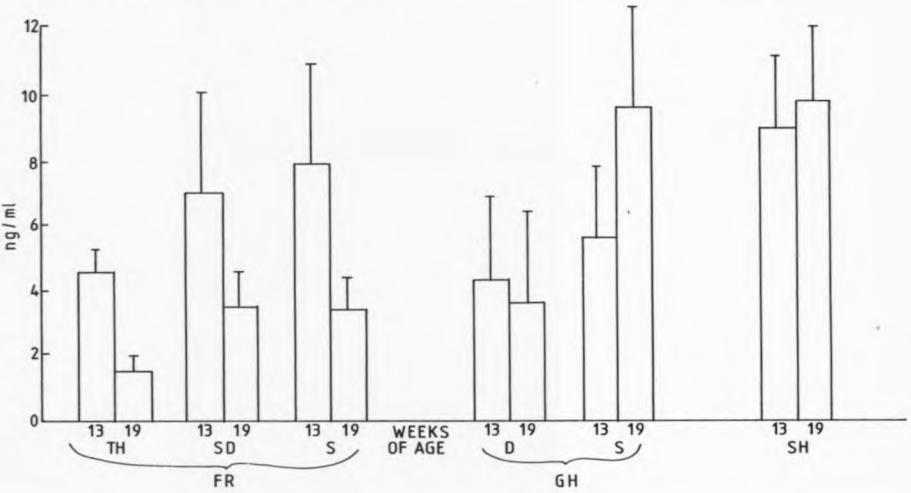
Figure 5:4 shows the means and standard errors for testosterone levels in these different groups. Table 5:C shows the 2-way ANOVA results for the data. There were no significant differences between or within the groups although comparison of the data for the free range mice for change over time just failed to reach significance. Territory holders were seen to possess the lowest levels of hormone both at 13 and 19 weeks. Consistently high levels were found in single caged mice.

Figure 5:5 gives the means and standard errors for the left testes weights as percentages of total body weight. Analysis of arcsine transformed data gave no significant differences between any of the groups although it was noticeable that free range territory holders, as well as showing reduced testosterone levels by nineteen weeks, also were found to have low testes weights at autopsy. The highest testes weights recorded were in singly caged mice.

These results were surprising in that they went against the

Testosterone levels at 13 and 19 weeks

Fig 5:4

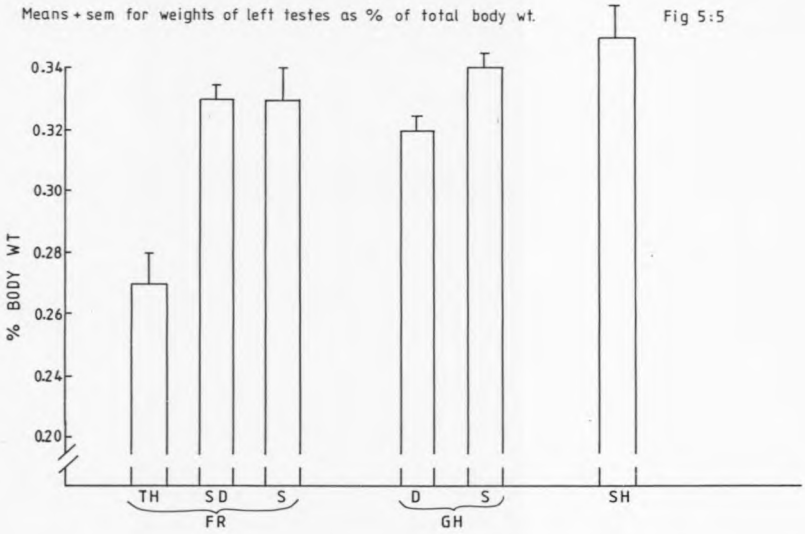


ANOVA TABLE 5:C

	Source	df	F-ratio	Probability
a) Single housed, caged dominants and free range territory holders	Trt. Groups	2+18	2.5283	0.11
	Time	1+18	0.1755	0.68
	Trt. x time interaction	2+18	0.6098	0.55
b) Free range territory holders, subdominants and subordinates	Trt. Groups	2+17	1.0145	0.38
	Time	1+17	3.6557	0.07
	Trt. x time interaction	2+20	0.0630	0.94
c) Free range and caged subordinates	Trt. Groups	1+20	0.0006	0.98
	Time	1+20	0.4614	0.24
	Trt. x time interaction	1+20	1.9311	0.18
d) Group caged dominants and subordinates	Trt. Groups	1+17	0.3258	0.58
	Time	1+17	0.0142	0.91
	Trt. x time interaction	1+17	0.0792	0.78

Means + sem for weights of left testes as % of total body wt.

Fig 5:5



original prediction made that free range territory holders would have higher testosterone levels than males of lower status as well as higher testes weights. As a consequence, further experiments were carried out to verify these findings.

Experiment FR5 In this experiment to examine testosterone levels, the first blood samples were obtained at ten weeks before differential housing, just prior to free range entry and single cage housing, at twelve weeks, three days prior to removal of the metal barriers and then four weeks later, at seventeen weeks of age. The following social categories of mice were examined:

Free range	Territory holders	n=3
	Subdominants	n=3
	Subordinates	n=13
Group housed	Dominants	n=3*
	Subordinates	n=16
Single housed		n=19

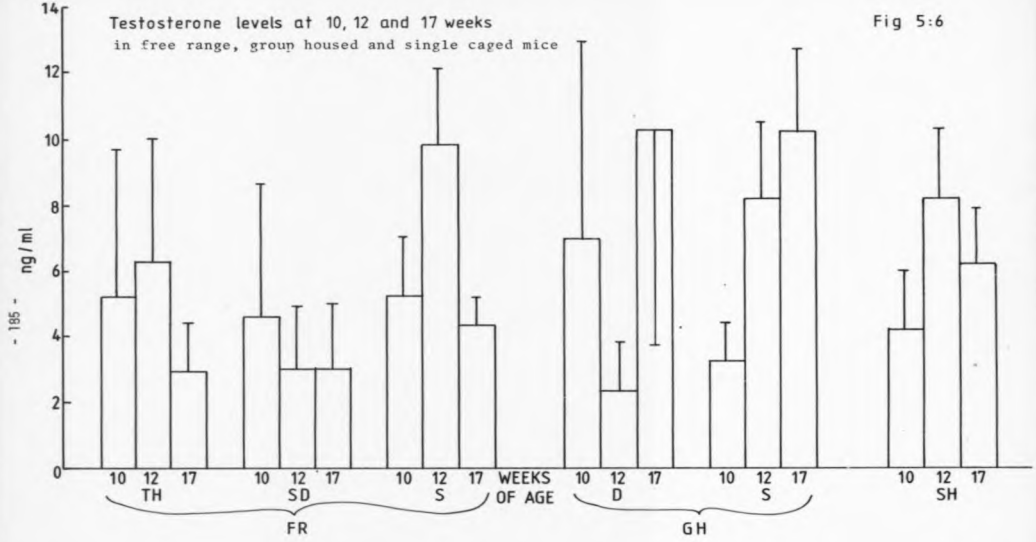
(*Difficulty was encountered in obtaining blood from a fourth caged dominant animal.) After each set of blood samplings, the status of the animals was verified by daily observations lasting 15-20 minutes. In week 19, free range and group housed mice were killed and the left testis of each animal removed and weighed as in the previous experiment. Due to an outbreak of cage mite among several of the single caged mice in the last two weeks of the experiment, this group was not autopsied.

Results

Levels of plasma testosterone between ten and seventeen weeks for the different groups are shown in Figure 5:6 and analysis of these data are given in ANOVA Table 5:D. The means and standard

Testosterone levels at 10, 12 and 17 weeks
in free range, group housed and single caged mice

Fig 5:6



ANOVA TABLE 5:D

	Source	df	F-ratio	Probability
a) Single housed, caged dominants and free range territory holders	Trt. Groups	2+22	0.1406	0.87
	Time	2+44	0.0548	0.95
	Trt. x time interaction	4+44	0.7155	0.59
b) Free range territory holders, subdominants and subordinates	Trt. Groups	2+16	0.6043	0.56
	Time	2+32	0.7770	0.47
	Trt. x time interaction	4+32	0.3294	0.86
c) Free range and caged subordinates	Trt. Groups	1+27	0.1895	0.67
	Time	2+54	2.5154	0.09
	Trt. x time interaction	2+54	2.2864	0.11
d) Group caged dominants and subordinates	Trt. Groups	1+17	0.0785	0.78
	Time	2+34	1.0131	0.37
	Trt. x time interaction	2+34	0.6843	0.51

TABLE FR5

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 10, 12 AND 17 WEEKS OF AGE

	ng/ml		
	10	12	17
Free range			
Territory holders	5.2 ± 4.4	6.3 ± 3.7	2.9 ± 1.5
Subdominants	4.6 ± 4.9	3.0 ± 1.9	3.0 ± 2.0
Subordinates	5.2 ± 1.8	9.8 ± 2.3	4.3 ± 0.9
Group Housed			
Dominants	6.9 ± 6.0	2.3 ± 1.5	10.3 ± 6.6
Subordinates	3.2 ± 1.2	8.2 ± 2.3	10.2 ± 2.5
Single caged	4.3 ± 1.7	8.2 ± 2.2	6.2 ± 1.7

errors are also shown in Table FR5. Again, no significant differences were found between any of the groups although by seventeen weeks, the lowest levels for free range mice were seen in the territory holders. Indeed, these levels were the lowest when also viewed in relation to other groups. Levels were seen to drop in the group housed dominants at twelve weeks but rose again by seventeen weeks.

Figure 5:7 shows left testes weights as percentages of total body weight. Only group caged subordinates, when compared to free range subordinates, showed a significant difference with the group caged mice having heavier testes ($df=30$, $p<0.05$). No other differences were found.

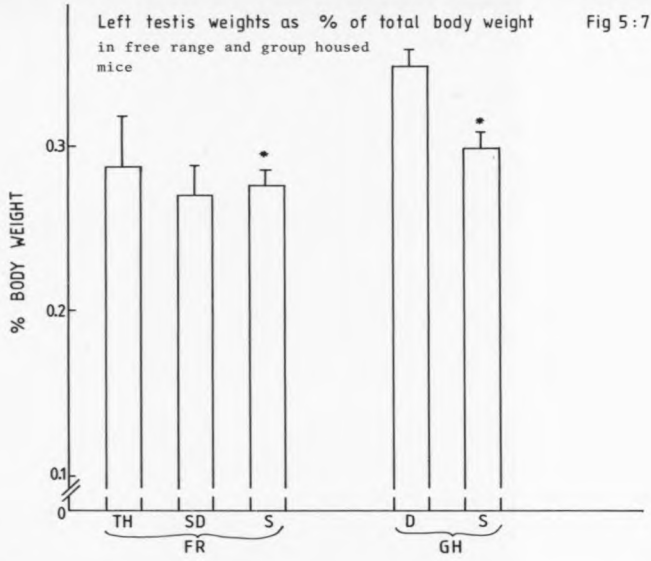
Experiment FR6 Levels of plasma testosterone were further investigated in free range and caged mice. Animals were blood sampled at twelve weeks, three days before removal of the barriers and then at fifteen and seventeen weeks of age (three and five weeks following their removal). At nineteen weeks, animals were killed and autopsied and the weights of left testes, preputials and adrenals were recorded. The following social groups of mice were examined:

Free range	Territory holders	n=5
	Subordinates	n=15
Group housed	Dominants	n=4
	Subordinates	n=16
Single housed		n=17

In this experiment, no subdominant mice were identified in the free range room.

Left testis weights as % of total body weight in free range and group housed mice

Fig 5:7



Results and Discussion

The means and standard errors of plasma testosterone levels for these different social groups are shown in Figures 5:8 and 5:9 and details of the data analysis are given in ANOVA Table 5:E. Means and standard errors are also shown in Table FR6. Significant differences were found between single housed mice, group housed dominants and free range territory holders ($p<0.001$) as well as there being a significant interaction between these groups over time ($p<0.04$, Table 5:E Anova a). Further analysis by Tukey Test at the 5% level of significance showed that the mean level of testosterone for group housed dominants at 17 weeks differed significantly from the means for the free range territory holders at weeks 15 and 17 and from the single housed mice at week 12, just missing significance at 15 weeks.

Among the free range mice, no difference was found between the groups (Figure 5:9) although within group analysis showed that levels differed significantly over time ($p<0.001$ Table 5:E Anova b). A further breakdown of this data across weeks adding the data together for the two groups, using Student's t-tests gave the following results:

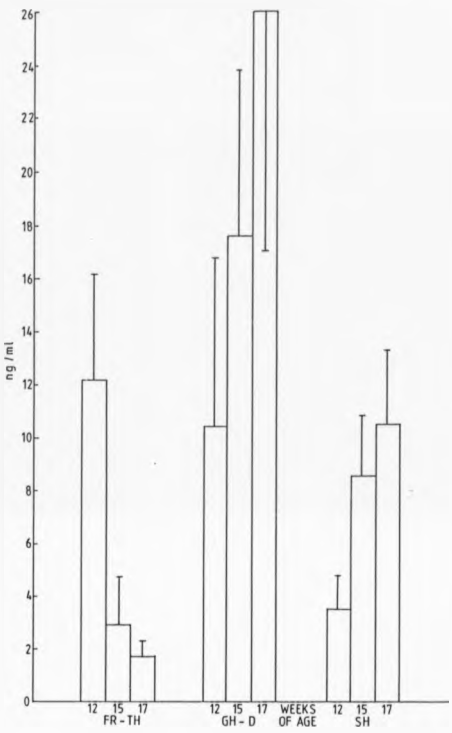
WEEKS	t-value	df	Probability
12 v. 15	2.92	38	0.005
15 v. 17	1.31	38	N.S.

A significant drop in levels was observed between weeks twelve and fifteen.

Data analysis for the subordinate animals, group housed and free range, showed significant changes in levels over time ($p<0.04$, Table 5:E Anova c). A comparison of levels at the three sampling times using Student's t-tests showed no significant difference between

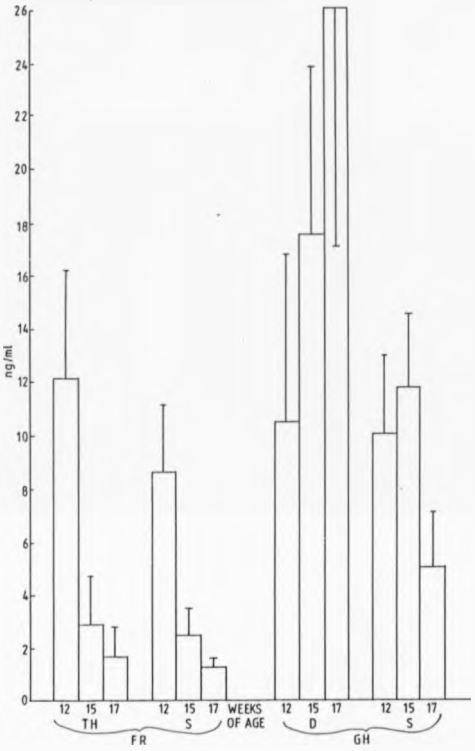
Testosterone levels at 12, 15 and 17 weeks in free range, group housed and single caged mice

Fig 5:8



Testosterone levels at 12, 15 and 17 weeks in free range and group housed mice

Fig 5:9



ANOVA TABLE 5:E

	Source	df	F-ratio	Probability
a) Single housed, caged dominants and free range territory holders	Trt. Groups	2+23	11.7992	0.001
	Time	2+46	0.7472	0.48
	Trt. x time interaction	4+46	2.6464	0.05
b) Free range territory holders and subordinates	Trt. Groups	1+18	0.5518	0.47
	Time	2+36	9.7910	0.001
	Trt. x time interaction	2+36	0.3503	0.70
c) Free range and caged subordinates	Trt. Groups	1+29	9.8356	0.003
	Time	2+58	3.3842	0.04
	Trt. x time interaction	2+58	1.4032	0.25
d) Group caged dominants and subordinates	Trt. Groups	1+18	13.3980	0.001
	Time	2+36	0.6250	0.54
	Trt. x time interaction	2+36	2.1927	0.13

TABLE FR6

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 12, 15 AND 17 WEEKS OF AGE

	ng/ml		
	12	15	17
Free range			
Territory holders	12.2 ± 4.0	2.9 ± 1.4	1.7 ± 0.9
Subordinates	8.6 ± 2.5	2.5 ± 1.0	1.3 ± 0.3
Group housed			
Dominants	10.4 ± 6.4	17.6 ± 6.2	26.0 ± 8.9
Subordinates	10.0 ± 3.0	11.8 ± 2.8	5.0 ± 2.1
Single caged	3.5 ± 1.2	8.5 ± 2.3	10.5 ± 2.8

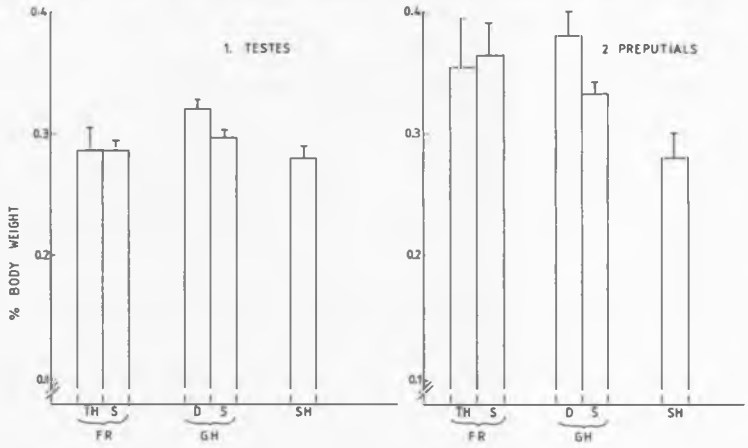
weeks 12 and 15 but a difference was found between weeks 15 and 17 ($t=1.95$, $df=60$, $p<0.05$). Levels also differed significantly between the two groups ($p<0.005$), and from the histogram (Figure 5:8) it can be seen that for the free range subordinates in particular, levels were very reduced by 17 weeks.

The final Anova result on Table 5:E compares the data for the group housed dominants and subordinates. Here, a significant difference was found between the groups ($p<0.001$) with levels for the dominant animals increasing over the seven weeks whilst those for the subordinates, although showing a rise between weeks 12 and 15, dropped back by week 17.

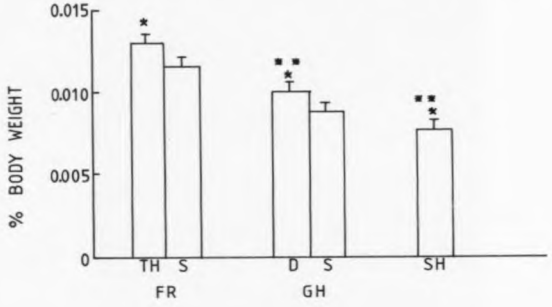
The means and standard errors for the weights of left testes, preputials and adrenals are shown in figures 5:10 and 5:11. Analysis of arcsine transformed data gave no differences between free range territory holders, group housed dominants and single housed mice for testes and preputial weights but territory holders had significantly higher left adrenal weights when compared with both group housed dominants ($t=2.66$, $df=7$ $p<0.025$) and single housed mice ($t=3.57$, $df=20$, $p<0.005$). Adrenal weights for group housed dominants were also significantly higher when compared with single housed animals ($t=2.58$, $df=19$, $p<0.02$, Mann-Whitney U Test: $U=6.5$, $p<0.01$).

Among the two social groups of free range mice, no significant differences were found for any organ weights. In the group housed mice, dominants were seen to have significantly heavier testes ($t=1.75$, $df=17$, $p<0.05$) and preputials ($t=1.75$, $df=17$, $p<0.05$) compared with subordinates but there was no difference in adrenal weights. Comparison of the data for the two groups of subordinate mice showed no differences for testes and preputial weights but free range subordinates were found to have significantly heavier adrenal glands ($t=3.81$, $df=28$, $p<0.001$). The weights of all three organs

Means and sem for left testes and preputials as percentages of total body weight Fig 5:10



Left adrenal weights as % of total body weight Fig 5:11



were seen to be lowest in single housed mice.

In view of the low levels of testosterone again recorded for the territory holders, together with the data showing raised adrenal gland weights, the following experiment was carried out which compared both gonadal and adrenal output.

Experiment FR8 This experiment examined both testosterone and corticosterone levels in free range and caged mice over a number of weeks in an attempt to find out if social stress, as indicated by raised plasma corticosterone levels, was associated with gonadal activity. The details of these tests which measured basal trough levels of corticosterone are already fully described in Chapter 4. Each plasma sample obtained from an animal was used to determine both corticosterone and testosterone levels. It will be recalled that for the purpose of this experiment, the lighting arrangements of the animal rooms were altered to facilitate blood sampling (red lights on on 3.00 hours, white lights on 13.00 hours). Blood sampling was carried out between 11.00 and 12.30 hours and samples were obtained within three minutes of cage disturbance or opening the door to the free range room. The first samples were taken at ten weeks of age, when all animals were still group caged, prior to free range entry and single housing then again at thirteen, fifteen and seventeen weeks, one, two and four weeks after the removal of the barriers. The following social groups of mice were used:

Free range	Territory holders	n=5
	Subdominants	n=4
	Subordinates	n=11
Group housed	Dominants	n=4
	Subordinates	n=16
Single housed		n=20

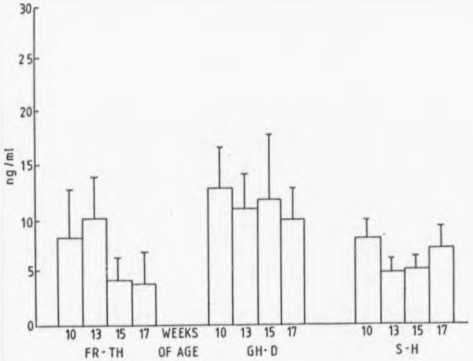
Results and Discussion

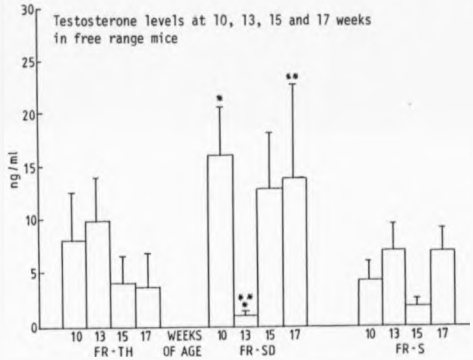
Testosterone levels for these groups are shown in Figures 5:12 and 5:13 and the analysis of the data is given in ANOVA Table 5:F. Means and standard errors are shown in Table FR8. No differences in the levels were found between free range territory holders, group housed dominants and single housed mice but territory holders again showed a trend towards a lowering of levels over time ($p<0.09$, Table 5:F Anova a) compared with the other two groups. Among the social groups in the free range, the between and within group analyses showed no significant differences but the interaction (groups versus time) was significant ($p<0.02$, Table 5:F Anova b). Further breakdown of the data using a Tukey Test at the 5% level of significance, showed that for subdominants, the mean level at ten weeks differed from that at 13 weeks. The mean level at week 17 also differed significantly from week 13. At seventeen weeks, the mean level for subdominants was different compared with the mean for the same group at thirteen weeks ($p<0.01$). No differences were found between levels for group housed and free range subordinates (Table 5:F, Anova c) but when group housed dominants and subordinates were compared (Anova d), these two groups differed significantly from one another ($p<0.05$) with high testosterone levels seen in the dominant mice compared with lower levels in the subordinates.

The results of these last three experiments were similar to those of experiment FR3. Testosterone levels were seen to drop over time in the territory holders, a result not observed in the other high status males although the data only reached statistical significance in experiment FR6. These levels were also reflected in the data for sex accessory gland weights although no statistical differences were found between the high and low status males of the free range, or among any of the high status categories.

Testosterone levels at 10, 13, 15 and 17 weeks in territory holders, group housed dominants and single caged mice

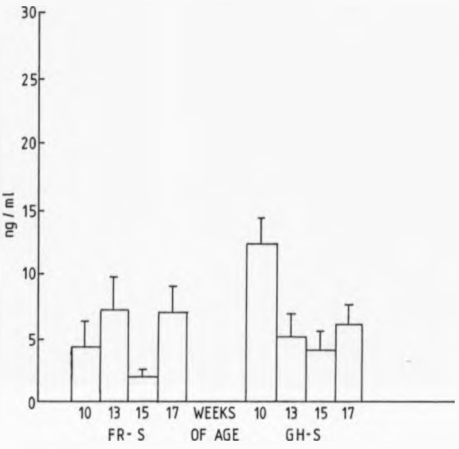
Fig 5:12





Testosterone levels at 10, 13, 15 and 17 weeks in free range and group housed subordinates

Fig 5:13



ANOVA TABLE 5:F

	Source	df	F-ratio	Probability
a) Single housed, caged dominants and free range territory holders	Trt. Groups	2+26	2.6988	0.09
	Time	3+78	0.6019	0.62
	Trt. x time interaction	6+78	0.4635	0.83
b) Free range territory holders, subdominants and subordinates	Trt. Groups	2+17	2.8723	0.08
	Time	3+51	0.8445	0.48
	Trt. x time interaction	6+51	2.7492	0.02
c) Free range and caged subordinates	Trt. Groups	1+25	2.8538	0.10
	Time	3+75	2.4465	0.07
	Trt. x time interaction	3+75	2.3443	0.07
d) Group caged dominants and subordinates	Trt. Groups	1+18	4.4991	0.05
	Time	3+54	1.0639	0.37
	Trt. x time interaction	3+54	0.4287	0.73

TABLE FR8

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 10, 13, 15 AND 17 WEEKS OF AGE

	ng/ml			
	10	13	15	17
Free Range:				
Territory holders	7.3±3.6	10.0±4.0	4.2±2.3	3.8±3.0
Subdominants	15.8±4.9	1.0±0.2	12.8±5.2	13.7±9.5
Subordinates	4.3±1.8	7.0±2.5	2.0±0.5	7.3±2.0
Group Housed:				
Dominants	12.8±4.0	10.8±3.2	11.6±6.0	9.7±3.0
Subordinates	12.4±1.9	5.0±2.1	4.1±1.5	6.2±1.8
Single Caged	8.0±1.7	4.9±1.2	5.0±1.3	7.2±2.1

The data for the corticosterone levels are presented in Chapter 4, Figures 4:9, 4:10, 4:11 and 4:12 and analysis of the data is given in Anova Table 4:C. No significant differences were found when the data from the various groups was compared.

A major problem with the experiments was the small number of high status males both in cages and the free range at any one time as well as the lack of control over the number of territory holders that were in the free range during an experiment. With very few subjects, obtaining statistical significance was extremely difficult. As a consequence, it was decided to pool the data from experiments FR5, FR6 and FR8 for the territory holders, the group housed dominants and for the singly housed males for the measurements made on testosterone levels at 12/13 and 17 weeks of age and these data are shown in figure 5:14. It is well appreciated that doubt can be cast on the validity of this exercise, particularly in view of the fact that besides the blood sampling that took place, the animals underwent other treatments in two of these three experiments (pain threshold tests in experiment FR5 and a reversal of the lighting regime in experiment FR8). Although the results of the analysis proved interesting, it would be unwise to place too much emphasis on them.

A two-way Anova analysis of the data gave the following results, and the means and standard errors for the combined data are shown in Figure 5:14 and in Table FR5-6-8.

Data collapsed together from FR5, FR6+FR8 Fig5:14

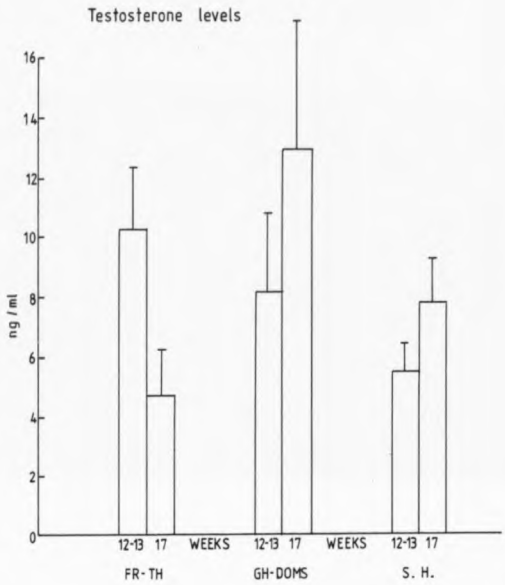


TABLE FR5-6-8

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS OF COMBINED DATA FOR 12/13 AND 17 WEEKS OF AGE

	ng/ml	
	12/13	17
Free range territory holders	10.3 ± 2.1	4.68 ± 1.5
Group housed dominants	8.2 ± 2.7	12.85 ± 4.4
Single caged	5.5 ± 0.9	7.8 ± 1.3

Source	df	F-ratio	Probability
Trt. Gropus	2+77	1.614	0.20
Time	1+77	0.058	0.80
Trt. x time interaction	2+77	3.035	0.05

The interaction was found to be significant with a strong downward trend in levels over time for the territory holders which was not observed in the other two groups. Levels for the territory holders also differed significantly between week 12/13 and 17, when Student's t-test was used ($t=2.14$, $df=22$, $p<0.025$).

An important difference between the free range territory holders and the group housed dominants was that in the cages, dominants only encountered other males that had been defeated and were submissive to them. Although this was true for the territory holders within their own areas and relative to the subordinates they lived with, other territory areas held mice that had not been defeated and therefore posed a threat. Fights between the animals of different groups took place but were generally inconclusive in outcome. Because territory holders had fights that they did not win but did not lose either, it was hypothesised that this might be having a depressive effect on testosterone levels. In the following experiment, a number of tests using controlled fights set out to test this.

Experiment 11 All tests were run on animals housed in cages: the free range room was not used. Ten, eight month old male mice that had been castrated at the age of three months, were used to train groups of intact male mice to fight. To achieve this, urine was collected individually overnight from five single housed, four month intact males that had been caged singly since the age of ten weeks. The method for urine collection is fully described in Chapter 6. After

collection, the urines were pooled, capped and stored at 4°C. Fighter mice were single housed and of four to six months of age, having been caged singly since the age of ten weeks. Four groups of fifteen mice were trained to fight over a twelve week period. This was done by painting collected urine on the backs of the castrated males and then placing them individually in the home cages of intact males. Animals were timed for 15-20 seconds after the resident mouse began an attack after which time, the castrate was removed. This procedure was employed as it is known that the urine of single caged males is able to promote aggression in other males (Mugford 1973, Mackintosh and Grant 1966), and it was carried out daily for fourteen days prior to each main test being run. After this time, mice that attacked the intruder within 1-2 seconds of it being introduced into the home cage, were designated as "fighter mice". Mice that did not meet this criterion were not used. No fighter mouse was used in more than one of the main test conditions.

Experimental male mice were aged between three and five months and housed in cages in groups of five. Fifty-eight cages of five mice were used over a twelve week period. Observations to determine which mouse was dominant in each group were carried out under red light between 14.00 and 15.30 hours for approximately 20 minutes on eight consecutive days. On day 9, at approximately 10.00 hours, a blood sample was taken from the dominant male in each group. A four day recovery period then followed during which time, daily observations were made to verify status. On day thirteen, groups of animals were placed in one of five experimental conditions.

Condition One A dominant mouse was removed from his home cage and placed in the cage of a trained fighter. The aim was to simulate an encounter with a neighbouring territory holder on an opponent's territory. Animals were timed for 5 seconds from the start of

fighting after which time the dominant male was removed and returned to his own cage. The same two animals were placed together between 11.00 and 12.00 hours on alternate days and seven encounters took place over a fourteen day period. Ten dominant males were used.

Condition two. Dominant mice were not engaged in fights against opponents but served as controls for Condition One, remaining in their home cages. Ten dominant males were used.

Condition three. Groups of five mice were housed in one half of a double cage (42x50x11cms) divided down the centre by a wire mesh barrier. A trained fighter mouse was housed alone in the other half. As described in Condition One, the dominant male of each group was placed on 7 alternate days in the half of the cage containing the fighter male and the animals were allowed to fight as before. The simulation this time included the permanent presence of an aggressive neighbour. They were then separated and the dominant neighbour was returned to his half of the cage. Fourteen dominants were used.

Condition Four Groups of five mice were again housed in one half of divided cages. In this condition, the fighter males that were housed in the other halves were removed and placed in the halves occupied by the dominant and his group. The procedure for fighting was the same as in Conditions One and Three. This simulated an encounter with an invading territory holder on a subject's home territory. Twelve dominant males were used.

Condition Five This served as a control condition. Fighter mice and mice in groups of five were again housed in the two halves of divided cages but fighters and dominants never encountered each in fights, and only had visual, auditory and olfactory contact. Twelve dominant males were used.

For each condition, every dominant male was again blood sampled two days after the final test day after which, these mice were killed

and the left testes, preputials and adrenals were removed and weighed.

The chart below provides a summary of the experimental regime used:

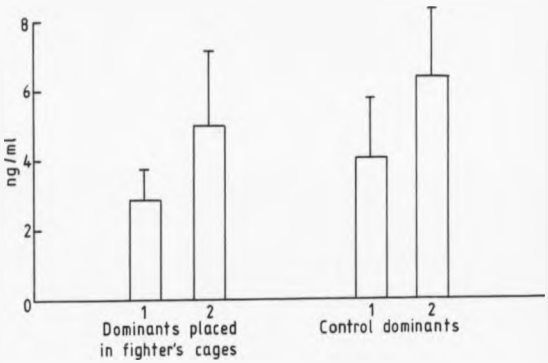
DAYS	1-8	Daily observations for dominance.
DAY	9	First blood samples taken followed by four recovery days.
DAY	13	Animals moved to experimental conditions.
DAY	14	First test day - controlled fights on seven alternate days.
DAY	28	Second blood samples taken, animals killed and autopsied.

Results Figure 5:15 shows the means and standard errors for levels of plasma testosterone in dominant mice for the five conditions detailed above. Data was analysed by 2-way ANOVA as follows:

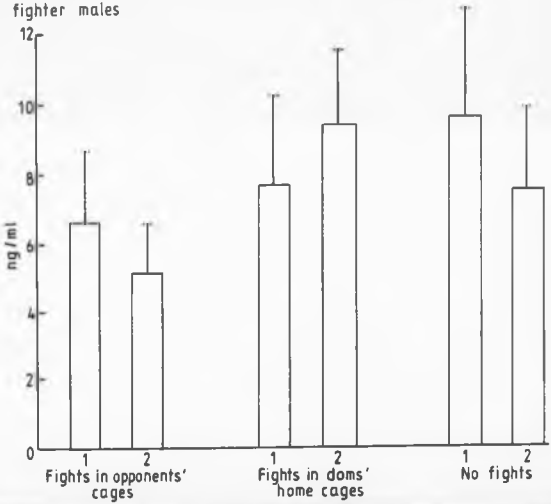
	Source	df	f-ratio	Probability
a) Large undivided cages-control vs. experimental	Trt. Gps.	1+18	0.52	0.48
	Time	1+18	1.57	0.23
	Trt. x time interaction	1+18	0.0005	0.98
b) Divided cages - one control vs. two experimental conditions	Trt. Gps.	2+33	0.82	0.45
	Time	1+33	0.09	0.75
	Trt x time interaction	2+33	0.38	0.69

Testosterone levels of dominant males over two weeks, housed in group cages - One group subjected to fights

Fig.5:15



Testosterone levels in dominant males over two weeks, housed in wire-mesh divided cages from trained fighter males



There were no significant differences within or between any of the groups.

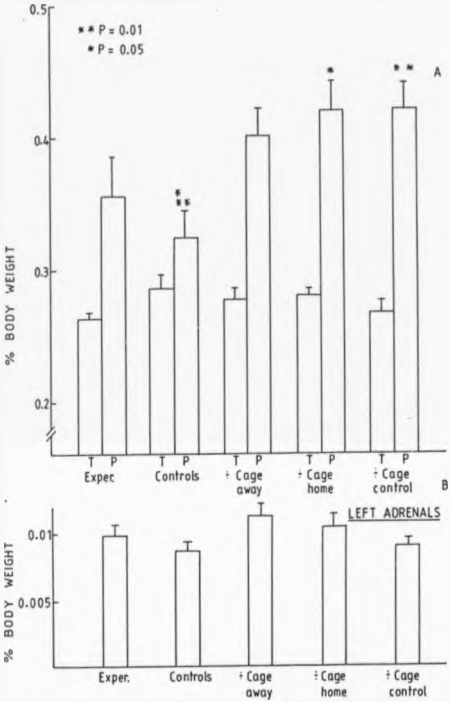
Figure 5:16 shows values for the post mortem organ weights as percentages of body weight. No differences were found between the groups for testes and adrenal weights but the data for preputial glands differed significantly ($F=4.73$, df 4+53, $p<0.002$). Further analysis by Tukey Test at the 5% level of significance showed that the mean preputial weights of dominants, housed in the divided cages, who either encountered a fighter animal in the dominants' home cage or had only visible contact with an opponent, were significantly heavier than the weights for other groups.

Experiment FR10 Based on a number of reports in the literature that the presence of females can stimulate a rise in testosterone levels, it was predicted that female mice, resident in the free range room for a number of weeks, would elevate testosterone output particularly among territory holders. Only mice housed in the free range room were used in this experiment.

Eight, four month old adult female mice of known fertility were placed, two per cage, into four cages of five males when these animals were nine weeks old. At ten weeks of age, seven mice (five males plus two females) were placed in each of the quarters of the free range room. The first blood samples were collected from the males at eleven weeks and the metal barriers were removed one week later. Further blood samples were collected at thirteen, fifteen and seventeen weeks of age, one, three and five weeks after the barriers were removed. At nineteen weeks, the males were killed and the left testes, preputial and adrenal weights were recorded at autopsy. During the experimental period, the females gave birth to litters during weeks twelve, fifteen and eighteen. Pups were taken out of the room around day sixteen after birth because several were killed

Left testes, preputials and adrenal glands as percentages of total body weight

Fig. 5 : 16



or injured by the adult males when they began to leave the nestboxes and because variables such as crowding or competition with other males were considered undesirable.

Results and Discussion

Figure 5:17 and Table FR10 show the means and standard errors for testosterone levels in the three social classes of males. Data was analysed by 2-way ANOVA with the following result:

Source	df	F-ratio	Probability
Trt. Groups	2 + 17	0.5977	0.56
Time	3 + 51	5.236	0.0032
Trt. x time interaction	6 + 51	1.9912	0.08

No differences were found between the three social categories but testosterone levels varied significantly over time ($p<0.003$). When the sampling times were compared using Student's t-tests, the following results were obtained:

Weeks	t-value	df	Probability
11 v. 13	0.5624	38	N.S.
13 v. 15	2.8489	38	0.005
15 v. 17	2.2531	38	0.025

Testosterone levels were found to differ significantly between weeks 13 and 15, and between weeks 15 and 17 when they rose in the territory holders and subordinates but then were lower again by week 17. For territory holders, levels were seen to be very low in week 11, rising in week 13 to a high mean level at week 15 before falling back by week 17.

Free range mice housed with females over 7 weeks

Fig. 5: 17

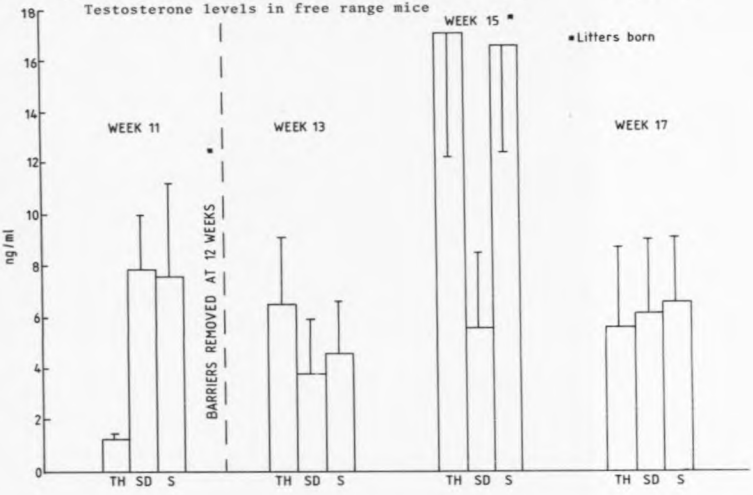


TABLE FR10

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 11, 13, 15 AND 17 WEEKS OF AGE

	ng/ml			
	11	13	15	17
Free Range				
Territory holders	1.2±0.2	6.4±2.6	16.8±4.7	5.5±3.1
Subdominants	7.7±2.2	3.7±2.2	5.5±2.9	6.1±2.8
Subordinates	7.5±3.6	4.6±1.9	16.5±4.2	6.4±2.6

The weights of organs from post mortems, expressed as percentages of total body weight are shown in Figure 5:18. One-way ANOVAs on arcsine transformed data for the three social groups gave the following results:

Organ	F-ratio	df	Probability
Preputials	1.102	2 + 17	0.355
Adrenals	0.801	2 + 17	0.465
Testes	1.198	2 + 17	0.326

There were no differences between any of the groups.

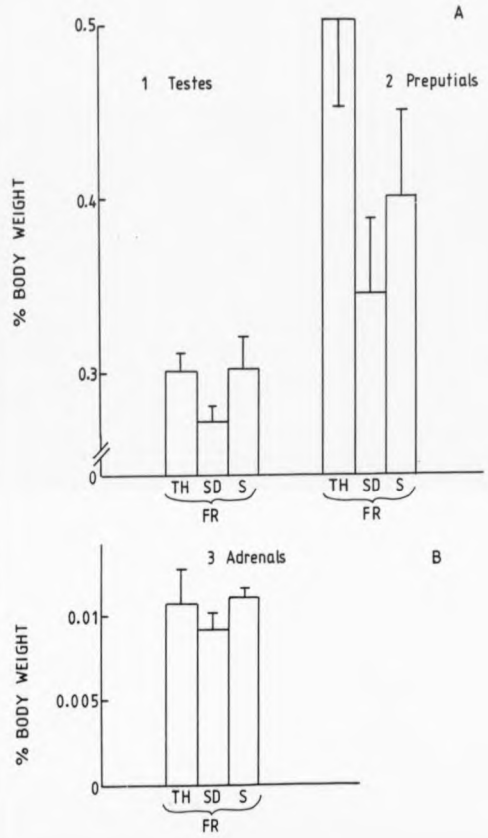
A comparison was also made of the data in this experiment with the results from experiment FR8 for weeks 13, 15 and 18 when no females were present in the room. The means and standard errors are shown in Figure 5:19 and also in Table FR8-10 and analysis by 2-way ANOVA gave the following results:

Source	df	F-ratio	Probability
Trt. Groups	1 + 8	0.914	0.37
Time	2 + 16	1.674	0.21
Trt. x time interaction	2 + 16	3.436	0.05

The interaction was found to be significant and from the histogram it is noticeable that a rise in levels at week 15 when females were present was not matched by the levels for territory holders in the all-male group. Indeed, in experiment FR8, levels dropped for territory holders by week 15 and were even lower at week 17. The high levels observed in week 15 in experiment FR10 coincided with the second litters being born and when females may have entered

Left testis, preputial and adrenal glands as percentages of total body weight

Fig. 5:18



Comparison of data for FR 8 + FR 10
(with + without females present)
Territory holders only

Fig 5 : 19

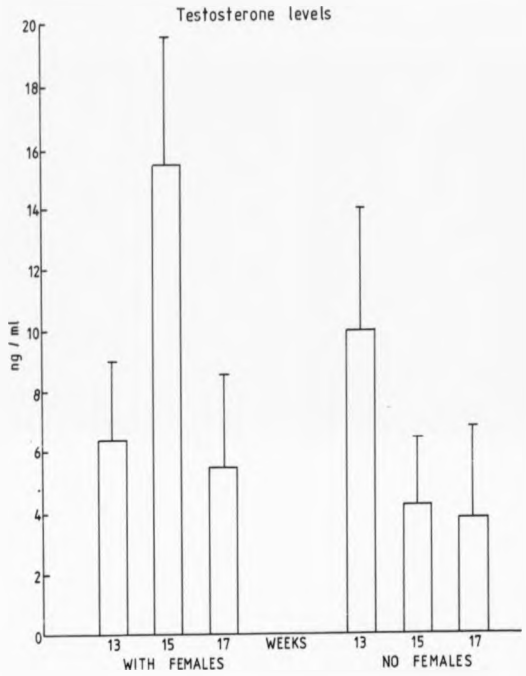


TABLE FR8-10

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 13, 15 AND 17 WEEKS OF AGE

	ng/ml		
	13	15	17
Territory holders			
Without females (EXP FR8)	10.0 ± 4.0	4.2 ± 2.3	3.8 ± 3.0
With females (EXP FR10)	6.4 ± 2.6	16.8 ± 4.7	5.5 ± 3.1

post-partum oestrus.

Experiment FR12

From the results obtained in experiment FR10, it was found that when free range mice lived together with females in the room, testosterone levels appeared to be higher particularly among the territory holders, compared with when females were absent. These levels were seen to be high particularly at week 15, when the females not only gave birth to their second litters, but quite probably entered post partum oestrus as well. As a consequence of these findings, this experiment, which only used free range animals, looked at whether the presence of females that had been induced into behavioural oestrus by hormone treatment, could influence levels of testosterone, particularly among the territory holders.

Pilot Study This was carried out to ensure that when females were "primed" and brought into oestrus, they were then sexually receptive to male mice. The females used were ten sexually experienced, four month old mice. They were brought into behavioural estrous using the method of Mosig and Dewsbury (1976) and were injected with 0.035mg I.M. estradiol benzoate (SIGMA) approximately 48 hours before testing and 0.1mg I.M. progesterone (SIGMA) 6-8 hours before testing. Other mice used in the study were ten, four month old males that had no previous sexual experience. A male and primed female were placed together in a large cage with bedding but no food or water and observed until the male was seen to demonstrate full copulatory behaviour (mount, intromission and ejaculation). Females that permitted sexual advances and these behaviours were considered to be receptive. A time limit of ninety minutes was set for each pair. Out of the ten males tested, seven reached ejaculation within thirty minutes, two others reached this stage within one hour and the final male was seen only to attempt to mount, the female he was with being

unreceptive to his behaviour. Tests were carried out under red light between 14.00 and 17.00 hours.

Main Study

Using the method described above, primed females were placed for a short period in the free range room where males were housed and the effects on levels of testosterone were examined.

Eight, six month old female mice with previous sexual experience were bilaterally ovariectomised by the dorsal route after first being anaesthetised using Sagatal anaesthesia, 60 mg/ml diluted in a 1:9:1 ratio in 10% ethanol injected intraperitonealy. Surgical equipment was sterilised using Hibitane. After a recovery period of ten days, vaginal smears were taken by the lavage method on the following seven consecutive days. The smears were dried, stained with diluted Giemsa stain (1:20) for thirty minutes, washed in water and dried again. Following the description given by Bingel and Schwartz (1969), they were then identified. Smears from all animals consistently showed an anoestrous appearance and from this, it was concluded that the ovariectomies had been successful.

As in previous experiments, a group of five male mice aged ten weeks old, was placed in each of the four quarters of the free range room. Following the usual period of observations, the metal barriers were removed at twelve weeks. At fifteen weeks, all animals were removed from the room, blood sampled and then returned to the room. This was carried out between 11.00 and 12.00 hours.

Eight days later, the eight ovariectomised females were induced into behavioural oestrus using the above method. At 10.30 hours they were placed in the free range room for thirty minutes after which time, all animals were removed. The males were then blood sampled and returned to the room. In week three, eight days later between 11.00 and 12.00 hours, all the males were again blood sampled. When

the females were placed in the room, it was noticeable that the males displayed high levels of aggression towards each other during the thirty minute period. Because it was considered possible that this aggression might in addition, influence testosterone levels rather than the presence of the receptive females alone, a further test was carried out to examine this possibility and the prediction was made that aggression levels due to the presence of intruder males, would not stimulate the same rise in testosterone output as was observed when females were present. A final study therefore, was carried out in week four when the males were eighteen weeks old, 8 days after the third blood sampling. Eight intact, four month old males, that had been previously group housed together, were placed at 10.30 hours in the free range room with the resident mice. After thirty minutes, all the mice were removed and the resident free range males were again blood sampled.

Results

Figure 5:20 and Table FR12 show the means and standard errors of testosterone levels for territory holders, subdominants and subordinates from the free range over a four week period. Comparison of the data for the three social groups in each condition, by one-way ANOVAS gave the following results:

WEEK	CONDITION	F-ratio	df	Probability
15	CONTROL	1.900	2+15	0.184
16	30 MINS WITH PRIMED FEMALES	7.744	2+15	0.005
17	CONTROL	3.532	2+15	0.055
18	30 MINS WITH MALE INTRUDERS	0.651	2+15	0.535

A significant difference was found at week 16 when males were exposed to the primed females ($p<0.005$). Further breakdown by Tukey

Testosterone levels in free range mice with male and female intruders

Fig. 5:20

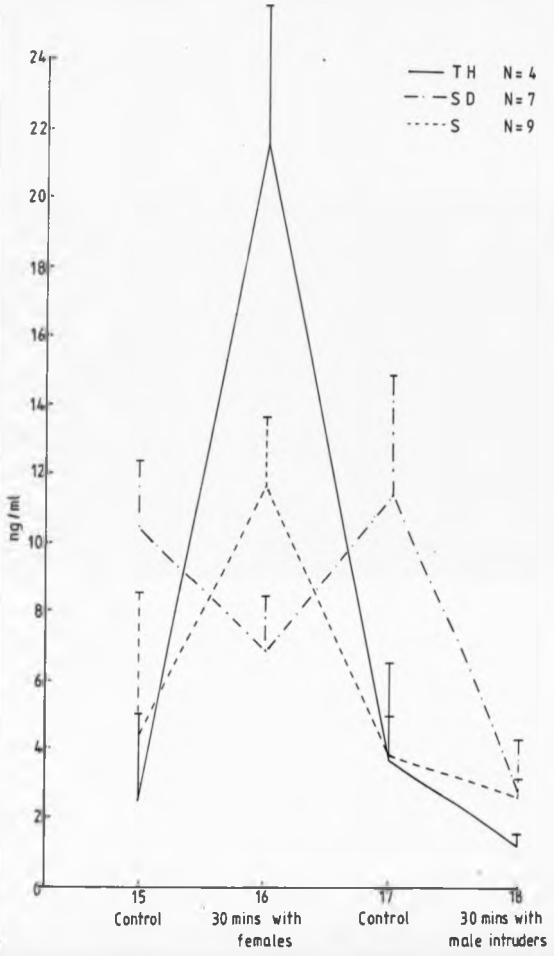


TABLE FR12

TESTOSTERONE LEVELS

MEANS AND STANDARD ERRORS AT 15, 16, 17 AND 18 WEEKS OF AGE

	ng/ml			
	15	16	17	18
Free Range				
Territory holders	2.5 ± 2.4	21.5 ± 4.1	3.7 ± 2.5	1.2 ± 0.3
Subdominants	10.3 ± 2.0	6.8 ± 1.6	10.4 ± 3.5	2.8 ± 1.4
Subordinates	4.2 ± 4.1	11.6 ± 2.0	3.8 ± 1.0	2.6 ± 0.6

test showed that the mean level of testosterone for territory holders differed significantly from both subordinates ($p<0.05$) and subdominants ($p<0.01$) but these two groups did not differ from one another. Territory holders were seen to possess the highest levels of testosterone in this condition.

There were no other significant differences although at 17 weeks, subdominants showed a strong trend towards having higher testosterone levels compared with territory holders and subordinates ($p<0.055$).

Experiment FR10/FR12

The final experiment described in this chapter was an attempt to quantify aspects of agonistic behaviour observed in mice housed in the free range room. Observations were made on the animals during experiment FR10, when females lived in the room throughout, and during experiment FR12, prior to the week in which the all-male group were exposed to receptive females. This work was carried out in order to try and answer certain questions:

1. What was the most usual/common form of aggression? Fights, chases or attacks?
2. Did most encounters take place on or off the territory of the attacker, irrespective of social status?
3. Which animals showed the most overall aggression?
4. Was the aggression observed mainly intra-group or did much of it take place between groups and which category of animals directed it?
5. Was the average length of a fight between two animals greater or shorter than 5 seconds?
6. Was there more overall aggression when females were present?

Aggressive behaviour was subdivided into three categories after the method of Clark and Schein (1966) as follows:-

a) Attack: a physical assault by one animal or another without the latter replying in kind

b) Chase: pursuit of one individual by another, with or without physical contact

c) Fight: a physical struggle between two animals and includes wrestling and rolling.

Cessation of these activities followed by recommencement again between the same two animals after five seconds was counted as two separate events. Observations were made on both groups over ten consecutive days and each observation period lasted sixty minutes. The start of recordings was made ten days following the removal of the metal barriers and were carried out under red light between 14.00 and 16.30 hours each day. The observer sat by the entrance to the room with the door open and a period of five minutes was allowed to elapse before recordings began each day in order to allow the mice time to become accustomed to the presence of the observer. Records were made using a checksheet and stopwatch.

The following details of events were noted:

1. the status of the initiator and receiver of aggression in each encounter
2. the location where the encounter began - on or off the territory of the "attacker" mouse
3. classification of the encounter - attack, chase and/or fight
4. the duration of fighting time - greater or less than five seconds

Results

Table 5:G shows a breakdown of the data gathered in both experiments. The data given in (1) was analysed by Sign Test (Siegel, 1956) and the number of encounters that took place with females present was compared with data from observations with no

females in the room. Significant differences were found for all three categories of aggressive activity: fights ($p<0.002$), chases ($p<0.001$) and attacks ($p<0.001$).

TABLE 5G

	Females Present		No Females	
	No.	%	No.	%
1. Number of encounters in each category of aggression:				
FIGHTS	73	11.1	27	19.1
CHASES	252	38.5	55	39.0
ATTACKS	330	50.4	59	41.9
2. Place of encounter				
ON TERRITORY		94.7		93.0
OFF TERRITORY		5.3		7.0
3. Incidents of aggression according to social category				
TERRITORY HOLDERS		55.0		50.0
SUBDOMINANTS		42.4		31.0
SUBORDINATES		2.6		19.0

TABLE 5G (cont...)

4. Aggressive behaviour given to and received by:	Females Present %	No Females %
a) TERRITORY HOLDERS to SUBORDS*	69.7	73.9
TERRITORY HOLDERS to INTRUDERS**	26.4	21.5
TERRITORY HOLDER to TERR. HOLDER	3.9	4.6
b) SUBDOMINANTS to SUBORDINATES	32.2	36.8
SUBDOMINANTS to INTRUDERS***	68.8	63.2
c) SUBORDINATES to INTRUDERS***	100.0	100.0
SUBORDINATES to SUBORDINATES	-	-
5. Between/within group aggression		
WITHIN	54.2	53.3
BETWEEN	45.8	46.7
6. Length of time spent fighting		
LONGER THAN 5 SECS	32.9	14.8
LESS THAN 5 SECONDS	67.1	86.2

* Subordinates - includes both subdominant and subordinate mice

** Intruders - mice that entered a strange territory area where they did not normally live, excluding territory holders

*** Intruders - all strange mice that entered a foreign territory including territory holders

Discussion

At the beginning of this chapter, the prediction was made that testosterone would be found to correlate positively with social status. It was anticipated therefore, that high levels would be observed in free range territory holders as well as group housed dominants and singly housed mice. In contrast, it was expected that these high levels would not be a feature of low status males and that levels of testosterone would be lower in subdominants and all subordinates. Data from a number of experiments have provided results which were the opposite of what was expected. In contrast to the dominant caged mice, levels in territory holders were found consistently to drop during the time these animals lived in the free range room. The data however, only reached statistical significance in one experiment (FR6). As a consequence, it will be recalled that data was pooled from three experiments for high status males (FR5, FR6 and FR8) in order to see if the overall trend across these tests was statistically significant. A significant interaction was obtained with a rise in testosterone levels occurring over time for group housed dominants and singly housed mice in contrast with the territory holders where levels were progressively reduced. The problems associated with the pooling of data have been discussed in the previous section, however this exercise was considered necessary because of an unavoidable weakness in the experimental design which allowed no control over the ratio of territory holders to subordinates in any free range experiment and numbers of high ranking males were always low. Despite this, a small significant difference was obtained in one experiment (FR6) and considering the small number of available subjects, to achieve even marginal significance is important. As results do show a consistent trend over four

experiments (FR3, FR5, FR6 and FR8) it is felt that taken together, these are indicative of a genuine effect, at least within the context of the free range room. The data shows that high status does not always correlate positively with high testosterone output.

The results from the testosterone measurements were confirmed by the data on sex accessory gland weights. Here there was a notable downward trend for territory holders and these findings go against those of other workers where observations have shown that gland weights are reflective of social status (Bronson 1973, Benton et al. 1978). In one experiment, (FR3) testes weights for territory holders were lower than the weights recorded in subdominants and subordinates. Only when females were resident in the room (Experiment FR10) were preputial glands found to be heaviest in territory holders although the data did no reach statistical significance. Bronson and Marsden (1973) showed that the preputial glands of mice actually grew in size during the establishment and/or maintenance of a dominant social position and they argued that this growth was due to increased androgen activity. Their experimental design was however, very different from the one in this study. Their male mice were paired and lived in cages in dominant-subordinate relationships for a total of fourteen days and so their results may reflect an acute response to the social conditions over a short time period. McKinney and Desjardins (1973) have suggested that this gland may reduce in size during periods when androgen output is low and the results of experiment FR3 support this idea.

Both the preputial gland and the testes are responsive to testosterone levels. For the testes this occurs via the action of LH on Leydig cells stimulating testosterone output. Experiments with hypophysectomised animals provide evidence that LH is the only hormone capable of stimulating testicular steroidogenesis in the

absence of other hormones (Bartke et al. 1978). But in addition, testes weight appears to be influenced by FSH. It is known that FSH can significantly increase testosterone production by perfused rabbit testes exposed to LH in amounts greater than that required to produce maximal steroidogenic response (Bartke et al. 1978). Cunningham and Huckins (1979) have reported that in rats where intratesticular testosterone was suppressed by testosterone propionate, levels of testosterone fell thirty fold whereas FSH was only partially suppressed and complete spermatogenesis was still apparent. It would appear that this activity in the adult rat is not dependent upon high intratesticular levels of testosterone and these authors suggest that FSH, which is known to regulate Sertoli cell function, may secondarily influence germ cell maturation and testes weight.

In order to answer the question of why testosterone levels in territory holders fell when in caged dominants they did not, a number of tests were carried out on caged males to examine levels of testosterone in relation to inconclusive fights (Experiment 11). Unlike the caged dominants, free range territory holders besides encountering mice that they lived with and continually won fights against, were also confronted with animals which they fought but did not win or lose against and the presence of these animals posed a constant threat. The prediction made was that failure to defeat an opponent could be related to the low testosterone output of territory holders, serving to depress levels. The aim of the cage experiments was to simulate this situation in a number of ways. There was simulated invasion of a dominant's territory by an aggressive male; invasion of another's territory by the subject dominant, coexistence with a neighbouring aggressive male with encounters between the animals and coexistence with no fight encounters. Testosterone levels in response to these various conditions were measured. No

differences in testosterone output were found when experimental and control conditions were compared although it was notable that all the "control" dominants, together with dominants that fought in their home cages, had the heaviest preputial gland weights. Overall however, the hypothesis was falsified and it can be concluded that failure to defeat all other males has no direct bearing upon circulating testosterone levels. A study by Leshner and Nock (1976) also examined the effects of complete and incomplete aggressive encounters in mice although endocrine parameters were not investigated. Male mice that had been housed for eight weeks in isolation were tested for aggressiveness in their home cages, once a day for three days, against an olfactory bulbectomised opponent. The aggressiveness of the experimental animals was rated using a composite aggression score (CAS) of different agonistic behaviours as a measure of basal aggressiveness (Svare and Leshner 1973). Mice were then assigned to matched pairs which fought on seven consecutive days, either in an "Interrupted Encounter" condition, where a pair was separated after one minute of fighting following the start of aggression, or a "Complete Encounter" condition where fighting was allowed to proceed until an animal had been clearly defeated by its opponent. Although all the animals used were matched in initial levels of aggressiveness, they differed in their agonistic experiences during the encounters. When the CAS's for each group in the two aggression tests were compared, the results showed that repeated experiences of complete victory had no effect on levels of subsequent aggression but that experiences of incomplete or interrupted victory increased aggressiveness. In the tests on caged animals in Experiment 11, it was noticeable that following an interrupted fight, dominant males frequently attacked the other males that were resident in the same cage. Leshner and Nock (1976) have

argued that a naive animal fights because it expects to derive some biologically important consequence from the event, such as dominance or access to females, and if fighting leads to victory, this expectancy is confirmed. They further argue that on this basis, there is no reason for the victorious animal to react more aggressively in future agonistic encounters although this line of argument does not take into account future threat of competition. For animals that experience incomplete victories, it is suggested that they seek "confirmation of their expectancies" by increasing aggressiveness towards opponents. In this study, these workers allowed their animals to fight for one minute whereas the fighting time in Experiment 11 was confined to five seconds - this being an average for the time one pair of males spent fighting together in the free range room, but it may have been too short a duration in these cage experiments to produce any significant changes in testosterone levels. Overall it appears that there is little evidence to show that stimulating aggressiveness produces parallel changes in testosterone. Clark and Nowell (1978) have shown that isolating mice results in an immediate rise in aggression levels which was only reflected in raised testosterone output seven weeks later, and these workers have argued that no apparent causal relationship exists between aggression and testosterone.

The fact that free range territory holders showed high levels of aggression even when testosterone dropped to low levels, is not entirely a surprise as it is known that even castrated mice with pre-operative experience of fighting, will continue to show aggression after surgery (Gandelman 1981). Edwards (1969) has put forward the view that endogenous testosterone influences the propensity to fight as a result of modification of central nervous function, but as to how testosterone affects the level of aggressive

behaviour is as yet unknown. From experiments in which testosterone or testosterone propionate crystals were implanted in the brains of castrated rats and mice, it is known that this steroid is behaviourally active in the central nervous system. In castrated rats, implants in the anterior hypothalamic preoptic area of the brain, activated intermale aggressive behaviour (Bean and Conner 1978, Bermond 1978 cited in Koolhaas 1980) and a similar result has been obtained for mice (Owen et al. 1974). The presence of testosterone receptors has been demonstrated in a number of brain structures such as the hypothalamus, preoptic area, septum, hippocampus and amygdala (Stern and Eisenfeld 1971, Sar and Stumpf 1972, Naess and Attramadel 1974, Greenstein 1979, Sheridan 1978). After the castration of male mice, testosterone is no longer produced yet a study of receptors in brain cytosol showed that these were emptied of testosterone only gradually over several days (Raab and Haedenkamp 1981). As an aside, testosterone-dependent behaviour, such as marking frequency in Mongolian gerbils, was diminished to 10% over a period of ten to fourteen days following castration although the half-life of plasma testosterone is only seventy-two minutes (Turner 1979). Despite extensive work, the specific role of testosterone in the functioning of brain structures in agonistic behaviour is also as yet, unknown.

Results from the tests on controlled fights together with results for free range territory holders and from a number of other studies on mice, suggest that gonadal response to agonistic experience has little or no behavioural significance (Nock and Leshner 1976, Maruniak et al. 1977, Leshner 1983). Studies on rats have also supported this by showing that fighting has no influence on testosterone output among winners and that the victors of serious agonistic encounters show neither an increase or decrease in levels

(Schuurman 1980, Koolhaas 1980). The results from experiment 11 fit well with these findings: whether mice fought under controlled caged conditions or in the context of the free range room, testosterone levels were uninfluenced by aggressive behaviour.

A wide range of evidence indicates that aggression may not directly influence testosterone output but the relationship between these two is a dual one and there are many studies which have demonstrated changes in levels of intermale aggression resulting from the manipulation of circulating testosterone concentrations. It appears that it may not be essential for testosterone to be present in the neonate in order for aggression to be displayed in adult life (Svare et al. 1974) but it must nevertheless be present at some stage for initiation of the behaviour (Gandelman 1981) although the behaviour may be demonstrated by appropriate environmental manipulation. Many studies on mice have shown that gonadectomy results in the decrease of inter-male aggression (e.g. Beeman 1947, Bevan et al. 1957, Leshner and Moyer 1975). Also androgen injections or implants can restore or maintain a normal level of inter-male aggression in castrated mice (Beeman 1947, Luttge and Hall 1973, Owen et al. 1974, Bowden and Brain 1978). Similar results have been found for rats although the number of studies is fewer (e.g. Barfield et al. 1972, Christie and Barfield 1979a).

For mice, Brain and Poole (1976) found that a daily dose of 50ug of testosterone maintained fighting in castrated, aggressive animals. When a wider range of doses was applied (Brain and Bowden 1979), a variety of results was obtained. In this study, castrated mice were given daily, intramuscular injections of oil containing 1, 5, 25, 50 or 100ug doses of testosterone for 17 days. Animals were tested for aggression using "standard opponent" tests (Brain and Poole 1974). One microgram of testosterone per day was relatively ineffective in

restoring fighting, but for other amounts, there was some evidence of a dose-dependency for all behavioural measures. It was also found that all the testosterone doses used significantly augmented the weights of sex accessory glands in a dose-dependent fashion. At the top end of the range, it was notable that fighting intensity rarely approached that of untreated, intact "aggressive" mice. The doses cited did not take into account loss of material by seepage, conversion of steroid, clearance of hormone or retention in injected tissue which could have been influential in this. It could be also that the stress of castration and injections reduced levels of fighting, or that the means of hormone application did not adequately mimic endogenous release of sex steroids. Although the data from this study provides interesting information, caution should perhaps be used when drawing correspondence between the levels of aggression induced by steroid treatment and aggression relative to testosterone output in intact animals.

It appears that an intact pituitary-gonadal axis is not necessarily a requirement for offensive behaviour. Castrated rats behaved less aggressively towards intruders in their home cage than before castration but almost all castrated rats became dominant over intruders. Indeed rats were still defending their cages two months after castration. Rather than there being a loss of offensive behaviour, it appears that castrated rats are still able to fight effectively and inflict defeat on intruders to the home cage (Barfield et al. 1972, Christie and Barfield 1979b, Koolhaas 1980). However, results from a recent study by Albert et al. (1986) are not entirely in line with this thinking. Using dominant rats housed in mixed sex groups in cages, males were either castrated and implanted with testosterone-filled Silastic tubes, castrated and implanted with empty tubes or sham-castrated. When tested for aggressiveness against

non-aggressive intruders to their cages, dominant males with no testosterone declined in aggressiveness while those with testosterone-filled implants showed levels of aggression close to those observed in sham operated controls. Subordinate males became dominant when dominant males were castrated and not given testosterone filled implants, similarly a decline in the aggressiveness of a dominant occurred in response to removal of a silastic capsule containing testosterone. In contrast to the conclusions drawn from studies discussed above, these workers have concluded that testosterone plays a primary role in intermale social aggression and that the decline in aggression following castration is typically accompanied by a loss of social dominance. It was also found that when castrate animals were residents, only one of six dominant males was substantially more aggressive than an intact intruder. They have suggested as a consequence, that social aggression should not necessarily be regarded as territorial defence at least in the rat. Instead, agonistic behaviour is dependent on the presence of testosterone and is regarded as a characteristic of and fostered by, the experience of living in a mixed sex social group. However, these workers do acknowledge that social aggression is more likely to occur in a familiar area and as a consequence, comes to be regarded as territorial defence.

Leshner (1975) has proposed that differences in the expression of aggressive behaviour by dominant and subordinate males is largely due to differences in androgen levels relative to social rank so that low levels of androgens, characteristic of subordinate animals may predispose them to behave unaggressively with the converse true of dominants. Results for testosterone measurements in free range mice do not support this view or the conclusion of Albert et al. (1986) that constant levels of testosterone play a vital role in social

aggression. Free range territory holders, despite very low levels of testosterone, were still able to attack and defeat both subordinates and intruders thus defending their home areas and maintaining their social position. It is considered therefore that low testosterone levels in the intact animal are sufficient for aggressive behaviour to be maintained.

Koolhaas et al. (1980) has proposed that the pituitary-gonadal system plays an important role in the adaptations of offensive behaviour following agonistic experience. Results from this study on rats have shown that a lowering of plasma testosterone levels, resulting from defeat of a dominant male, was accompanied by a decrease in offensive behaviour in an unfamiliar environment but not in the home area. From this, it was argued that it benefits a temporarily defeated or weakened male to avoid threats or risky encounters outside the centre of its territory for a time until it has recovered from its behavioural or physical weakness. In the meantime, the animal must continue to defend his home area both by defeating the subordinates he lives with as well as intruders. In the wild, losing the territory would reduce chances of survival. Extending this argument, it is therefore proposed that within the free range room, lowered gonadal hormone secretion serves to reduce aggressiveness in a territory holder outside of the territory area he controls whereas no such effect takes place in terms of the aggressive behaviour demonstrated within the territory towards low status males and intruders. Indeed the data gathered on fighting behaviour (Table 5:G) supports this view and clearly shows that the percentage of aggressive encounters that took place both between animals from different territory areas and also away from a home area, particularly between territory holders themselves, was very small when compared with the percentage of intra-group aggression.

Further, the observed absence of aggressive behaviour by a territory holder and the adoption instead of defensive behaviour when outside the home territory, such as fleeing, may reduce the likelihood of a serious aggressive encounter when in the area of another male. In comparison with dominants in cages with high testosterone levels, who never encountered males other than those they had already defeated, for the free range territory holders who did meet with strange males, there may be an advantage in having low circulating levels of testosterone. It is proposed therefore that these low levels are sufficient to maintain territory ownership by intra-group aggression and the defeat of intruders but are also influential in the behaviour of territory holders outside the home area. As a consequence, when a resident territory holder leaves his own area and in so doing encounters strange males, a serious aggressive encounter does not usually take place. Indeed as frequently witnessed, fights were either short and incomplete or more commonly, no aggression took place and instead, the original territory holder was seen to show defensive behaviour and retreated back to the home area. It may be the case that high levels of testosterone could be influential in stimulating intermale aggression whereas low levels by contrast permit an animal a certain flexibility of behaviour to adopt what may instead be a more advantageous activity, such as retreating, in certain circumstances.

In summing up, from the data in the experiments of this study it is concluded that the drop in testosterone to low basal levels following the establishment of territories and social order may have important adaptive advantages both within an environment such as the free range and maybe also under natural conditions. Towards the end of the discussion in Chapter 1, it was suggested that the free range, rather than attempting to copy conditions in the wild, bore a closer

approximation to more enclosed environments such as store houses, attics or cellars which are recognised habitats for wild mice. Under these conditions, it would seem likely that territory areas would be close together and even border onto one another. It is suggested therefore that a testosterone profile, similar to that observed for free range territory holders, could aid in both territory maintenance and general social stability within such habitats where serious and continual fighting between high status males of different groups would result in risks to survival. Long-term stability between and within groups was a feature not only of this study but in others also (Crowcroft 1966, Reimer and Petras 1967) and may be related to endocrine changes.

In considering further the direct links between testosterone levels and social status, a number of studies mentioned previously have found a positive correlation between dominance and androgen output: in humans (Mazur and Lamb 1980, Elias 1981), Talapoin monkeys (Eberhart et al. 1980, Keverne et al. 1982), rats (Albert et al. 1986) and mice (Lloyd 1971, Bronson and Marsden 1973, Lee and Naranjo 1974). The results from the present study for free range territory holders however, go against these findings and territory holders were seen to hold high status irrespective of whether androgen levels were high or low. This work is supported by the findings of other workers who have shown that high testosterone levels may not be associated directly with high status on a simple, direct basis. In a study on mice, serum testosterone levels in dominant inbred males were not always elevated compared with subordinates (Barclay and Goldman 1977a). Other work using laboratory mice (Dessi-Fulgheri et al. 1976a, Selmanoff et al. 1977) has also failed to demonstrate a relationship between testosterone titres and a male's social status. In the study by Selmanoff et al. (op.cit.) male mice of the highly

aggressive DBA strain when housed in pairs, showed no correlation between testosterone levels and status. When housed in group numbers of eight, high testosterone was correlated with subordination and low testosterone correlated with dominance in one set of cage results although in another, high testosterone did correlate with dominance and low testosterone with subordinance. In groups of male and female guinea pigs, housed in $3.00m^2$ enclosures, where the dominants were challenged and fighting occurred, these high ranking males had the highest testosterone levels but in other groups where no fighting took place, low levels of testosterone were found among high status males and yet these males showed a high degree of courtship behaviour (Sachser and Prove 1986). These findings suggest that endogenous testosterone levels only reflect the highest ranking males' social status when their position is challenged. Taken together, these studies suggest that a consistent association of testosterone with high or low status is doubtful across a number of rodent species.

In birds, a not dissimilar result has also been demonstrated for male song sparrows (Wingfield 1985). During the agonistic phase of territory establishment, testosterone levels were seen to be high, dropping back once ownership was established suggesting that the social interaction coupled with territory formation acted as the stimulus to hormonal change rather than just ownership *per se*.

In a recent study, Mendoza (1984, pp.20-21) has argued that "relationships between males therefore influence each individual's psychological state rather than the individual's physiological state determining the nature of the relationship to be formed." In this same study, it was demonstrated that in well-established groups of monkeys, dominance and testosterone levels were not correlated. In general, a number of the animal studies indicate that it is not dominance *per se* that leads to elevated testosterone levels but the

acquisition of it, a point also noted by Henry (1980) whereby a rise in androgen occurs with the attainment of dominance whereas a decline is noted with reduction in status and/or with submission.

Further to this, Rose et al. (1975) have argued that the decrease in androgen levels found following defeat and loss of status, may decrease the probability of aggressive action on the part of the individual, thereby precluding the likelihood of instigation of additional combat and repeated defeats. However, whether this consideration is applicable to similar endocrine and behavioural events among rodent groups is uncertain. Other studies on primates have produced contrasting results: among Japanese macaques, studied both as a natural troop and as a laboratory group (Eaton and Resco 1974), although dominance and levels of aggression correlated highly, testosterone titres did not. For the laboratory group of males, blood sampled every fifteen minutes over two hours, multiple, apparently random, peaks of testosterone were observed although the mean levels of 11.73ng/ml for the group, did not vary substantially throughout this 120 minute period. Among stable, social groups of vervet monkeys of mixed sexes, testosterone concentrations were also found to be unrelated to dominance rank (Steklis et al. 1985) when dominance was measured on the basis of success in inter-male aggression. Further, this work showed that within subject variations in testosterone concentrations fluctuated five to ten fold over successive days. Endocrine responses to social stability and instability in the olive baboon point to high testosterone titres among high ranking males that were found exclusively during a socially unstable period with elevated testosterone and high levels of aggression being unrelated during periods of social stability (Sapolsky 1983). Over a three year period, a lack of correlation between rank position and testosterone levels has also been

demonstrated in rhesus monkeys by Gordon et al. (1976).

In the light of comments made earlier, together with the studies reported above, to consider that a simple and direct relationship exists between circulating testosterone levels and high status is a naive assumption. Status alone may not in itself be a predictor of circulating androgen levels in a number of species, but in combination with other factors, such as attainment of status or a challenge to position associated with aggression, dominance may then be correlated with androgen output.

During the establishment of dominance, factors such as body weight and size may be influential; heavier, larger animals being the most likely winners of fights (Barclay and Goldman 1977a) although the weights recorded for high status males in the present study go against this particular idea as these animals were not always the heaviest animals in a group. Lloyd (1971) has proposed that rank plays an important role in determining androgen secretion in groups of mice. However, in a long-term study over several months, Selmanoff et al. (1977) found no significant correlation of rank with serum testosterone levels. A number of studies though suggest that high testosterone levels are important for the initiation of dominance behaviour. The data from the present study would tend to support this Further Eberhart et al. (1980) in long term studies on Talapoin monkeys, have stressed the importance of taking into full account the dynamics of behavioural integration when studies are made on dominance rank and testosterone levels. Indeed, a number of the above studies note that social interaction coupled with the establishment of dominance status and/or territory formation, act as the stimulus to hormonal changes rather than just the possession of high status per se.

Among free range territory holders, high testosterone levels

were found on the whole, at times of social disturbance, during territory formation and when the barriers were removed so that different groups of mice met for the first time. These findings fit well with the studies mentioned above and it appears that in the free range environment, high testosterone titres in high ranking males are a reflection of socially unstable periods.

In contrast to the territory holders, testosterone results for the group housed dominants were similar to those from the number of studies that have found a direct correlation between rank and testosterone output. Sex accessory gland weights for this group on the whole reflected androgen output and although these data did not reach statistical significance, there was a strong trend in this direction. The reasons why these animals had fairly consistently high testosterone levels over the experimental time periods are not entirely clear. For humans, Mazur and Lamb (1980) have suggested that the link between status and testosterone is contingent upon mood and mood changes. Although this can be investigated in human subjects, it is impossible in animals as their subjective state cannot be determined. Nevertheless, an animal's perception of its social position relative to other group members may serve to influence endocrine changes. These dominant mice lived constantly with subordinates that they had defeated and presumably they recognised this fact and in addition, there was never any outside challenge to their social position. In the cages, in contrast to conditions in the free range, the close proximity of individuals made it impossible for animals to avoid contact and it is possible that this could have resulted in more frequent bouts of aggression than took place in the larger, free range territory areas. If subordinates on occasion fought back, rather than just submitted, this may have provided a form of challenge and stimulus to testosterone production

in the dominants.

An alternative explanation is that urine odour in the bedding materials of the caged mice was associated with testosterone output. All animals in this housing condition were placed each week in clean cages with fresh bedding. By contrast, mice living in the free range room had no changes of bedding material throughout the duration of an experiment. This was for two reasons: first, the number of animals in the room relative to the space available meant that bedding did not become badly soiled quickly and secondly, replacing the old bedding was considered undesirable because of the potential disturbance to established territory boundaries through the removal of odour cues. Although the group caged mice were not at risk of territory disturbance, unlike the animals in the free range, clean bedding each week may have led to dominant males reasserting their status through fresh deposition of urine odour and this activity could have been associated with the high output of testosterone in this group, although this requires experimental verification.

A third possible explanation for these high testosterone levels is that these dominant males, rather than being socially established, were instead insecure within their high status positions. As a consequence, constant reassertion of their status through aggression provoked steady high androgen output. This idea is however not given too much credence. Poole and Morgan (1973) investigating social stability relative to small and large group numbers in mice, showed that in the two largest groups of mice and twelve males, five to six changes in dominance were observed over an experimental period of twenty-one days. The smaller the groups, the more stable the social hierarchy. Five animals to a cage was found to produce a socially stable environment and it was on the basis of these data that units of five animals were chosen for the experiments in the present study.

As a consequence, it seems unlikely that lack of stability among the groups of caged males was the stimulus to high testosterone output. It would seem most likely that a combination of factors which could include odour and aggression, contributed to the levels observed in the caged dominant males.

Testosterone output in singly housed mice was not found to be as high as levels in group housed dominants, a result opposite to the findings of McKinney and Desjardins (1973). Levels for the singly housed animals were nevertheless much higher than those measured in free range territory holders. However the data for sex accessory gland weights is conflicting. In both experiments FR3 and FR8, testosterone levels were found to rise in singly housed mice over six and five week periods respectively and in the first of these two experiments, mean testes weights were found to be the highest in this group of animals. By contrast, in experiment FR8, both testes and preputial weights for this group showed the opposite result to the one previous. It would appear that high androgen output is not necessarily associated with high sex accessory gland weight in this group but the reasons for this are not clear from this study. It has been theorised that single housing produces responses that are behaviourally and physiologically similar to dominant or territory holding mice (Brain 1975, Benton et al. 1978, Benton and Brain 1979). If this holds true, then the expectation would be that testosterone levels for these animals would be similar to levels for the free range territory holders. Results from these experiments however, showed that levels for these two groups differed in every test. Valzelli (1973) and others have suggested that isolation results in hyper-irritability which is a product of the stress of individual housing, an environment which may be equated with a form of social deprivation. Brain (1975) arguing against this, has instead related

single housing to the feral state and isolation of territory holders in the wild. However, in contrast to this, the idea proposed here is that single housing is an artificial and unnatural condition and cannot be compared to a natural environment. The availability of space is the first striking difference between the two categories and although the sizes of territory areas in the wild differ according to location, a number of reports indicate that defended areas are considerably larger than the space provided by a cage for a single mouse. A number of field studies have revealed a variety of territory or home range sizes in natural mouse populations: chicken barn, 1.9m^2 (Selander 1970 cited in Berry 1981), Oxford cellar, 4.6-5.6m^2 (Southern 1954 cited in Berry op.cit.), open field with voles, 122.3m^2, open field without voles, 364.6m^2 (Quadagno 1968 cited in Berry op.cit.).

Secondly, the versatility of this species has aided adaptability to habitats of great variety and size and to directly equate the behaviour of single housed mice in small laboratory cages with the behaviour of feral mice is to ignore the flexible range of behaviour that these mice adopt depending on changes in social and environmental circumstances, opportunities which the caged mice never experience. It is concluded that the high testosterone levels observed in the singly housed mice may be associated with hyperirritability and high levels of aggression witnessed in animals in this housing regime, all three of which may be associated with the artificial conditions rather than anything else.

Brain (1975) discussing single housing in terms of isolation, has made the point that these animals, although they do not defend boundaries, are not subjected to defeat by conspecifics in the home cage either. However, if feral mice in the wild are "isolated", the form that this takes may be quite dissimilar to cage conditions.

Feral mice that hold territories may indeed be geographically "isolated" from one another by the boundaries of home ranges but this may not mean that they are therefore isolated socially from other mice. It is known that wild mice live in demes comprising a dominant male together with one or a number of females and their litters at various stages of development. These females and immature animals pose no threat to the dominant male's position and so their presence is tolerated whereas a strange intruding adult male is not. Single housing not only separates and "isolates" adult males but also disallows the more natural condition of mixed sex groups that provide the normal forum for social behaviour to take place.

Taken together, evidence from the experiments in this study suggests that the natural condition for established dominant territory holders in a more natural environment is to have low circulating levels of testosterone which are sufficient to maintain high status through aggression directed towards other male residents in the territory or intruders. However it is also proposed that certain stimulations result in the elevation of testosterone output from a basal level such as the presence of receptive females or periods of social instability such as the organisation or reorganisation of territory areas and the acquisition of dominance status. It was noticeable that in the free range, levels of testosterone for territory holders were generally at their highest between weeks ten and thirteen prior to entry to the room, during the period when territories were established and (week 12) when the metal barriers were removed and the four groups of mice encountered each other for the first time and inter-group fighting was particularly intense. When social order was established and maintained during the subsequent weeks, during this time, testosterone dropped to low basal levels in the territory holders. The data indicate that testosterone

essentially plays a permissive role in that only basal levels are required for effective fighting behaviour to occur.

The establishment of high status through aggressive encounters although related to testosterone levels, would also appear to be dependent upon learned success and positive feedback. An animal that wins a fight against an opponent gains the experience of winning which leads to expectancy during subsequent encounters. If expectations are fulfilled, then status becomes established. However, this cannot take place in isolation because just as a potential dominant gains positive feedback through winning fights, so the animals that are defeated also derive feedback about losing and develop behaviours associated with submission which also reinforce the winner. The establishment therefore of a social hierarchy is a two-way event with both upward social mobility of dominants together with downward conditioning of subordinates. Leshner (1975) however has argued instead that the prime influence on social differentiation is hormonal change and he does not consider learning from experience as a principal cause.

The data for corticosterone and adrenal weights in chapter 4 showed that low status males were not chronically subordinated and indeed low testosterone levels were not a marked feature of this group. This is considered good evidence to support the view that subordinates particularly in the free range room, learned to cope with their social conditions despite being frequently attacked and submitting to the territory owners they lived with. Indeed from the data gathered from observations on aggression in the free range (Experiments 10 and 12), 93-94% of all aggressions took place on the territory area and 70-74% were directed by territory holders towards low status males. Although these animals were both under threat from and attacked by territory holders and showed the lowest levels of

aggression (2.6% when females were present, compared with 19.0% in an all-male environment) they were nevertheless capable of directing aggression towards intruders from other territories and indeed 87.5% of all subordinate aggression was in this form of encounter. All other aggression in this group was directed at other subordinates. When this occurred, it was notable that encounters were due to competition for food. Mice that huddled on bricks had to make short forays to the ground to retrieve food pellets whilst avoiding attack. Aggressive behaviour took place within a group of mice over the food that was obtained. Although the data for corticosterone levels did not support the original prediction that these animals would be chronically socially stressed, testosterone levels were found to be low at different times and data from rats suggest that a depressive effect on these levels may not necessarily be influenced by pituitary adrenal activity. Gray et al. (1978) examining mechanisms involved in the reduction of testosterone levels due to chronic surgical stress by gauze implantation, found that the suppressive effect of surgical stress on LH and testosterone levels was apparently not mediated by the pituitary adrenal system. Corticosterone returned to basal levels within twenty-four hours of gauze implantation whereas LH and testosterone levels remained suppressed. A similar pattern of response has been reported in mice after crowding (Bronson 1973) and it is possible that this could have occurred in free range subordinates also, though the mechanisms operating to suppress androgen output are not clear.

In a study by Maruniak et al. (1977) on levels of testosterone in dominant-subordinate relationships in castrated mice with testosterone implants, it is of interest to note that there was no evidence to show that submissive behaviour was directly related to a reduction in circulating testosterone levels. Data for the free

range subordinates in this study supports this view. Both high and low levels were recorded at weeks 12 and 13, times of social instability when these animals were subjected to numerous attacks and defeats. The raised levels of testosterone recorded for both free range subordinates and subdominants may have reflected periods of acute stress which can produce elevations in this androgen (Frankel and Ryan 1981, Pitzel et al. 1984). This event would seem to be mediated via the pituitary, bypassing output from the adrenal gland. In a recent study on the effects of acute ACTH administration (Armario et al. 1986), serum testosterone levels in intact rats were raised without modification to LH levels. Since this rise was not observed in castrated animals, it was assumed that the increase in testosterone was of gonadal origin. Data for rabbits also shows that ACTH, but not cortisol, was able to increase circulating testosterone levels (Fenske 1980). The physiological significance and the mechanism whereby ACTH can increase testosterone secretion are unknown. From the literature it seems that there is no evidence for a direct positive action of ACTH on androgen secretion at least in rats, and it may be that the effect is indirect. It is suggested by Armario et al. (1986) that steroid precursors from the adrenal may be used by the testes to produce testosterone, enhanced by ACTH activity. From these studies it would appear that the pituitary-gonadal system may reflect conditions of acute stress at least as accurately as the pituitary adrenal system and if ACTH is able to stimulate testosterone output without a parallel rise in corticosteroids, this may go some way towards explaining why raised testosterone levels in low status free range males were not mirrored by raised adrenal output.

This topic of acute stress and its possible influences on endocrine levels has been considered in the light of data gathered

for both subdominant and subordinate animals. Although the endocrine profiles for these two groups shared certain similarities, differences in behaviour, particularly aggression, were very marked. Subdominant mice in the free range were considered to hold a social position below that of the territory holders and were submissive to them but showed aggression towards the subordinates in a territory and any intruders. Because of their aggressiveness but lack of dominance status or territory ownership, they may be considered as a "super-class" of subordinates. When females were resident in the free range (Experiment FR10), 42.4% of all aggression came from subdominants compared with 31% in an all-male setting (Experiment FR12). It is of interest that only 32.0 to 36.8% of attacks by subdominants (depending on whether or not females were present) were directed towards subordinate mice they lived with. All other aggression was against intruders to the home area. Testosterone output from this group showed no consistent pattern, following both a similar picture to that of the territory holders with raised levels at the outset of an experiment which dropped later and also lowered levels in the early weeks of an experiment (Experiments FR5 and FR8), which may reflect varied response to periods of social upheaval which differed between groups.

In two experiments, testosterone levels were measured in response to the presence of females. At the outset of the experiments on androgen output, inclusion of females did not form part of the original predictions concerning levels of testosterone relative to social status. However, the data has shown that high testosterone output in caged dominant males was not matched in free range territory holders. The data for this group provides evidence to support the idea that low levels of testosterone are normal for high status males living in stable social conditions in a territorial

environment and that these levels are only raised in response to certain stimuli such as periods of social organisation. From reports in the literature for a number of species (Macrides et al. 1974, 1975, Batty 1978a,b, Bronson and Desjardins 1982) it is apparent that the presence of females can influence testosterone and gonadotrophin secretion and it was therefore predicted that the presence of females in the free range would also serve as a stimulus to testosterone output in territory holders. During experiment FR10, female mice lived in the room for the duration of the experimental period. Levels of testosterone in the territory holders were extremely low one week after the mice entered the room (week 11) and before the barriers were removed but these levels were higher in week 13 and then at their highest in week 15 which coincided with the time when the second litters were born and the females presumably entered post-partum oestrus. Levels of testosterone may also have been raised at twelve weeks when barriers were removed and the first litters were born but this was not tested for. The data show that testosterone levels were high for territory holders and subordinates, but not as high in subdominants at week 15 and it is possible that the receptive state of the females was influential in raising testosterone levels rather than simply their continual presence.

The influence of females on male testosterone levels in mice has been investigated by Macrides et al. (1975) who found that when male mice were paired with a normal female for one week, levels did not differ from those of males housed in all-male groups. However, when the resident female was replaced by one that was unknown, elevations in testosterone were found after thirty to sixty minutes of exposure. These workers showed that this effect was not dependent upon copulation taking place, and was still found under housing conditions where there was continuous exposure to the odours of other females.

The surge in testosterone was not found to occur, however, when the resident female was replaced by a strange male so it would appear that the elevation may be a particular endocrine response to an encounter with a strange female. Results from experiment FR10 would support the findings for the first part of this study as overall, levels of testosterone did not appear to be greatly influenced by the continual presence of the females.

However as levels were found to be high at the time when the second litters were born and the females may have been receptive, a second experiment was carried out to examine the influence of receptive females on testosterone levels using ovariectomised mice that were brought into behavioural oestrus by hormone treatment. These animals were placed in the free range room with the resident males for a period of thirty minutes and then removed. The results (Figure 5:18) showed a surge of testosterone among the territory holders which was not matched in either subdominant or subordinate animals. These results for the high status males agree well with those of Macrides et al. (1975) for single housed mice. This effect has also been demonstrated in bulls on presentation of a teaser cow (Katangole et al. 1971) and also in rats (Purvis and Haynes 1974, Kamel and Frankel 1978, Koolhaas et al. 1980).

In order to demonstrate that it was the presence of females that was responsible for this elevation in testosterone output and not simply the high levels of aggression that accompanied their presence, a second test in Experiment FR12 examined the effects of placing strange male intruders in the free range also for a period of thirty minutes. The results showed no elevation in testosterone levels despite the aggressive behaviour that was observed when these animals were present. Again, these findings agree well with those of Macrides et al. (1975). Data for the three categories of social

status indicate that the pituitary gonadal axis differs in its response to stimuli depending on factors such as social position which may affect the way a stimulus is perceived. Taken together, the results from this experiment and for experiments on all-male groups, showed that high status territory holders under stable social conditions possessed low basal levels of testosterone that were responsive to social stimulation such as hierarchy formation and the presence of receptive females. As copulation was not seen to occur during the thirty minute period, this could not have been an influential factor in raising testosterone levels. In guinea pigs, it has been demonstrated that the rise in testosterone that occurs following exposure to females, does not differ from levels recorded in animals that experience sexual activity (Harding and Feder 1976), and it may be that endocrine changes are therefore responsive to vaginal discharge (Macrides et al. 1974) or urine odour (Maruniak and Bronson 1976) mediated in mice by the vomeronasal organ (Wysocki et al. 1983).

In summing up, from the experiments discussed above in association with results from other studies, a number of conclusions are drawn. Firstly it is evident that elevated testosterone output is unrelated to high status in territory holders within a stable, social environment but instead is a feature associated with high ranking males during social organisation or periods of social instability. Testosterone seems to be important for the establishment of territories but may play only a permissive role in their maintenance. Secondly, aggression, in terms of both complete and incomplete encounters, was found to have no influence on testosterone levels but it may be the case that levels are important for regulating aggression particularly between rival territory holders and low levels of testosterone may be advantageous within this context.

Finally, despite low testosterone levels, the presence of receptive females can stimulate a surge in androgen output in territory holders, an event not observed in low status males.

From these three main points, it is therefore proposed that the normal state for a territory holder, established in the free range and perhaps also under more natural conditions, when at rest, unstimulated by females and with no competition from rivals, is a low resting basal level of testosterone output which is sufficient to allow aggressive behaviour when needed as well as to maintain other aspects of dominance such as odour cues and urine marking patterns. From these data, it seems feasible to consider the emergence of a new model, not based simply on a system of dominance equating with high testosterone output and subordinates with lower output but where true territory holders have low resting testosterone levels but are more responsive to certain stimuli when compared with subordinates or subdominants. As a result of this, it is perhaps worth asking whether animals such as the territory holders therefore show some degree of emancipation from rigid endocrine control and this idea may be worth future consideration.

CHAPTER 6

URINE ODOUR AND URINE MARKING PATTERNS

Introduction

This chapter describes a series of experiments which investigated the properties of urine in mice from the three housing conditions and, in particular, an aversive factor which is known to be present in the urine of male mice of certain social categories. Studies were also made of urine marking patterns together with measurements of plasma testosterone levels. Some of the work reported here is in press, and due for publication in Behavioural Processes, Vol. 15, No. 2, 1987.

The data are discussed in terms of a relationship between the existence of an aversive cue and production of particular marking patterns for its dispersal; these factors were not always found to be associated. These results together with those for testosterone levels suggest that odour properties and deposition patterns of urine may not be controlled by a single physiological factor.

Considerable evidence has been accumulated which points to the importance of olfactory cues in the regulation of social interaction among mammals. As knowledge in this area has increased, attempts have been made to develop a system for the classification of social odours. One early attempt was made by Bethe (1932) (cited in Macdonald and Brown 1985) who made a distinction between 'endohormones' and 'ectohormones', the former being secreted into the individual's body and the latter, excreted from the exterior of the body. He further divided the ectohormones into two sub-groups, homoiohormones if the action was intraspecific and alloiohormones if interspecific.

It was Karlson and Luscher (1959), (cited in Macdonald and Brown, op cit.) who used the term pheromone to stand for the homoiohormones described by Bethe. The term was originally introduced by Karlson and Butenant (1959) in discussing communication among insects but since then, it has been extended to include chemical communication among mammals and is also used in relation to mammalian behaviour.

The action of pheromones may be through the central nervous system bringing about a rapid change in the behaviour of the recipient, in which case it is said to have a "releaser" or signalling effect (Wilson 1963). Releaser pheromones are believed, among other roles, to influence the onset of mating behaviour, acting as sex attractants (Keverne 1978). Odour cues may also act through the neuro-endocrine and anterior pituitary system with the principal effect of stimulating physiological changes which may then secondarily result in altered behaviour in the animal. The behavioural effects due to these "primer" signals, come about slowly and involve prolonged stimulation of the central nervous and endocrine systems; these signals are known to influence aspects of rodent reproductive physiology.

The expressions, "primer" and "releaser" were originally given to the effects of pheromones and it was believed early on that the same pheromone could be both a primer and a releaser (Wilson and Bossert 1963). Later they were applied to the pheromones themselves rather than to their effects (Bronson 1968, 1971). In the knowledge that both priming and releasing functions could reside in a single factor, it was a mistake at that time to apply the term to the factors rather than to the responses. The term pheromone was initially used to describe the effects of substances on insect behaviour whereby chemicals secreted by an individual externally are

"received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process" (Karlson and Luscher, 1959 p55.).

Criticism has resulted however, when use of the word "pheromone" has been used to describe mammalian chemical signals (Beauchamp, et al. 1976). In the context of insect behaviour, "pheromonal response" implies a rather rigid set of programmed responses by individuals which results in conceptual problems arising when the term is applied to odour signals in mammals. The fact that responses to these odours are not stereotyped in mammals and that a significant feature of mammals is the ability of individuals to respond differentially to complex signals must therefore mean that in a strict sense, the value of the term within this context is questionable.

Secondarily, the behavioural responses of mammals are rarely completely independent of some learned element and so this results in a range of behaviours that are more variable and so less stereotyped than those of animals from the lower phyla, such as insects. Nevertheless for the mammals, their physiological responses, such as in the Whitten effect (Whitten 1956), are more reliable and use of the word pheromone may be justified here.

It is also true that many of the odours that mammals use as signals for behaviour are known or believed to be mixtures of substances which may be bacterial in origin and therefore of varied composition. As a result, these compounds are distinct from the single chemical substances used by insects. Although the pheromone concept has nevertheless served a useful function, to avoid these conceptual problems, the term has been avoided in what follows, as it is believed that the same information may be conveyed without using an expression which leads to unwarranted implications.

In most mammals, except the higher primates and man, olfactory

signals are dealt with by a system that has dual components. These consist of a main sensory olfactory pathway leading from the nasal epithelium, via the cribriform plate, to the olfactory bulbs from where further neural connections are made with the thalamus and orbital frontal cortex. Another set of receptors is located in the epithelial lining of the vomeronasal organ (or Jacobson's organ) to form the accessory olfactory system. Axons run between the main olfactory bulbs and synapse in the accesory olfactory bulbs which are both spatially and histologically distinct from the main ones. Projections go directly to the amygdala and from there, pass on to the hypothalamus, preoptic area and septal nuclei in the limbic system. (Keverne 1979, Quay 1983, Scalia and Winans 1975). This accessory system is known to transmit the "priming" effects of "pheromones" (Keverne and de la Riva, 1982) as it connects to the seat of neuroendocrine control in the hypothalamus. Thus the main system may be seen as being for sensory processing and the accessory for eliciting physiological change.

Recent research in rodents on gender recognition and determination of sexual state by conspecifics, suggests that responses to sex chemosignals are elicited primarily by stimulation of the vomeronasal organ and accessory olfactory bulb (Lepre et al. 1985) whereas responses to non-sexual odours such as food, are served by the main olfactory system (Wysocki et al. 1982). The vomeronasal organ may play an important role in detecting non-volatile molecules of high molecular weight and has been implicated in arousal and stimulation of mating behaviour (Steel and Keverne 1985, Winans and Powers 1977).

Among rodents, the roles played by odour cues have been intensively studied, and the list of odours involved in their social behaviour continues to grow. Stoddart (1974) has said "if odours are

used for social purposes, one might expect that the range of messages to be exchanged would be as large as the range of visual signals observed in social groups of large mammals".

The odour signals used so extensively are contained mainly in the urine in the house mouse and although attempts have been made to identify the chemical nature of these components (Evans et al. 1978, Novotny et al. 1985, Jemiolo et al., 1985) it seems possible that a single odour may possess two functions depending on the context of use (Ropartz 1977).

The need for individual recognition among members of a social group of rats and mice is important for the maintaining of social relationships. The ability of mice to do this has been demonstrated by Bowers and Alexander (1967), work that was later extended by Hahn and Simmel (1968) who showed clearly the important role of olfactory cues in this context. In gerbils, Halpin (1974) found evidence of individual discrimination in faeces and ventral gland secretions as well as in urine. Olfactory recognition of conspecifics in mice remains feasible up to a distance of 17.5cm (Kalkowski 1967), an important capacity for communication among members of the same social unit or between small groups that belong to the same population.

Ropartz (1968) has shown that between two groups of male mice exists two odour types: the first, "planta factor mice" is produced as a secretion from the paws of male mice when isolated or grouped. The second has been named "urinary factor mice" and comes from the coagulating gland of the male, and is secreted by grouped males only when able to exchange tactile stimuli. These results are in line with the ideas of Barnett (1963) who concluded that among rats, two types of smell might explain the relations between members of the same group and between strange groups. A "type odour rats" allowed recognition of an individual, its age, sex and perhaps physiological

state also, and a "colony odour rats" which enabled an animal to differentiate between a member of its own social unit and strange rats. It appears that mice also use these two odour types to determine if an odour emanates from known or strange individuals.

For maintenance of social stability, the ability to recognise at an individual level is essential but equally important is recognition of social status. There is evidence to suggest that dominant individuals produce odours that differ from those of subordinate animals (Carr and Martorano 1967). Male rats living in groups are able to distinguish between odours from submissive strange males against those from dominant strangers (Krames et al. 1969). Female bank voles show positive responses to the odours of dominant versus subordinate males in choice chambers (Hoffmeyer 1982) and using similar test conditions, comparable results have been found for male and female lemmings (Huck et al. 1981) and hamsters (White et al. 1984). More recently, McGlone (1985), working with swine, has postulated the existence of a submissive odour cue in the urine of subordinate pigs which appears to be ACTH-induced and is released towards the end of agonistic encounters. Among rodents, stressed mice emit a specific odour (Muller-Velten 1966, cited in Ropartz, 1977; Lane-Petter 1967) and so do rats (Valenta and Rigby 1968).

In contrast to the signals of subordinates, the urine of dominant male mice is more attractive to oestrous females than the urine of subordinates (Jones and Nowell 1974d). As well as attractiveness, the urine of highly aggressive male mice stimulates aggression in trained opponents when painted on the backs of castrates (Mugford and Nowell 1970), the reverse of which happens when the urine of recently defeated or submissive males is used and levels of fighting are diminshed. The urine of females also suppresses attack and also serves to attract male mice who will

select urine from oestrous versus dioestrous females, (Davies and Bellamy 1972) an effect which is lost if the males are castrated.

The preputial glands are believed to be responsible for production of the attractant odours released in mouse urine (Bronson and Caroom 1971, Gawienowski et al. 1975). In addition, Jones and Nowell (1973b) have shown that the contents of the preputial glands mixed with water, induce aggression in trained opponents, when painted on the backs of castrates, while those of the coagulating gland inhibit or reduce fighting.

In mice, Bronson and Marsden (1973) showed that the preputial glands are heavier in dominant individuals than in subordinates, and the effects of the secretions of these glands are known to be androgen dependent. Castration inhibits the production of aggression-promoting cues, an effect which is reversed when hormone-replacement treatment, testosterone propionate (TP) is given (Lee and Brake 1972). This same inhibition also results when progesterone is administered to TP treated animals (Lee et al. 1976); progesterone is a known androgen inhibitor and can arrest TP-stimulated aggressive behavior in mice (Lee and Griffo, 1974) and possibly in hamsters (Payne and Swanson 1972). Lee et al. (1976) have postulated that the action of progesterone is peripheral, influencing androgen dependent tissue such as the preputial glands and seminal vesicles, by competing with androgens for binding sites and blocking enzymes or causing rapid catabolism of testosterone itself. Certainly these tissues are sensitive to the effects of other anti-androgens also. Preputial glands undergo marked atrophy following treatment with cyproterone acetate, which results in effects similar to those of castration, and both of which are reversed by androgen treatment (Pandey and Pandey 1984).

Overall, it appears that the production of aggression-promoting

cues is linked with androgen levels and both of these are considered to be features of high status in males. In contrast it is thought that androgen-linked substances are not found in males of low status due to the stress often suffered by these animals who may be forced to live in close proximity with aggressive, high status males (Jones and Nowell 1973c).

A characteristic of male mouse urine which is closely related to its apparent aggression-promoting properties, is it aversiveness to other males (Sandnabba 1985). This urinary feature deters other males from prolonged investigation of a marked area (Jones and Nowell 1973a, 1974b), and the exploratory behavior of a male mouse can be attenuated by the presence of a strange male's urine. As with the aggression-promoting odour, the urinary aversive cue is not found in all males. In a series of papers by Jones and Nowell, the aversive factor was shown to the present in the urine of singly housed males (1974b) and of dominant males (1973c) but not of group housed (1974b) or subordinate animals (1973c). Castrated males do not possess it (1974a) and anti-androgens can suppress its production (1974c). The coagulating gland is considered to be the source of this substance (1973b) although neither bladder urine, nor the product of the gland alone nor these two materials placed in close proximity has any effect (Albone 1984). The aversive function arises from contact between the product of the coagulating gland and bladder urine which would indicate that the aversive signals arise from a chemical reaction between components of these two materials.

These details, taken together with evidence that high social status is also correlated with high levels of androgen secretion (Bronson and Marsden 1973, Lee and Naranjo 1974) have led to the conclusion that dominant status results in the production of an "aversive factor". In part, this theory includes the idea that

single housing influences the physiology and behaviour of male mice in the same direction as dominant status (Benton and Brain 1979). As male mice are territorial and are known to exclude all other adult males from their living area (Crowcroft 1966), housing them alone is thought to produce similar changes, making them resemble territory holders in their behaviour and physiology. Single housing certainly increases the aggressiveness of male mice (Valzelli 1973, Leshner et al. 1973, Brain and Benton 1977, Goldsmith, Brain and Benton 1976) but the idea that this is solely due to raised androgen levels is now recognised as an oversimplification (Clark and Nowell 1978). Nevertheless, it is believed that territory-holding male mice under natural conditions, may well produce urine that signals territory occupancy and this influences the behaviour of investigating conspecifics.

In view of the evidence, the experiments described in this chapter were carried out to investigate further the social conditions relating to the presence or absence of the urinary aversive cue and employed male mice both caged and housed in the free range room.

Besides the odour properties of their urine, dominant male mice are reported to distribute it differently from subordinates. With the use of ultra violet visualisation, over a period of twelve hours, dominant males have been shown to produce large numbers of marks which are widely distributed whereas subordinate mice tend to void their urine in a few large pools, particularly around the periphery of a test arena (Desjardins et al. 1973). Using testing times of one hour and also thirty minutes, Powell and Wolff (1982) have shown that levels of marking are lower in females compared with males and that castration drastically reduces the numbers of urine spots male mice deposit, at least in the presence of female odours (Wolff and Powell 1984).

A number of other studies have demonstrated the dependence of scent marking activity on androgen secretion (Griffo and Lee 1973, Mykytowycz 1970, York and Thiessen 1972). Testosterone propionate (TP) and estradiol benzoate (EB) given to castrated male mice restores marking to pre-castration levels, a result which does not occur if dihydrotestosterone (DHT) is given (Kimura and Hagiwara 1985). Testosterone is aromatized to oestradiol but as both TP and EB can restore marking when used alone, this effect is not therefore thought to be due to aromatization of androgen. In the same study, the lower levels of marking in females compared with males, is shown to be further reduced if females are ovariectomised. Females given androgens neonatally mark at much higher levels in adulthood compared with control females. It would appear that the sexual dimorphism shown in the urine marking behaviour of mice may be determined by the hormonal environment during the early postnatal stages and by the competence to respond to androgens which play a regulatory role.

Similar results to those of Kimura and Hagiwara (1985) have been shown in gerbils (Turner 1975, Turner and Carbonell 1985). In this species, mid-ventral sebaceous gland marking can also be stimulated by testosterone implants to the hypothalamus, and this marking activity is blocked by Actinomycin D which suppresses transcription of RNA from DNA (Thiessen et al. 1973, Thiessen and Yahr 1970). These authors have suggested that the behaviour is centrally controlled in this species. Testosterone treatment in castrated rats also restores levels of marking of objects as well as of conspecifics (Price 1975). The degree of marking intenstiy was investigated in a study which included house mice, deermice, gerbils and hamsters together with the propensity to mark in each species (Maruniak et al. 1975). Frequency of urine deposition varied between the species with housemice and deermice producing numerous small spots and streaks over regular

short distances, whereas gerbils only produced large pools and hamsters released little or no urine during the two hour testing periods. Hamsters and gerbils are known to use exocrine glands for scent marking and are not known to possess urinary signals (Thiessen et al. 1971a) which may account for the scant and restricted deposition of urine that was seen.

The means by which numerous tiny urine drops are produced is of some interest: the penis sheath of the adult male mouse is long relative to body stance and the hairs at the prepuce may act as a wick for the deposition of urine on the substrate. Maruniak et al. (1975) have postulated that the design of a long preputial sheath may be an energy-saving adaptation which permits frequent deposits of urine while other home-range or territorial activities are undertaken and the need for specific muscular involvement during marking is therefore reduced.

Scent marking is recognised as playing an important part in mammalian communication although at one time, the chief role was considered to be territorial marking (Hediger 1949 cited in Ralls 1971, and Gosling 1982) where marks serve to keep away potential rivals. However, such a theory has been refuted by observations of undeterred intruders in a variety of species and generally, evidence for this idea is hard to come by (Johnson 1973). Nevertheless, the investigation of a marked area by strangers may not mean that the scent marks are ignored, nor that they do not function in the context of territorial defence; indeed the marks may serve to decrease the probability of a fight or bias the outcome in favour of the resident (Yahr 1983). In as much as marking is concerned with territory occupancy, it may only possess this function by virtue of the behavioural characteristics of a species and certainly Mackintosh (1973) in experiments with mice housed in enclosures, found that

visual cues played as important a role as scent marks in the maintenance of territory boundaries.

There is certainly a strong correlation between high frequency of marking and high social status or dominance. This has been shown for rabbits (Mykytowycz 1965) and mice (Desjardins et al. 1973) although in gerbils housed under semi-natural conditions (Roper and Polioudakis 1977), territories were not always defended by the animal that marked the most. Although it has been argued that saturating a territory with the animal's scent serves to make the area familiar (Mykytowycz 1968), scent marking may also have functions other than the determination of territories or the maintenance of dominance orders. Ralls (1971) has stated that even if a dominant animal marks frequently, it may not necessarily maintain its status by marking; rather the aggressiveness of dominant animals keeps them in a dominant position regardless of whether or not they mark. She also suggests that males mark frequently in any situation where they are both intolerant of and dominant to other members of the same species and are likely to attack.

In criticism of Ralls's argument, Eisenberg and Kleiman (1972) state that only situations producing high levels of marking are considered, thereby ignoring the motivation for less vigorous marking. Also emphasis is only given to marking in males with no consideration of females in whom marking levels vary with the reproductive cycle, with high levels bearing no relationship to attack propensities. Although marking may well occur when an animal is aggressively motivated, it does not follow that marking in itself is an expression of aggressiveness.

In a discussion of scent marking in Canidae, Kleinman (1966) defines marking as the means by which an animal maintains familiarity with its environment so that odour placed on specific landmarks

familiarises an animal with new areas as well as reaffirming recognition with old terrain. In addition, the marking of a territory may act as an extention of the animal (Geist 1964) enhancing the confidence of the resident and providing information to others about strength, stamina, state of arousal and motivation (Clutton-Brock and Albon 1979). For these reasons territory owners mark their areas to provide intruders with a means of assessment.

In conclusion, the evidence for scent marking in mice suggests that among males, it is only territory holding individuals or those in a similar physiological condition, that urine mark prolifically. The experiments described in this chapter were conducted to test this hypothesis using both caged and free range mice together with investigations into the presence of the aversive odour signal described above. Evidence that these characteristics are androgen dependent has resulted in the inclusion of certain tests to measure plasma levels of testosterone.

Summary of Experiments

FR2a: Test for the presence of an aversive factor in the urine of three free range dominant territory holders. One of the urines was found to deter investigation by test subjects.

FR2b: Urine marking patterns in mice of differing social status and housing conditions were found to be similar to those observed by Desjardins et al. (1973). An optimum time length of twelve hours was found to be suitable for production of urine patterns.

FR2c: Marking patterns in the presence of a stranger resulted in subordinate mice producing patterns similar to those generally associated with high status males.

FR3a: Tests for urinary aversive cues and marking patterns were carried out on six categories of mice. Only free range territory holders were found to possess an aversive factor in their urine. Single caged males produced the largest number of urine marks compared with other groups although dominants marked more heavily than subordinates.

FR9a: A series of tests carried out at two four-week intervals examined aversive urinary cues, urine marking and levels of plasma testosterone. Urines of both free range and caged dominant mice were found to contain an aversive factor on both test occasions. Levels of urine marking were again found to be highest in single caged animals. In the second set of tests, marking levels were also found to be extremely high for free range subordinates.

Testosterone levels in the second tests were significantly lower in single caged males compared with dominant animals but significantly higher for free range dominants compared with free range subordinates.

FR8a: Due to changes in the social hierarchy in the free range room, three territory holders were deposed by three subordinate males. Urine tests two weeks later showed an aversive factor to be present in the urine of ex-dominants only. No differences were found in urine spot numbers nor again, when tests were repeated two weeks later when the original three mice had been reinstated as territory holders. Levels of plasma corticosterone differed significantly, but this was not so for levels of testosterone.

Methods

1. Urine Odour Tests

Collection of urine was carried out by placing donor animals invidually in wire-mesh bottomed cages (30x13x11cms) over funnels with collecting vessels. Donors were acclimatised to the urine-collection apparatus with food and water ad libitum for twenty-four hours before collection began, in order to minimise stress. Although King and Pfister (1975) have reported that odours generated by stressed rats do not carry alarm signals, other studies have shown that the urine of stressed mice may contain a substance which induces fear responses in other conspecifics (Carr, Martorano and Krames 1970, Rottman and Snowdon 1972).

The collection apparatus was a modification of that used by Jones, Dilks and Nowell (1973) (Figure 6). The mesh-bottomed cage rested in the top of a rectangular aluminium funnel. A small glass orb hanging from the base of the funnel diverted faeces, and urine ran over the orb into a collecting vessel. Orb and collecting vessel were enclosed in an outer beaker to reduce evaporation. Collections were carried out overnight from 17.00-9.00 hours. Water was supplied freely throughout, but food was restricted to reduce contamination of

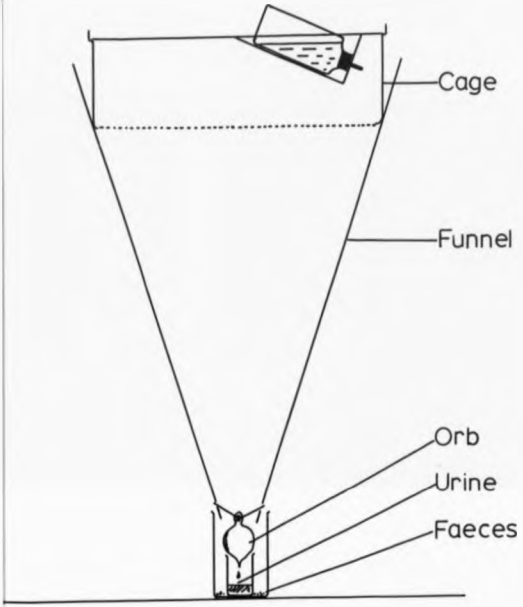


Figure 6 Urine collection apparatus

the urine by crumbs. After collection, the urine was capped and stored at $4^{o}C$. It was used between four and eight hours after the end of collection. Each mouse produced 4.5 ± 0.5ml urine during the collection period, and entire samples from all individuals in a donor category were pooled.

Tests on urine were carried out under red light beginning four hours after collection ended. In all experiments except FR2a, test animals were five to seven month old TO males which had been caged together in groups of eleven since weaning, and were thus socially stable, and were drawn from a separate pool of animals from the urine donors. On each of the three days prior to testing, these subjects were observed for fifteen minutes for fighting. In each cage, only one animal was ever seen to initiate fights and these were always quickly terminated by submissive responses before injury occurred. Visual inspection revealed no scars or other signs of injury, and in no other way could the subjects be distinguished. The identity of the individual seen to initiate attacks was confirmed on the test day, and this animal was not used. The subjects used were thus all socially subordinate and with previous experience as uniform as possible. Subjects were selected non-systematically for tests, which employed a method after Jones and Nowell (1973a) with some modifications. Tests were carried out in a polythene tank (36x43x34cms) which contained a sheet of white blotting paper (42x36cms) divided into twenty-four rectangles (9x7cms) with faint pencil lines.

A drop of urine was placed centrally in each of the twelve rectangles in one half of the paper and twelve spots of tap water were put on the other half. A test animal was then placed on the centre of the paper and observed for five minutes. Timing began fifteen seconds after entry to the test box. The number of seconds

spent in the urine half of each paper was recorded. The Wilcoxon-Matched Pairs Signed Ranks Test was used to determine differences between the amount of time spent on each side of the papers. Also, as an approximate measure of mobility, the number of rectangles entered on each paper was recorded. All four feet in a rectangle was used as the criterion for scoring activity. Data for mobility scores were analysed by Kruskal-Wallis analysis of variance and Mann-Whitney U tests. Water and urine sides were alternated to eliminate position bias and the scorer sat symmetrically between the two halves of the tank. Between tests, the tank was cleaned with a weak solution of disinfectant as water is known to be ineffective in removing mouse odours (Whittier and McReynolds 1965). When urine collections were done on free range mice, all animals were removed from the room and singly caged with food and water freely available until space was available in the collection apparatus. This was to avoid the possibility of low status males taking over territory areas in the absence of dominant animals. It was possible for animals to be removed from the free range room for periods of up to 52 hours. To ensure that dominant territory holders retained their position, the precaution was taken whereby these animals were replaced in the room thirty minutes before the rest of the mice. Following this, 15-20 minute observations were made on five consecutive days, between 14.00 and 15.30 hours to verify that status was unchanged. On no occasion was social position found to have changed and mice were seen to return to the areas they had lived in previously.

2. Urine Marking Tests

Sheets of polythene-backed absorbant paper (Benchcote) (27x45cms) were laid below bottomless metal cages (86x25x11cms) which were divided by partitions to form two cages, each 43x25x11cms, and

were stood on clean formica worktops. Animals were placed on the Benchcote sheets, one mouse per cage and left undisturbed for twelve hours (14.00-2.00 hours, red lights on 12.00-22.00 hours). Food and water were supplied throughout. The mice were then removed and the papers were air dried. These methods were used in all tests except FR2b, for which the method is described separately.

Where quantitative measurements were made, urine spots were counted by dividing each paper into fifteen boxes (9x9cms) by pencil line and recording the spot numbers in each box. Three boxes in the centre of each paper were bordered by twelve around the periphery. An ultra-violet lamp (Hanovia U.V. Lamp, 240-360nm, broad spectrum U.V.) was used to visualise the urine marks which were counted by an observer who was unaware of the identity of each paper until after the count was complete. The ultra-violet lamp was also used for qualitative assessment of urine patterns. Data were analysed by one-way and two-way analysis of variance (mixed design) and Student's t-tests.

Experiments

FR2a:

A preliminary pilot study was carried out to determine whether the urine of free range dominant territory holders possesed aversive properties. Using the method described above, the urine of three territory holders was collected three weeks after removal of the barriers in the room, when the animals were fifteen weeks old. For this experiment, the test animals used were eight subdominant and eight subordinates that lived in the free range room together with the urine donors. The tests investigated whether the urine of a familiar territory holder was less aversive in quality than that of an unfamiliar territory holder. It was therefore noted which

subdominants and subordinates lived with particular high status males so that, as test subjects they could be tested against both familiar and unfamiliar urine. Each test subject underwent the test procedure described above three times; once for familiar urine and twice against unfamiliar ones. Subordinate and subdominant mice were tested alternately and the urines were rotated after one animal of each category had been tested. The identity of the urines in terms of familiarity or unfamiliarity for each subject was unknown until all the tests were complete. Table 6:A shows the rotation order of urines against the different test subjects. When tests were concluded, the mice were returned to the free range room. The data scores for the two unfamiliar urines for each animal were summed and a mean score obtained to give a single set of scores for unfamiliar urine. All data was then analysed using the Wilcoxon Test.

Results

Figure 6:1 shows the means and standard errors for time spent in the urine and water halves of papers by subdominant and subordinate test mice when tested against familiar and unfamiliar urine. Subdominant mice spent significantly more time in the halves of papers spotted with familiar urine than in the water halves ($T=6$, $p<0.05$) but spent less time in the halves of papers spotted with unfamiliar urine although this result just failed to reach significance. Scores for subordinate mice failed to reach significance for both urine categories although the data showed a slight trend towards more time spent in the urine halves of papers for both urine types.

Test for aversive properties in familiar and unfamiliar urines

Fig. 6:1

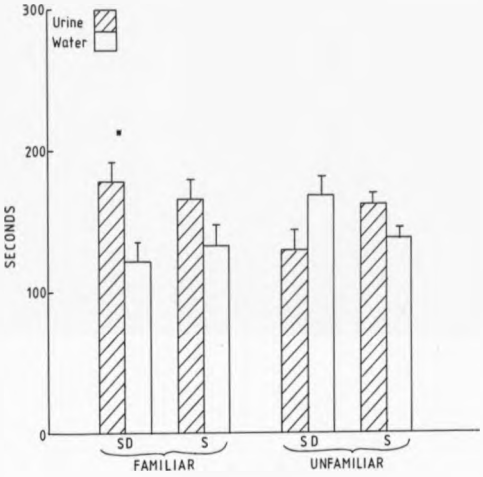


TABLE 6:A

	Test Subjects	Urine No.		
	SD1	1	3	2
	S1			
	SD2	2	1	3
	S2			
ORDER				
OF	SD3	3	2	1
TESTING	S3			
	SD4	1	3	2
	S4			
	SD5	2	1	3
	S5			
	SD6	3	2	1
	S6			
	SD7	1	3	2
	S7			
	SD8	2	1	3
	S8			

SD = Subdominant

S = Subordinate

FR2b:

A second pilot study examined the urine patterns produced by TO strain mice and was carried out over a period of one week commencing two weeks after the urinary aversive test described in FR2a above, at seventeen weeks, five weeks after removal of the barriers.

For this urine marking experiment only, sheets of white blotting paper (42x25cms) were attached to the bases of large plastic cages with adhesive tape. The urine patterns of eight single caged, four group caged territory holders, eight group caged subordinates, three free range dominants, eight free range subdominants and eight free range subordinates were investigated. Animals were placed one per cage and left to urine mark for one hour and then for twelve hours. Fresh paper was laid down at the start of each test period, and food and water were freely available.

Results

Very few urine spots were found to be present on any of the papers after one hour. Several animals, notably subordinates from the free range, had produced no marks at all. However after twelve hours, a pattern of marks was clearly detectable on every paper. Animals were removed from the cages and returned to their previous housing conditions.

Illumination with ultra-violet light showed that single caged and all dominant mice had produced urine patterns of numerous small marks spread widely over the area of paper provided. By contrast, the subdominants and all subordinates showed a tendency to produce urine in a number of pools mainly at the periphery of the paper and to mark with far fewer small spots. (Photographs show details of the patterns produced.)

A number of animals destroyed parts of the papers they were marking and this, together with the practical difficulties of

sticking paper in each box, resulted in a change in both the type of paper used and the design of the test cage. Details of the bottomless cages and polythene-backed paper used in susbequent experiments have been given above.

FR2c:

This pilot study on urine marking patterns sought to extend the findings of Experiment FR2b. The aim was to find out whether the patterns observed in the previous test were fixed with respect to the social status of an animal or whether as a response to a particular stimulus, the pattern could be altered irrespective of status. For these tests, the stimulus was the presence of a strange, adult male mouse. Testing took place one week after the end of the previous study, using the same animals as before in FR2b.

Using the methods described above, mice from the three housing conditions were placed in one half of the metal bottomless cages which for this experiment, were divided into two halves by wire mesh barriers. Five month old adult males which had previously been singly caged, were used as strange male subjects to stimulate urine patterns and were placed in the other half of the divided cages, one per cage. All animals were then left undisturbed for twelve hours, before being removed and returned to their previous housing conditions.

Results

Visualisation of the urine patterns showed that in the presence of a strange male, almost all test subjects produced a pattern of widely dispersed small urine marks regardless of social status or housing. Only three subordinate mice continued to produce a pattern of large peripheral pools (one group caged and two free range subordinates). The intensity of marking by single caged and dominant mice was seen to be of greater intensity when compared with the

previous test papers.

Despite the changes seen in the patterns produced by subdominant and subordinate mice, intensity of marking was of a lower level compared with single caged and dominant mice. No attempt was made to quantitate the results of these two pilot studies on urine marking.

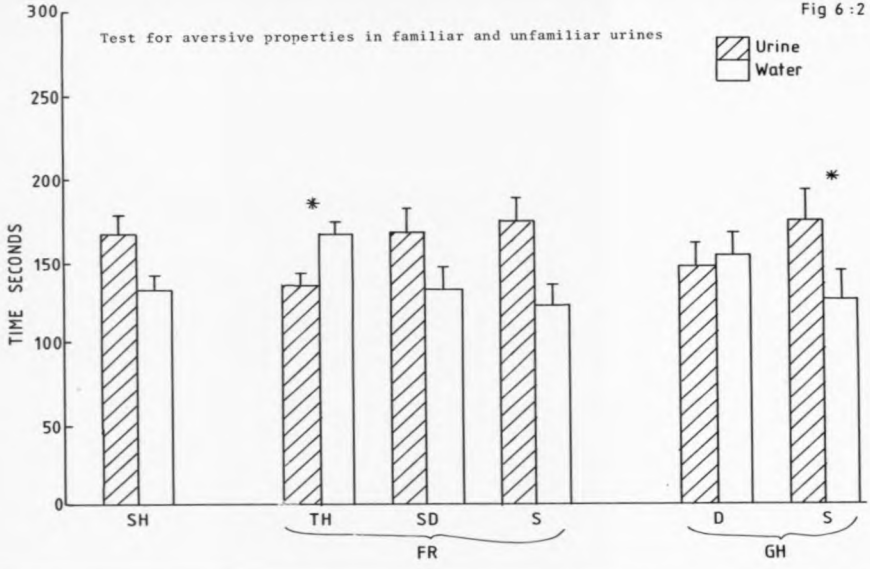
FR3a:

Animals from the three housing conditions were used to test for the aversive properties of urine. This was collected from six groups of individual donors, six single caged, four group caged dominants, six group caged subordinates, six free range territory holders, six free range subdominants and six free range subordinates using the method described previously. For the subdominants and for the subordinates in both the free range and group cages, and for the singly housed mice, six urine donors were chosen at random; in the other categories, all available mice were used. Collections were made when the mice were approximately fourteen weeks old, two to three weeks after the barriers in the free range were removed and the territories formed. Urine samples from a particular donor category were pooled and testing followed the procedure detailed above. Fifteen test animals from the pool of animals described above, were used against each urine type and no animal was used twice. A record of the number of rectangles entered by each test animal (i.e. a mobility record) was also made.

Results:

Figure 6:2 shows the means and standard errors for the times spent by test subjects in the two halves of the papers for each donor category. Test mice spent significantly less time in the half of the area marked with the urine of free range territory holders than in the water half ($p<0.025$). By contast, they spent significantly more time in the halves of the papers spotted with group caged subordinate

Fig 6 :2


Test for aversive properties in familiar and unfamiliar urines
Urine
Water
TIME SECONDS
300
250
200
150
100
50
0
*
*
SH
TH
SD
S
FR
D
S
GH

urine ($p<0.025$). No other donor category produced a significant result.

Figure 6:3 shows the mobility results. No significant differences were found between the free range groups nor in the free range versus group caged dominants but test animals showed reduced mobility when exposed to group caged subordinate urine compared to urine from free range subordinates ($p<0.025$).

FR3b:

Animals were returned to their housing conditions at the conclusion of the first part of this experiment. Observations were made on five consecutive days for 15-20 minutes between 14.00 and 15.30 hours and it was verified that their social status was unchanged. One week later, urine marking tests were carried out using the same individuals; marks were recorded and quantified as described above.

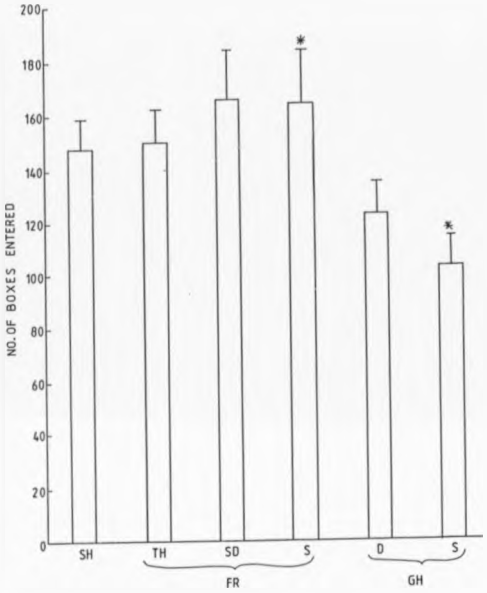
Results:

Figure 6:4 shows the means and standard errors for the numbers of urine spots produced by each category of animals over a twelve hour period. Singly caged mice produced significantly more spots than free range territory holders or caged dominants ($F=11.67$, $df=2,12$, $p<0.002$). Within the free range animals, the territory holders marked to a significantly greater extend than subdominants and subordinates ($F=3.399$, $df2,16$, $p<0.05$). No differences were found between group caged dominants and subordinates nor between caged and free range subordinates.

The data in Figure 6:5 are the mean ratios of edge to centre marking for each group. These figures were obtained by first determining the average number of spots per box in edge and centre boxes for each animal in a group. The ratio of edge to centre marking was then calculated for each subject by dividing the average

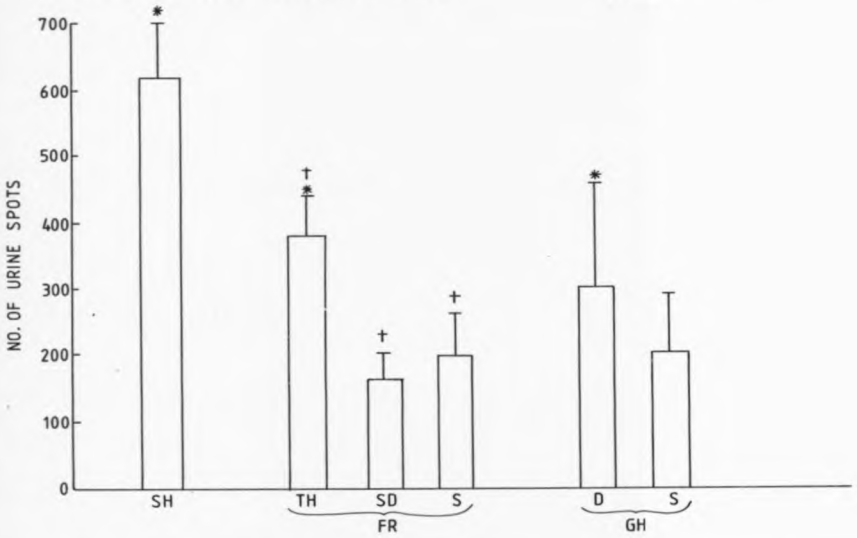
Mobility scores

Fig 6:3



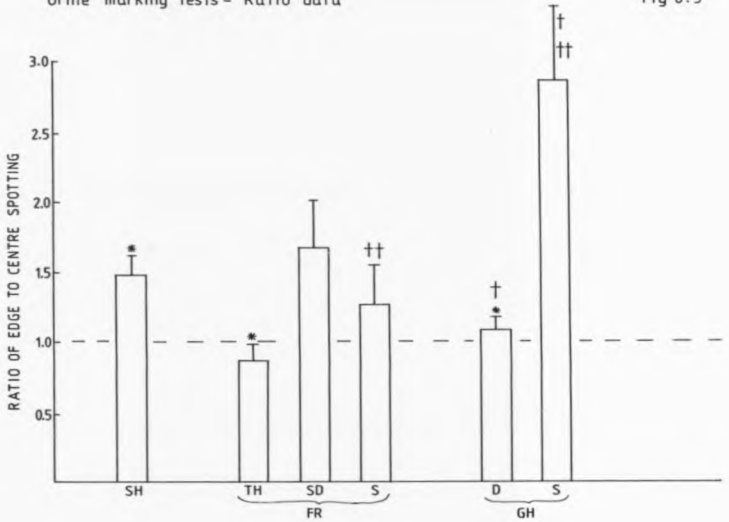
Urine marking tests Total spot numbers

Fig 6:4



Urine marking tests - Ratio data

Fig 6:5



for edge boxes by that for centre boxes and the mean ratios for each group derived. Values of greater than 1.0 indicate a trend towards edge marking and below 1.0, towards centre marking. This method takes into account the 12:3 ratio of edge to centre boxes and is a measure of spotting density.

Between group analysis of the data employed on way ANOVA and Student's t-tests on arcsine transformed ratio data. Single caged mice showed a significant degree of edge spotting compared with free range territory holders and group caged dominants ($F=6.906$, $df=2,12$, $p<0.01$). No significant differences were found between the ratios for the free range mice. The ratio was significantly higher in group caged subordinates than either group caged dominants ($t=2.61$, $df=8$, $p<0.05$) or free range subordinates ($t=2.68$, $df=10$, $p<0.06$).

FR9a:

In this experiment, animals from the three housing conditions were again used to test for the aversive properties of urine, numbers of urine marks and also plasma levels of testosterone.

The previous experiment demonstrated the presence of an aversive factor in the urine of free range territory holders, two to three weeks after removal of the barriers. The tests here aimed to extend these results and examine whether the aversive cue was present in urine before the barriers were removed at twelve weeks of age and then again four weeks later, at sixteen weeks of age. Because the presence of an aversive factor together with high levels of urine marking are thought to be androgen-dependent, plasma levels of testosterone were recorded also at twelve and sixteen weeks.

Five groups of individual donors were used: six single caged, four group caged dominants, six group caged subordinates, four free range territory holders and six free range subordinates. Subordinates were again drawn at random from the two housing

conditions. No subdominants were identified during observation periods. Collection and testing of urines together with scoring for mobility levels followed the procedure detailed above. Urines from a particular donor category were again pooled. Twelve test subjects were used against each urine type and no subject was used twice.

Immediately after urine collection was complete, donor mice were placed individually in bottomless cages as described above and left to urine mark for twelve hours (9.00-21.00 hours). Following this, approximately 300µl of blood was taken from each animal using the method outlined in Chapter 2. Plasma levels of testosterone were measured using the RIA procedure described in Chapter 5. Using the precautions mentioned in the methods section, animals were then returned to their previous housing conditions. The same experimental animals were used in both sets of tests. After the first tests, social status was verified by observations of 15-20 minutes on five consecutive days and then twice weekly prior to the second set of tests at sixteen weeks.

Results:

Figure 6:6 shows the means and standard errors for the time spent by test subjects in the urine and water halves of the papers for each category of urine donor when testing was done at twelve weeks, before barriers were removed. Test mice again spent significantly less time in the halves of papers spotted with the urine of free range territory holders ($p<0.01$). This result was also seen in caged dominant mice with test mice spending significantly less time in the halves of papers spotted with their urine ($p<0.005$). By contrast, significantly more time was spent by test subjects in the halves of papers spotted with the urines from both group caged ($p<0.025$) and free range ($p<0.025$) subordinate mice. No significant difference was found for single caged mice.

Test for aversive properties in familiar and unfamiliar urines at 12 weeks

Fig 6:6

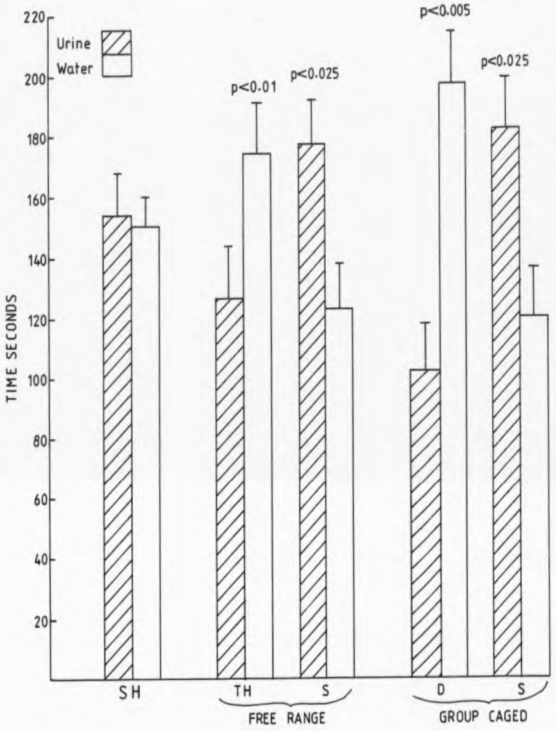


Figure 6:7 shows the results for the aversive factor tests carried out at sixteen weeks, four weeks after the barriers were removed, and territories formed. Results are similar to those for the tests at twelve weeks. The urines of free range and caged dominants deterred prolonged investigation by test-subjects to a significant extent ($p<0.005$, $p<0.05$). Again, test subjects spent significantly more time in the halves of papers spotted with urine from both caged ($p<0.025$) and free range ($p<0.025$) subordinates. No difference was found for the time spent in the halves of the papers containing urine from single caged mice.

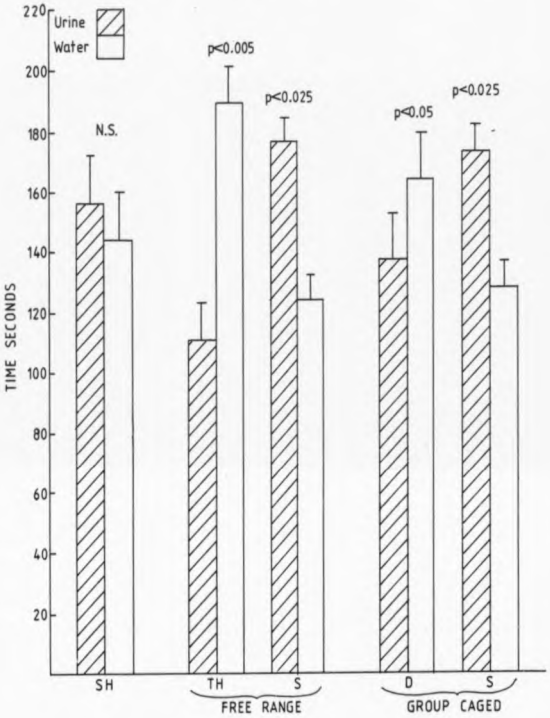
Figures 6:8 and 6:9 show the mobility results. No significant differences were found between any of the groups at twelve weeks nor at sixteen weeks.

Figure 6:10 gives the means and standard errors for the total numbers of urine marks produced in the tests at twelve weeks. Single caged mice spot marked with a great intensity but this result was not significant when compared with free range territory holders and group caged dominants. Both caged and free range subordinates showed high levels of marking but numbers did not differ significantly. Levels of marking were not significantly different between the groups of free range mice either.

The total numbers of urine marks for the tests carried out at sixteen weeks are shown in Figure 6:11. Again analysis of the data shows no significant differences in numbers when single caged, group caged dominants and free range territory holders are compared. Results for the categories of free range mice show high levels of marking for both groups and no significant difference in numbers. However, group caged dominants marked significantly more than group caged subordinates ($df=8$, $t=9.16$, $p<0.001$), as did free range subordinates compared with the same category of animals in group

Test for aversive properties in familiar and unfamiliar urines at 16 weeks

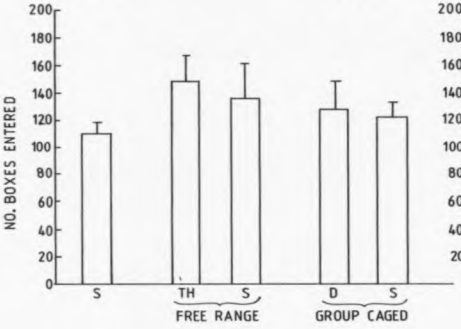
Fig 6:7



Mobility scores for aversive factor tests

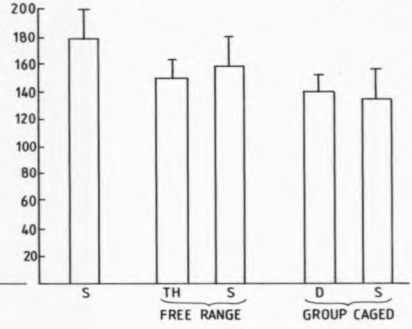
12 weeks

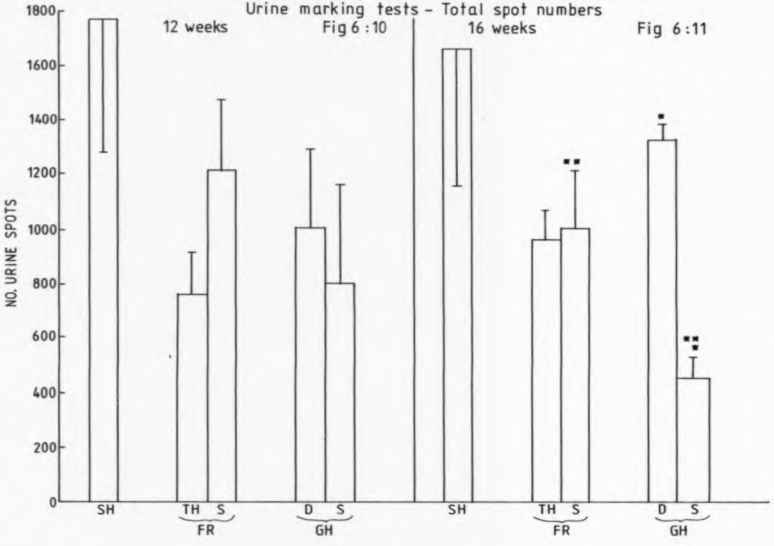
Fig 6:8



16 weeks

Fig 6:9




Urine marking tests - Total spot numbers
12 weeks
Fig 6 :10
16 weeks
Fig 6 :11
NO. URINE SPOTS
1800
1600
1400
1200
1000
800
600
400
200
0
SH
TH
S
FR
D
S
GH
SH
TH
S
FR
D
S
GH

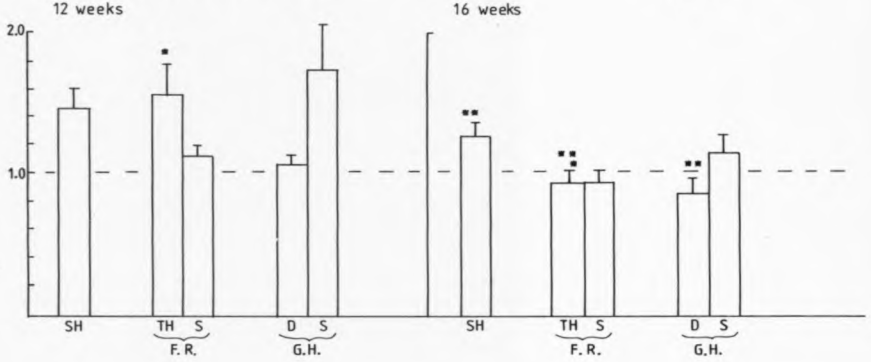
cages (df=10, t=2.53, p<0.025). When the data are compared for each category across both test times, no significant differences in the amount of spotting are found.

The means and standard errors of edge to centre ratio marking for each group at twelve and sixteen weeks, are shown in Figure 6:12. Analysis of arcsine transformed data shows no differences between the groups at twelve weeks. Results for the data at sixteen weeks show that single caged mice have a higher level of edge spotting compared with group caged dominants and free range territory holders (F=4.721, df2,11, p<0.033). Further breakdown by Student's t-tests showed that the density of edge to centre spotting was significantly higher in single caged mice compared with group housed dominants (df=8, t=2.74, p<0.05) but not when compared with free range territory holders. When the ratios are compared across both test times, territory holders show a significantly greater tendency towards edge marking at twelve weeks compared with data for sixteen weeks (df=6 ,t=2.78, P<0.05). No other category showed a significant difference.

Figure 6:13 shows the means and standard errors of plasma testosterone levels for each of the five groups at twelve and sixteen weeks. Data were analysed by two-way analysis of variance and Student's t-tests. Significant differences were found both between and within the groups of single housed, group housed dominants and free range territory holders over both times of sampling.

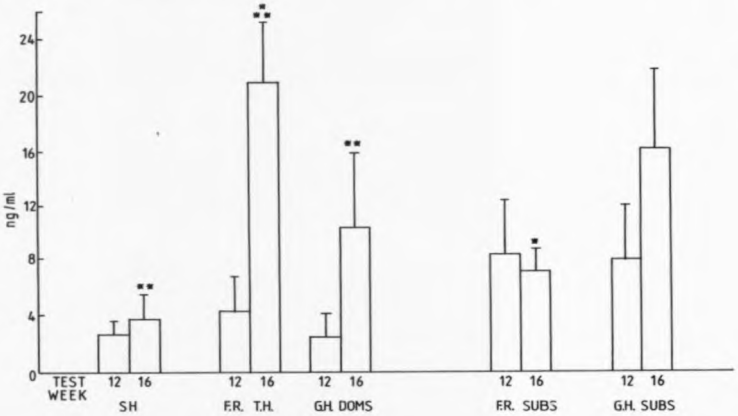
Urine marking tests - Ratio data

Fig 6 : 12



Testosterone levels after aversive factor and urine marking tests at 12 and 16 weeks

Fig 6 :13



2-way ANOVA

Source	df	F-ratio	Probability
Trt. Groups	2+11	9.1270	0.0046
Time	1+11	8.5132	0.0140
Trt. x time interaction	2+11	2.5816	0.1204

Further breakdown showed that levels for free range territory holders differed significantly from those of both single caged mice (df=8, t=5.38, $p<0.0005$) and group caged dominants (df=6, t=2.31, $p<0.05$); these two groups did not differ from one another. Levels for week twelve versus sixteen weeks also differed significantly (df=26, t=2.46, $p<0.025$). Free range territory holders were also found to have significantly higher testosterone levels compared with free range subordinates at sixteen weeks (df=8, t=3.16, $p<0.01$). No other between or within group differences were found.

FR8a:

The final experiment described here was an impromptu one, carried out after an estabilised social hierarchy in the free range room underwent changes which resulted in altered social status for a number of mice. Four dominant mice had held established territories for seven weeks. Three of these animals were deposed by subordinate mice when an experimental procedure left the territory holders in a state of physical disadvantage. Following the experiment that examined trough levels of corticosterone in free range mice, described in Chapter 4 (FR8), a single test then followed which sought to investigate whether the presence of intact adult female

mice, placed in the free range for 30 minutes, would in any way alter levels of testosterone in territory holders. After the thirty minute period, these animals together with the females were removed from the room and the males were blood sampled, and then returned to the room. Unfortunately, these animals, due probable to the combined effects of ether anaesthesia and being blood sampled, were defeated in fights that took place soon afterwards. Out of four territory holders, three were deposed and their positions taken over by other males. Daily observations verified that these "deposed" mice were subordinated, being seen to huddle with other low status mice and showing no resistence to being attacked. A week later, when the three new dominants were established, tests were carried out to investigate which, if any of the new and deposed dominants possessed the aversive factor in their urine, and after a further week, urine patterns were examined together with plasma levels of corticosterone and testosterone.

Collection of urine followed the method already described. Urines from the three mice of each donor category were pooled and after collections were completed, animals were returned to the free range room. Fifteen test subjects were used against each urine type and no animal was used twice. The urines were tested by an observer who was unaware of the identity of donor category until after testing was completed.

One week later, urine marking tests were carried out on these six animals using the same method as before. At the end of the twelve hour marking period, a blood sample of approximately 300µl blood was taken from each animal within three minutes of disturbing a cage, and levels of testosterone and corticosterone were determined by the methods described in Chapters 4 and 5. Animals were then returned to the free range room.

Results

Figure 6:14 shows the time spent by test mice in the two halves of the papers for urine of both new and deposed territory holders. Significantly more time was spent in the water sides of papers spotted with urine from deposed animals ($p<0.05$), but no differences was found for the urine of the new high status mice. Figure 6:15 shows the mobility results and no differences were found for either urine type.

The means and standard errors for total numbers of urine marks are shown in the first two columns of Figure 6:16. Deposed mice marked less than the new dominants but results did not differ significantly. The third pair of columns in Figure 6:16 shows the ratios for edge to centre marking. Deposed dominants had a stronger edge marking tendency than the new dominants but results were again, not significantly different from one another.

Figure 6:17 shows levels of plasma corticosterone after two weeks of reversed status. When levels for new dominant territory holders were compared with those of deposed animals, they were not found to differ significantly. The levels for testosterone, shown in Figure 6:18, were also not significantly different.

Following these tests, it was further decided to investigate the effects of reversing the social roles of these mice by allowing the original dominants the opportunity of regaining their areas thereby replacing the new dominants with the old ones, with the new ones therefore becoming subordinate once more to them. The aim was to see if this in any way would alter urine marking patterns together with circulating levels of plasma testosterone.

Manipulation of the social arrangements was carried out when the new dominant mice had held and defended their teritories for three weeks. These three mice were removed from the room and were caged

Fig 6:14

Urinary aversive tests in new and deposed free range dominants

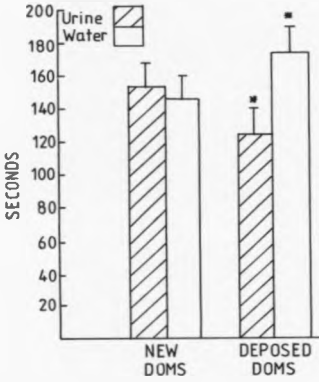
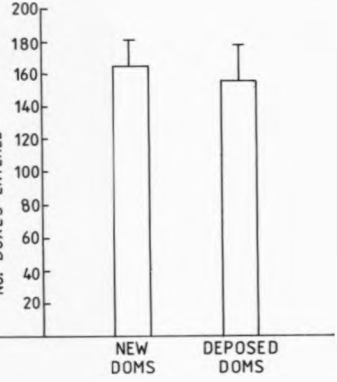


Fig 6:15

Mobility levels - new and deposed free range dominants



Urine marks of free range territory holders - deposed and reinstated Fig 6 :16

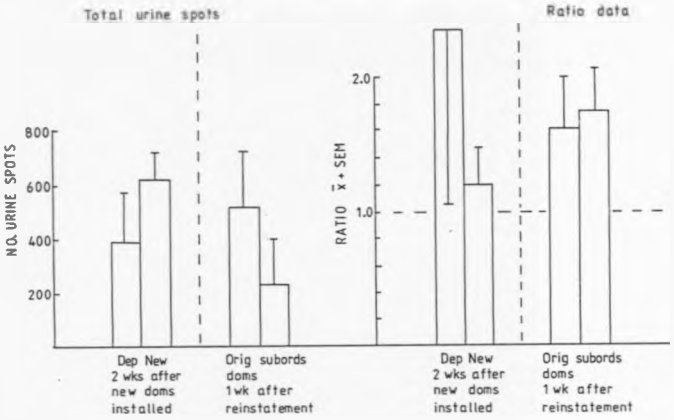


Fig 6:17

Corticosterone levels in new and deposed dominants

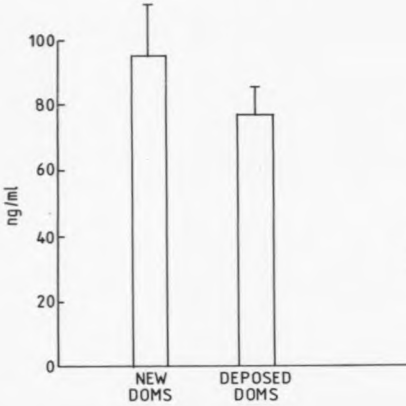
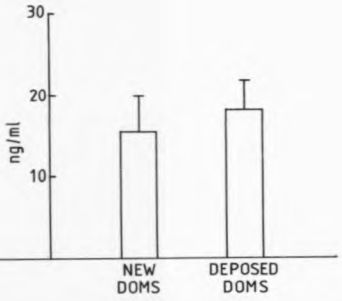


Fig 6:18

Testosterone levels in new and deposed dominants



singly with food and water for twenty-four hours. During this period, the three ex-dominants were seen to have regained their former territories and were observed to fight and defeat other mice in the groups where they lived. The three single caged mice were then returned to the room where they were also seen to be defeated by the original dominants and showed submissiveness to them. The animals were then left undisturbed for one week except for daily ten to twenty minute observations during the red light period to affirm that the social conditions had not changed.

After one week, the three dominants and three subordinates were removed from the room and placed in the test arenas to urine mark as before. Afterwards, a blood sample was taken from each animal before the mice were returned to the free range room.

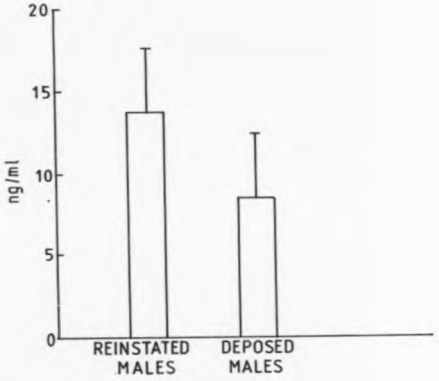
Results:

Spot marking data for total numbers produced is shown in the second pair of columns of Figure 6:16 and the ratio data is given in the fourth pair of columns. Although the overall number of marks is higher for the reinstated dominants, the data does not reach significance. Also a shift towards greater edge marking is seen in the subordinates but this again does not differ significantly from the data for the dominants.

Figure 6:19 shows the plasma levels of testosterone. Reinstated dominants were found to have higher levels than subordinates but results did not differ significantly.

Fig 6 :19

Testosterone levels in reinstated and deposed dominants



Discussion

The results from experiments FR3 and FR9 confirm the prediction that territory-holding dominant males produce in their urine an odour which deters other males from prolonged investigation. Although in experiment FR3a, the urine of caged dominant males did not show this aversive factor, these animals were shown to produce this odour characteristic in later tests (FR9a). The data thus support the hypothesis that a urinary odour is used by male house mice to serve a territorial marking function. Overall, the results for free range territory holders agree with and extend those of Jones and Nowell (1973c), who found the aversive factor to be present in the urine of caged dominants, similar to the finding in experiment FR9 although their experiment was rather different in design: their dominant animals had been isolated for two months and were then housed in pairs to form dominance-subordinate relationships only three days before the experiment. Thus the social status of the urine donors was of a much briefer standing than in these experiments. When considering the first result from the group caged dominants when no aversive factor was found (FR3a), it is arguable that in comparison, Jones and Nowell's results reflect an acute response to a change of housing and status. Results for the tests which examined the aversive properties of familiar and unfamiliar urine in territory holders when the test subjects used were free range subdominants and subordinates, are perhaps harder to explain. On the whole, familiar and unfamiliar urine appeared to be neither attractive nor repellent to test subjects and if anything, there seems to have been a trend towards the familiar urine attracting test mice, particularly for subdominant mice, where the result was statistically significant. It is possible that low status animals become familiar with the odour characteristics of the territory holders they live with and whereas

for outsiders to the group, these scents may carry aversive properties, to the group members, they are not perceived as threatening. It is however, possible that the test environment may pose as threatening to an animal, and in this context, the tendency is shown towards selecting an area where the odour appears not to be threatening within the context of that environment, particularly if the odour has been encountered previously.

The failure throughout all the experiments to show the aversive factor in single caged males is at variance with the results of Jones and Nowell (1973a, 1974b) which is hard to explain as these experiments closely resembled theirs. Even the genetic strain used (TO) is the closest now available to the TT strain which they tested. Although there were minor differences in respect of age of subjects and duration of individual housing, the most likely explanation is that the effects of individual housing are notoriously variable between laboratories. Jones and Nowell (1974b) refer to their animals as "isolated" but in almost all animal houses, caging animals singly leaves at least some degree of auditory and olfactory communication, and it may well be these uncontrolled factors which, together with genetic and other differences give such varied results. Studies with bladder urine and coagulating gland tissue from isolated and grouped male mice showed no differences existed in the ability to generate the aversive signals from the two groups, using any combination of urine or coagulating gland tissue (Albone 1984), so the strength of the signal must be linked with whether a particular mouse releases signal-generating material within its coagulating gland.

A further difference in the results here from those of Jones and Nowell is that test subjects were apparently attracted to the urine of both caged and free range subordinates (FR3a, FR9a). There is a

strong trend in the results towards attraction to the urines of all except dominant mice, though this only reaches statistical significance for the free range and group caged subordinates. No attraction of males to the urinary odours of other males is described in any of Jones and Nowell's experiments though there are reports of this phenomenon (Whittier and McReynolds 1965, Thiessen, Lindzey and Nyby 1970, Daly 1977) and in field studies, mice are known to be attracted to traps containing odours of the opposite sex (Rowe 1970). An odour which is perceived as threatening in the context of one environment, such as the home cage where it may be associated with the presence of an aggressive dominant animal, may seem attractive in another environment which itself appears threatening due to novelty. So an aversive scent in one context, may for the receiver appear attractive under a different set of circumstances. Indeed subordinate test animals have been shown to prefer areas marked by conspecifics (Baron 1973). Experiments such as those described here, are entirely dependent on the responses of subordinate test subjects and it is very likely that subjects drawn from cages where they had experienced much intra-group fighting would react more vigorously to odours which might for them, be associated with the threat of attack than would subjects from more peaceful cages. While care was taken to control the prior experience of the test subjects as far as possible, as no doubt Jones and Nowell did, differences between the subjects of these tests and theirs could not be eliminated and probably account for the discrepancy. It is very likely then that the scent of an animal acquires aversive properties through a learning process (Johnson 1973). Lee (1976) has argued that although social interactions take the form of two-way traffic, very often rodent behaviour studies fail to analyse responses for both sides of these social encounters which may therefore lead to incomplete

conclusions. Although these experiments did not directly address the question of whether avoidance responses to odour are learned, as Sawyer (1981) has shown that they can be, it is worthwhile noting that the test subjects in our experiments had had no opportunity to learn to avoid the specific odour of any of the urine donors. Being subordinate to the dominants in their own cages, they may well however, have learned to associate his odour with attack and defeat. If the avoidance of urine of dominant males that they showed here was learned, that implies that the "aversive factor" is a general property of high status males.

In the final experiment where the urine of new and deposed dominants was investigated two weeks after the changes in social status, it was surprising to find that the deposed males showed the aversive factor in their urine which was not present in the urine of the new territory owners. The deposed males continued to live in their original quarters and their proximity to the new dominants together with the aversive cue in their urine may have in some way influenced its production in the males with newly-acquired high status, although this possibility requires further investigation. It may also be the case that the relatively high levels of corticosterone in the newly dominant males may also have influenced the quality of their urine making it non-aversive to test subjects. Recent tests with gerbils (Fullenkamp et al. 1985) failed to demonstrate avoidance of ventral gland odours from dominant males that had recently won aggressive encounters compared with odours from other males when presented to test subjects.

Across all the tests, the mobility results differ from those of Jones and Nowell (1974a), with the presence of the aversive cue having apparently little influence in decreasing levels of activity among test subjects. It seems that under some conditions, rodents

respond to mildly threatening circumstances by reduced locomotion (Baron 1964, Kumar 1970) but in others, the opposite may be the case (Bronson 1971). Here, reduced mobility was only found when a mildly attractive odour was present (FR3a). By comparison, the urine of some other groups induced more movement (FR3a, FR9a) and this may represent fear-motivated attempts at escape.

The first urine marking tests (FR2b) show that qualitatively, the patterns produced are in line with those described by Desjardins et al. (1973); also the result that quantitatively, dominant males mark more than subordinates (FR3b) is also compatible with their results and agrees well with findings for the hamster (Drickamer, et al. 1973) and gerbil (Thiessen et al. 1971). The highest levels of marking were observed in single caged animals, a feature which has been attributed to the novelty of a new environment (Maruniak et al. 1974) although the results of Powell and Wolff (1982) disagree with this. More recently Brown (1985) in a study using male rats, found that one effect of sixty days of isolation was a drastic reduction in overall marking in these animals.

Urination, self-evidently, serves other functions than odour deposition, whilst gland rubbing in hamsters, gerbils and rabbits presumably does not. It is quite possible that the natural mode of urination for male (and for that matter, female) mice, is to release drops as and when produced by the kidneys and not to withhold substantial quantities in the bladder. Male mouse urine is known to elicit attack in conspecifics (Mugford and Nowell 1970, Archer 1968, Mackintosh and Grant 1966) and it is more than probable that subordinate males learn to urinate as infrequently as possible as a strategy for avoiding attack, leading to pools of urine produced in males of this social category. Although there was no satisfactory way of quantifying this observation, it was very noticeable that

animals, particularly free range subordinates, often voided relatively large quantities of urine soon after being placed on the Benchcote papers. In contrast, dominant and single caged mice, in close agreement with the findings of Desjardins et al. (1973), rarely produced large pools. On the assumption that all mice deposit similar total quantities of urine during the test period, the differences in spot numbers illustrated in Figure 6:4 were inevitable. Post-mortem examination of free range subordinates has often shown them to have enormously distended bladders (see photographs, Chapter 4), while this has never been seen in territory holders or caged dominants and only to a minimal extent in caged subordinates. J.P. Henry (personal communication) has also observed this phenomenon in his specialised population cages (Henry, et al. 1982) and believes that it leads eventually to kidney damage through reflux nephropathy. Inhibition of urination then, may be a learned phenomenon peculiar to subordinate laboratory mice forced to live in proximity to aggressive dominant individuals.

The spatial distribution of urine marks too, may not really carry the significance of genuine active scent-marking; it is possible that it is determined by and secondary to, the locomotory patterns of subjects during the test period. The peripheral patterns seen in single caged and subordinate mice in FR3b and FR9a then, would reflect the wall-seeking behaviour of emotional or fearful animals (Powell and Wolff 1982), a pattern not evident to any extent in dominants. It is thus possible that all aspects of the differences in spatial distribution and in marking patterns according to housing condition and social status, as reported here, are determined by the subject's emotional responses whether learned or otherwise. This is perhaps given further weight by the results of FR2c where subordinates were found to alter their patterns of large

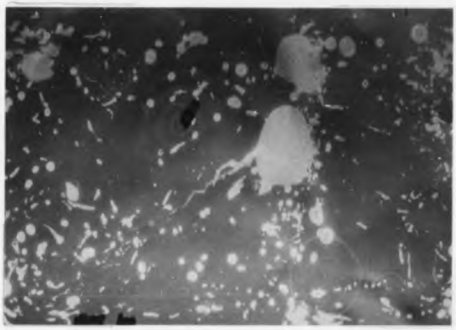
pools to those of small, widely dispersed marks in the presence of a strange male. Again at twelve and sixteen weeks in experiment FR9a, free range subordinates were found to produce marking patterns that were similar to those seen in the dominant mice. These subordinates had already spent thirty six hours away from their housing condition before urine marking tests were carried out. This may have been sufficiently long to allow for any behaviourally repressive effects on marking that dominants have over subordinates, to be removed so that the more natural mode of urination was able to emerge. It is proposed therefore that low social status may not rigidly confer a certain marking pattern on an individual at least under laboratory conditions, but instead the ability remains to alter the pattern depending on particular circumstances that arise.

Studies on the relationship of urine marking to sex differences and to endocrine status (Bronson 1976, Brown 1978, Wolff and Powell 1984, Powell and Wolff 1982, 1984) would support the original conclusion, namely that urine marking does, in the male mouse, have a genuine role in controlling odour deposition. The fact that in order to safeguard the stability of the free range social structure it was only possible to use each individual mouse once, meant that it was not possible to take account of the "within mouse" variation noted by Powell and Wolff, op cit.

Previous workers have suggested that both odour production and urine deposition are under androgen control. There is evidence for this in a number of species: mouse (Bronson 1976, Jones and Nowell 1974a) gerbil (Griffo and Lee 1973, Thiessen, et al. 1968, Turner 1975) rabbit (Mykytowycz 1970) rat (Price 1975) and dog (Ranson and Beach 1985). Results from the experiments here however show little relationship between levels of testosterone and the presence or absence of the aversive factor together with varying levels of

marking. The intense marking seen in single caged males is not reflected in similarly high levels of testosterone and despite this degree of marking, the aversive factor was not present in this category of animals.

By contrast, in experiment FR9a, at twelve and sixteen weeks when both free range and group caged dominants were found to have urine containing the aversive cue and both groups of animals produced marking patterns associated with high status, testosterone levels were seen to be low at twelve weeks yet raised four weeks later. From the results of experiment FR8a, marking patterns and the presence of an aversive cue again appear to bear little relation to the circulating levels of testosterone. Unfortunately, subject numbers in all the groups tested here were too low to provide sufficient data for correlational studies. It should be said that the studies that have examined the association of odour marking and androgen levels to one another have on the whole employed the use of hormone replacement therapies in castrated animals whereas this work here has only used intact animals with respect to these phenomena. Probst (1985), in an investigation of marking activity and testosterone levels in male gerbils, found a high correlation between weekly mean values for testosterone and marking behaviour which was not evident at an individual level. It is arguable that testosterone concentrations do not determine the level of behavioural activity in different individuals although the presence of testosterone is necessary for the expression of the behaviour, and indeed to initiate the onset of the behaviour, but low circulatory levels may be sufficient for maintaining it.



Urine marking pattern typical of free range territory holders, caged dominants and single housed mice.

Marking pattern observed in subdominant and all subordinate mice.

Both photographs are the same scale

CHAPTER 7

FINAL DISCUSSION

The foregoing work has used both traditional laboratory cages and the more open environment of a free range room to examine certain behavioural and physiological characteristics of male mice under stable, social conditions. A number of predictions were tested. Briefly, it was anticipated that a number of these males would establish and maintain distinct territory areas in the free range with other mice in a social hierarchy, subordinate to them. Likewise, the same social conditions would exist among the caged groups even if the establishment of actual territory areas was not considered to take place in a comparable way. Secondly, it was predicted that these mice, in particular the subordinates, would demonstrate physiological evidence of social stress: high corticosterone levels and adrenal weights, and also raised pain thresholds and blood urea levels. These animals were also expected to have reduced testosterone output.

In contrast, it was anticipated thirdly, that the high status mice - territory holders and caged dominants together with the singly housed males - would have consistently high plasma testosterone levels together with heavier sex accessory glands when compared with the subordinates, with little increased adrenocortical activity. Finally, it was predicted that one influence of social status would be on the presence or absence of an aversive factor in urine and that urine marking patterns would also be dependent upon the social standing of individuals. Overall, the behavioural predictions were supported but in general, except for the urine factor properties, the physiological data did not confirm expectations.

From observations made on the free range mice, findings compared

well with the work of Crowcroft (1966) and Mackintosh (1981). Territories were established and maintained and with minor exceptions, remained stable throughout the different experimental periods. Stable social order was also observed among the group caged mice. A social category of subdominant mice was observed among the free range mice, a group which Evans and Mackintosh (1976) also identified and which they considered to be of midway status between their territory holders and subordinates. This social category was not observed among the caged groups where only dominants and subordinates were ever identified.

Although the work was concerned with the deleterious effects which could arise from the establishment of dominant-subordinate relationships within all-male groups of fixed numbers, it was not possible to show that subordinate mice, either in the free range or in group cages, had prolonged, raised glucocorticoid levels at trough or peak sampling times, nor were adrenal weights raised. However, in every experiment, a good many of the free range subordinates were seen to fall into very poor physical condition with numerous bites on the rump and tail together with extensive scarring around the genital area, findings which closely match those of Henry et al. (1982) and other workers. Also in parallel with Henry's findings was the observation at post mortem that many of these animals had bladders enormously distended with urine which indicated an inhibition to urinate. This behavioural trait among subordinates may serve as an attempt to reduce the frequency of attack by dominant animals; by witholding urine, odour is also witheld and so the amount of attention an animal attracts is thereby lessened. Although it was not a frequent finding, a number of grey and pitted kidneys were found among this group indicating some kidney damage, although this was not further investigated. Caged subordinates also suffered attack by

dominants, however these animals were never seen to fall into the poor condition of the free range mice nor was gross bladder distention ever a feature of this group. The endocrine data suggest that the lack of pronounced pituitary-adrenal response was indicative of non-stressful conditions but the possibility that the free range conditions were provocative of psychosocial stress cannot be totally negated. Munck et al.(1984) have argued that subordinates produce physical responses to psychosocial stress which are independent of the adrenocortical response which has the primary function of protecting the individual against these initial, normal defence reactions. The subordinates in the free range apparently learned to cope with their situation and as a consequence, the glucocorticoid response may have been switched off and offered these animals no protection, hence the poor quality of their coats and the scarring which failed to heal.

Although the corticosterone response was the only hormone system measured, other systems could well have been activated as part of the stress response. As Mason (1975) demonstrated, hormones such as thyroxin and insulin also play important and separate roles during stress, as indeed do a number of peptides such as α-MSH, β-endorphin and prolactin (Smelik 1985, Dijkstra et al., 1984, 1985, Keverne et al. 1982, Keverne 1985.). Placing too great an emphasis on measured results from a single system may result in a distorted interpretation of events. It is therefore of interest to note that the data from the pain threshold tests (chapter four) support the corticosterone findings. There was no evidence to show that the subordinates, particularly those from the free range, had raised pain thresholds when compared with other social groups, in other words, absence in these mice of stress-induced analgesia supports the idea that they were not stressed, but coping.

The data showing raised blood urea levels remains unexplained however. Work by Henry (Henry and Ely 1980, Henry et al. 1982) has shown that raised urea levels and hypertension are features of socially stressed mice and although this latter physiological parameter was not measured in this study, the possibility that the free range mice had elevated blood pressures cannot be ruled out.

The most surprising result to emerge from the study was the finding that the high status territory holders in the free range did not possess continual high levels of testosterone as was predicted. The general picture given by these animals was one where levels at the outset of an experiment were high relative to other social groups but then the trend was for output to be reduced over the ensuing weeks. Low androgen activity was also reflected by the results at post mortem where there were no statistical differences in the weights of sex accessory glands between high and low status animals. This fall off in testosterone levels was not seen among the caged dominants or singly housed mice and so it is concluded that the circumstances of the free range in some way, influenced the endocrine state of these mice.

It is suggested from these results, that territory holders have low resting values of testosterone which are sufficient to maintain dominance- type behaviour and therefore, status. From this, it is concluded that the main difference between high and low status males, at least in the context of the free range, is not simply whether one group has high or low circulating levels but rather the degree of endocrine responsiveness that occurs when certain stimuli are presented. Two of the experiments carried out support this theory. It was noticeable that when females were present in the room throughout an eight week period (experiment FR10) and for a single half hour period (experiment FR12), testosterone levels were generally raised

among the territory holders (although there were fluctuations during experiment FR10). In particular, the short exposure to primed females in experiment FR12 produced an acute rise in testosterone among these animals only, which was not considered to be due solely to the increased intensity in fighting that accompanied the presence of the females. Alien males also provoked fierce fighting but this was not accompanied by any rise in testosterone levels.

However, the argument that the absence of females may have resulted in lowered androgen output in these high ranking males is not seen as valid as these low levels were solely a feature of the high status males of the free range and were not paralleled by results for the caged dominants or singly caged males, neither of which were ever given access to females. It is worth restating that females used for breeding were kept in cages in the same animal room as the group housed and singly housed mice but to ensure that female odour would not bias results, group cages of adult females were also kept on shelves in the free range room.

Low testosterone levels over time did not result in the loss of status by the territory holders which indicates that the hormone played a permissive role once territories and social order were established. However, it is thought likely that at some earlier stage, high levels existed, being intimately linked with establishing the foundations of dominance behaviour. At which stage of development this could have occured is a matter of speculation but data from work done on the intrauterine hormone environment, suggest that certain physiological traits may be laid down prenatally, at least in this species (vom Saal 1979).

The results of this work are recognised as relating to the conditions used here but they are worth considering in general terms for the dependence of aggression and dominance on high testosterone

output. It would certainly appear that earlier studies have placed too great an emphasis on the need for high levels in order for these traits to be present. As stated, raised levels are probably required for the establishment of these behaviours but then low levels are sufficient for their maintenance. Castration certainly abolishes them eventually, but then has the added effect of removing the source of even low circulating levels. Early studies which employed castration with hormone replacement therapy could have, because of the treatments used, failed to recognise that the links between the hormone and the behaviours were not just a consequence of either having high circulating levels or an absence of the hormone, but instead depended upon an intermediate state where lower levels were sufficient to sustain particular behaviours.

Despite low androgen levels among territory holders, it was found that these animals, together with the group housed dominants, produced in their urine a factor that deterred test males from prolonged investigation. This was not observed among the singly caged males. Again in terms of testosterone responsiveness, the indication is that low androgen output is sufficient to maintain odour quality and its production. It is possible that all dominants share common features of which one is the urinary factor and although this may be a particular testosterone metabolite in urine, it does not follow that this automatically serves to deter subjects from investigation, animals instead respond to urine odour because they learn to avoid both dominants and the odours associated with them.

The work of Jones and Nowell showed clearly that castrated animals do not possess the urinary factors of dominant animals. Whether stress has the effect of suppressing odour production is not fully clear and there are no data from sham-castrated animals to show if the stress effects of surgery are a sufficient influence. Indeed

the odour may be dependent on the presence of androgens other than testosterone, which were not measured in this study but which could have been present in high levels in the dominants and territory holders but not in the singly caged mice. If this is the case, then the test procedure used would not have detected it as results were based on response to total urine content regardless of which androgens were present. Another point worth considering is that the factor may be a feature of all mice but among subordinates, a component may be present which has a masking effect or may even be attractive to other animals in contrast to the unmasked aversive odour of a high status male. Again, the test used would not have shown this.

All high status mice together with the singly caged animals produced a urine marking pattern of intensive overall spotting similar to that observed by Desjardins et al. (1973). This behaviour probably serves a number of functions being used not only to delineate territory boundaries and to signal occupancy, but also to increase an animal's confidence and sense of status (Ewer 1968). It can be argued, however that placing animals in the test arenas promoted intensive marking due to encountering a novel environment as has been observed for gerbils (Gallup and Waite, 1970) but if this were the case, it would be expected that all animals, regardless of status, would mark intensively under these conditions and this was not observed. That the territory holders possessed low androgen levels appeared to have no influence on their marking patterns and it is suggested therefore, that the previous literature may have implied too simple a relationship between androgens and urine marking. The production of intensive spot marking may be an overall male characteristic irrespective of status. However, within the context of a social group, lower status males may have to learn a new pattern of

pooling as part of the coping response to their situation, an event which a number of workers believe is enhanced by the presence of high corticosterone levels (Brain 1971). From the results of this study, one conclusion drawn is that the suppression of intensive marking in subordinates can occur without the suppression of testosterone levels and indeed, the reverse of this is also possible. Also, from the data for singly housed mice, it is concluded that possession of an aversive urinary factor and an intensive marking pattern do not necessarily go together but that their joint presence is dependent upon certain social circumstances.

When considering the work above as a whole, the question that arises is whether the form this took can be considered an advancement on other semi-natural studies. Because this work employed physiological measurement in a system such as was used by Mackintosh for behavioural measurements, it is believed that new directions have been opened up for this type of research. It can be argued of course, that a room such as the free range provided few similarities to the wild state due to the absence of predators, females and opportunity for emigration so that there was no possibility of testing the fitness of animals in Darwinian terms. However, I would argue that despite its restrictive nature, the free range allowed the animals that lived there greater physical freedom than is offered by the laboratory cage and therefore, a wider expression of behaviour and although it may not have mirrored the truly wild state, it may have more faithfully reproduced certain intermediate environments (eg. grain stores and chicken houses) that many wild mice, being opportunists, do colonise.

The house mouse is a peculiar species when compared with other rodents because of its greater association with man than is generally found in other non-domesticated animals. It has adapted its habits

and social behaviour to accommodate and exploit new and unusual habitats. As a consequence, the structure of house mouse populations living in places such as cellars and other domestic environments, may be totally unlike that of the mouse in true feral conditions, and so to draw behavioural comparisons between animals even of the same species but living under very different conditions, may not be to compare like with like. It is felt that the free range has certainly gone some way to providing an environment for a better understanding of the social and physiological responses that take place in mice housed in more confined conditions because of the continual and dependable supply of resources that these other environments provide. In places such as grainstores, few predators probably threaten the colonies of resident mice and so, as was seen in the free range, subordinate animals probably do exist under these conditions and if these mice are especially good at coping with their status, this may explain why numbers increase dramatically in confined areas with plentiful resources. The free range is also considered to be an advancement on the population cages of Reimer and Petras (1967) and Henry and Stephens (1977). These workers have employed cages interconnected with tubes to form runways for the animals, often leading to a central area containing food and water. Although territories were satisfactorily established under these conditions, the choice of direction available to the mice to move in and for exploration is very restricted. In the free range, animals were free to move about as they chose and it was social rather than physical restrictions that reduced mobility.

It would appear that the choice of the TO outbred strain used in this study was a fortunate one as the work of Bisazza (1981) has indicated that not all laboratory strains so readily form territories together with social hierarchies. The mice used here quickly

established social order and escalated fighting was very rare once stability was achieved and despite the poor condition of many of the subordinates, there were very few deaths.

The layout of the room closely followed the design of Mackintosh (1970, 1973) with bricks arranged in repeat formation to aid with the establishment of territory boundaries and their recognition although the question of whether these served more as visual than as olfactory cues as Mackintosh (op cit.) has claimed, was not answered.

The openness of the free range in contrast to the population cage inevitably produced problems in catching and handling the animals whilst attempting to cause as little disruption as possible. On the whole, this was achieved successfully as removal of the animals from the room did not result in loss of status or rearrangement of the territories although there was some evidence to suggest that a long absence from the social environment in one experiment (FR9), to some extent affected urine marking patterns.

From the work already done, two areas in particular are considered important for further study. The first would be to develop a better understanding of the physiology of the subdominant mice relative to their social position and behaviour. From the endocrine data and from results on odour tests and marking patterns, the indications are that these mice are a sub-group of subordinates yet it is felt that there are other areas that require testing before this is conclusive. One suggestion is to look at the effects that the urine of this group has on the development of young, female mice with an examination of whether their urine contains oestrus-inducing factors, comparing results with those for the urine of dominants and subordinates. The prediction would be that the urine of dominant males would advance female puberty in contrast to the urines of both subdominants and subordinates.

Secondly, tests to examine the endocrine characteristics of subordinate mice in response to the aggressiveness demonstrated by individual territory holders are proposed. For statistical purposes and in order to gain an overall perspective, data in this study were accumulated from the subordinates as a total group within the context of one experiment. However, certain important information may be lost when this method of data collection is employed because from observation, it was apparent that certain territory holders were more "tolerant" of the subordinates that lived with them in their areas than others. Therefore, subordinates living in a territory occupied by a highly aggressive male may show a greater physiological response to social stress than animals living in more peaceful areas. Endocrine profiles of these smaller groups of subordinates together with measurements of aggression in territory holders would provide a clearer picture of the physical effects of social interaction in these groups.

Although many of the findings from this study are inconclusive, a number of interesting results have emerged and it is concluded therefore that the environment of the free range did indeed provide wider scope for investigation, not permitted by the confines of the laboratory cage.

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