Stress and pituitary-adrenocortical manipulation during late pregnancy: Effects upon offspring development in Mice

by

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ABSTRACT

This thesis describes the effects of crowding during the final third of pregnancy upon development of the offspring in mice, and examines the role of the pituitary-adrenocortical axis as the physiological mechanism of mediation. In an attempt to confine the effects of the various experimental manipulations to the prenatal period, and eliminate postnatal variables affecting development, all litters were fostered at birth to control mice. Offspring in litters from crowded mice showed increased perinatal mortality rates and reduced birth weight. Later in development, female offspring from crowded mice show retarded onset of puberty, which is not due to delayed postnatal body weight gain, and in adulthood these animals show disruption of the oestrous cycle typified by shortening of the pro-oestrus stage. Adult male offspring from crowded mice show impaired copulation and reductions in aggression compared with offspring from control mice. Testosterone propionate therapy in adulthood improved copulation in these animals, but the aggressive responses of fighting male offspring from crowded mice were still deficient compared with those of control offspring even after testosterone propionate treatment. The causes of the syndrome evident in offspring from crowded mice is discussed, and hypoprolactinaemia has been postulated as a general underlying cause of pathology.

Crowding did not severely reduce maternal food intake or shorten the length of pregnancy, but was found to increase plasma corticosterone concentrations during pregnancy. The hypothesis that the effects of crowding during pregnancy upon offspring development are mediated by the maternal pituitary-adrenocortical system was tested. Hormones known to be secreted from this system (e.g. ACTH, corticosterone, progesterone and androstenedione) were administered singly to pregnant mice in an attempt to reproduce the effects of crowding during pregnancy upon offspring development. Evidence that the maternal adrenal is required for producing the effects of crowding was inconclusive.

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For Denise Stirrup, William and Vivienne Harvey

and to the memory of Pam

CHAPTER 1

INTRODUCTION

The pathology of stress continues to attract intense research interest although it is nearly 50 years since Selye's (1936) seminal study. In the general field of stress research, two distinct areas of study have evolved. The first concerns the advancement of knowledge of the mechanisms producing stress pathology in adult animals. The second and more recent area of study concerns the "pathological" effects of stress during early, and even prenatal life, on the development of the embryo, foetus or young animal. This thesis is a contribution to the second area of study and investigates the consequences of environmental stress during pregnancy upon development of the offspring, and on the identification of factors which may mediate the described effects of "prenatal stress".

In the context of this study, the terms "stress" and "stressor" are used as originally defined by Selye (1936, 1950). Stress is the descriptive term applied to the physiological response to adverse environmental conditions. The stressed animal typically shows changes in the activity of endocrine glands and particularly in the activity of the hypothalamo-pituitary-adrenocortical system. Stressors are the agents which activate the physiological stress response and are usually nocuous agents, or adverse environmental conditions that cause disturbance, irritation or pain. Stressful conditions may occur in natural circumstances, for example during resource shortages, intraspecies and other conflicts or in over-crowded conditions. In the laboratory, crowding, restraint, intense illumination, temperature extremes, avoidance conditioning,

noise and food restriction have been employed as stressors. More recent definitions and concepts of stress, re-affirm the importance of the hypothalamo-pituitary-adrenocortical axis in the endocrine response to stressors (e.g. Mason, 1968; Allen, Allen, Greer and Jacobs, 1973; Brain, 1975; Smelik and Vermes, 1980; Nichols, 1980) and further discussion of the endocrinology of stress is given in Chapter 2.

Selye's (1936, 1950) studies were important because they showed a relationship between environmental conditions, an internal physiological response and stress-related pathology. The endocrine response to environmental conditions was considered to be a mechanism of phenotypic adaptation. Selye recognised a general adaptation syndrome characterised by changes in the activity of the adrenal gland. In conditions of chronic stress the adrenal cortex can increase and sustain steroid output for as long as metabolic resources will allow, until adrenocortical exhaustion results. Certain pathological conditions are correlated with adrenocortical activity, and in some cases stress-related pathology is thought to be mediated by the action of adrenocortical products. Pathological conditions reported to be associated with chronic environmental or social stress in studies on various mammalian species include loss of body weight, gastric erosions, arteriosclerosis, inhibition of reproductive development and function (Evans, 1959; Christian and Davis, 1964; Christian, Lloyd and Davis, 1965; Burchfield, Woods and Elich, 1980) and there is even a report of stress causing death by renal failure (Holst, 1972).

A problem exists in explaining the effects of stress during pregnancy upon the development of the offspring. Many environmental stressors to which the maternal organism is exposed

cannot directly contact the foetus, although electric shock, temperature extremes or undernutrition may directly influence foetal development. However, development of the foetus may be influenced by the action of hormones released by the maternal organism during the stress response. Ward (1972) and Dahlöf, Hard and Larsson (1977) have suggested that the effects upon development and behaviour detected in offspring from rats crowded or restrained during pregnancy are mediated by exposure to adrenocortical products of maternal origin. There is only limited support for this hypothesis, and this endocrine mechanism has only been applied to account for certain effects of stress during pregnancy, such as impaired copulation in male offspring. There are in fact many reported effects of stress during pregnancy upon offspring development and behaviour and these will now be briefly reviewed. They are summarised in tabulated form in literature summary 1.

The first studies to report that exposure of the maternal organism to stressful conditions during pregnancy could influence the offspring were performed by Thompson (1957) and Kaplan and Thompson (1957) who showed that offspring from rats that were avoidance conditioned during pregnancy, were less active compared with offspring from undisturbed controls. This study has been repeated in both rats and mice using the stressors of crowding, handling, avoidance conditioning, electric shock and forced swimming (Morra, 1965; Ader and Belfer, 1962; Thompson, Watson and Charlesworth, 1962; Thompson and Quinby, 1962; Hutchings and Gibbon, 1962, Keeley, 1962; Ader and Conklin, 1963; Defries, 1964; Hockman, 1961; Defries, Weir and Hegmann, 1967; Ader and Plaut, 1968; Smith, Joffe and Heseltine, 1975; Chapman, Masterpasqua and Lore, 1976;

Joffe, 1977; Rohner and Werboff, 1979). In contrast, Ader and Plaut (1968) and Lieberman (1963) have reported increased activity levels in the offspring of rodents stressed during pregnancy. This apparent contradiction of the effects of stress during pregnancy upon activity and performance of the offspring may simply be due to differences in animals' initial arousal states, which is well known to affect performance (Yerkes and Dodson, 1908). Archer and Blackman (1971) and Joffe (1978) have reviewed the effects of stress during pregnancy upon activity, arousal and emotional responses.

More recent studies of the effects of stress during pregnancy upon the offspring have concentrated on sexually dimorphic patterns of behaviour, and in particular, sexual behaviour in male offspring. Ward (1972) reported that male offspring from rats restrained during the final third of pregnancy showed impaired masculine sexual responses and augmented feminine sexual responses. This finding has since been confirmed (Herrenkohl and Whitney, 1976; Chapman, Masterpasqua and Lore, 1976; Dahlöf, Hard and Larsson, 1977; Chapman and Stern, 1978; Dunlap, Zadina and Gougis, 1978; Meisel, Dohanich and Ward, 1979; Rhees and Fleming, 1981). Similarly, the stressors of avoidance conditioning (Masterpasqua, Chapman and Lore, 1976) crowding (Chapman, Masterpasqua and Lore, 1976; Dahlöf, Hard and Larsson, 1977) and malnutrition (Rhees and Fleming, 1981) of the pregnant rat have been shown to either impair masculine patterns of sexual behaviour, or augment feminine patterns of sexual behaviour in the male offspring. There has been only limited study of the mouse, and evidence that stress during pregnancy affects male offspring sexual behaviour is conflicting. Allen and Haggett (1977) report that male offspring from mice

chronically crowded during pregnancy show impaired copulatory responses, as evidenced by lengthened ejaculatory latencies. In contrast, Politch and Herrenkohl (1984a) found no impairment of the sexual responses of male offspring from mice restrained during pregnancy.

With reference to the abnormalities in the sexual responses of male offspring from rodents stressed during pregnancy, the terms "demasculinisation" and "feminisation" have been used to describe respectively the reductions in masculine behavioural responses, and the increments in feminine behavioural responses. In the male, a demasculinisation is the loss of male characteristics and a feminisation is the appearance of female characteristics. One effect of stress during pregnancy upon aspects of sexual behaviour in male offspring, which is difficult to classify as either a demasculinisation or feminisation, is the increased incidence of homosexual responses reported by Götz and Dörner (1980) in male offspring from rats restrained during pregnancy. With reference to the female offspring from rodents stressed during pregnancy, the term "defeminisation" has been used to describe the loss of female characteristics in these animals. The effects of stress during pregnancy upon the behavioural responses of female offspring will now be reviewed.

There has been limited study of the effects of stress during pregnancy upon sexually dimorphic patterns of behaviour in female offspring. Allen and Haggett (1977) reported that female offspring from mice crowded during pregnancy were less sexually receptive than offspring from non-crowded mice. In contrast, Beckhardt and Ward (1983) found no apparent losses of sexual receptivity in female offspring from rats restrained during pregnancy. However,

such females are less likely to show maternal aggression (Politch and Herrenkohl, 1979) and this finding, together with that of Allen and Haggett (1977) does indicate that stress during pregnancy defeminises female offspring behaviour patterns.

In addition to the reported effects of stress during pregnancy upon the behavioural responses of male and female offspring, other consequences upon the endocrinology and physiology of these animals have been reported. These physiological consequences of prenatal stress may be the cause of the effects upon behaviour and this is reviewed later in this and in other chapters. Schnurer (1963) first studied the maternal and foetal endocrine pathology resulting from stress during pregnancy and reported atrophy of the foetal adrenals, thyroid, thymi and testis. Unfortunately, Schnurer injected formaldehyde into the pregnant rat and although this may have activated the stress response, this substance may have also been directly teratogenic. Similarly, severe undernutrition during pregnancy has been shown to produce severe foetal adrenal, testis, thyroid and thymus atrophy and mild ovarian underdevelopment in rats (Barry, 1920) but it is unclear what contribution was made to this result by maternal stress, as undernutrition was not recognised as a stressor. Clearer evidence of endocrine pathology in both male and female offspring from rodents stressed during pregnancy has since been obtained using the stressor of restraint. Dahlöf, Hard and Larsson (1978) report reduced adrenal and testis weight in foetal male offspring from rats restrained during pregnancy. That stress during pregnancy influences the development of both the pituitary-gonadal and pituitary-adrenal systems of male offspring has since been confirmed. Male foetuses from restrained rats show an acceleration of the

normal prenatal testosterone surge (Ward and Weisz, 1980) and such animals when born have decreased plasma testosterone concentrations (Dörner, 1980) compared with those from control rats. In adulthood, male offspring from rats restrained during pregnancy also show lower stress secretions (induced by ether exposure) of prolactin and corticosterone (Politch, Herrenkohl and Gala, 1978). Similar endocrine pathology has been reported in female offspring: adult female offspring from rats restrained during pregnancy show lengthened oestrous cycles (Herrenkohl and Politch, 1978) decreased fertility and fecundity (Herrenkohl, 1979) and reductions in prolactin levels after themselves becoming mothers (Herrenkohl and Gala, 1979). Additionally, female offspring from mice restrained during pregnancy are slower to achieve sexual maturation as evidenced by delayed vaginal opening (Politch and Herrenkohl, 1984a) and this result can be taken to indicate an underlying endocrine abnormality.

Closely related to the effects of stress during pregnancy upon the endocrinology of the offspring are the effects upon offspring brain biochemistry. The neurochemical effects of prenatal stress can be divided into two categories: those influencing nucleic acid concentrations in brain tissue and those affecting neurotransmitter concentrations. Petropoulos, Lau and Liao (1972) report reduced brain protein and decreased concentrations of nucleic acids in the hypothalamus, cerebellum and cerebral cortex of juvenile offspring from rats handled in pregnancy. Similarly, undernourishment of rats during pregnancy has been shown to reduce nucleic acid concentrations in the brain stem of neonatal offspring (Hammer and van Marthens, 1981). It should be noted that these reports supply evidence that stressful conditions during pregnancy can damage the structure, as well as the neurochemical integrity,

of the brain of the offspring. A reduction of brain DNA is indicative of reduced brain cell number. In addition to the studies previously mentioned there are reports suggesting that offspring from rodents stressed during pregnancy show other neurochemical abnormalities. Moyer, Herrenkohl and Jacobwitz (1978) found decreased noradrenaline in the nucleus preopticus medialis and median eminence of adult male offspring from rats restrained during pregnancy, and increased noradrenaline concentrations in the entorhinal cortex, decreased dopamine concentrations in the nucleus paraventricularis, and increased dopamine concentrations in the arcuate nucleus of their female litter mates in adulthood. Similarly, Rohner and Werboff (1979) report reduced dopamine concentrations in the corpus striatum (but found no differences in the hypothalamus) of offspring from mice subjected to the stress of avoidance conditioning during pregnancy. Sobrian (1977) found no differences in brain concentrations of serotonin, noradrenaline or 5-hydroxyindoleacetic acid in neonatal or juvenile offspring from rats subjected to electric shocks during pregnancy.

The final major category of the effects of stress during pregnancy upon the offspring is concerned with general development and morphology. Included in the general development category are neurological development, somatic development and sexual maturation (which is partially controlled by body weight) and the morphological effects include such phenomena as cleft palate, hydrocephaly and abnormalities to the form of the genitalia. These latter consequences of stress are such as to be considered as teratogenic in the normally accepted sense.

Neurological and neuromuscular development, as assessed by the development of reflexes, has been examined in the offspring from

rodents stressed during pregnancy. Sobrian (1977) reported accelerated development of spontaneous motor activity in juvenile offspring from rats subjected to electric shock during pregnancy. This treatment did not affect development of the auditory startle or freefall righting reflex, eye opening (a correlate of brain maturation) or incisor eruption. In contrast, offspring from rats restrained during pregnancy show retarded development of the auditory startle reflex (Barlow, Knight and Sullivan, 1978) but again, eye opening and limb grasping was not affected. However, more chronic stressing procedures have been shown to retard a variety of correlates of neurological development, at least in the mouse. Chevins (1981) has shown that neonatal and juvenile offspring in litters from mice chronically crowded during pregnancy, exhibit impaired development of the limb grasp, body righting and auditory startle reflexes. As most reflexes depend for their operation upon muscular, as well as neural development, it remains difficult to separate the effects of prenatal stress upon body and muscular development, from those influencing the nervous system alone. The auditory startle reflex can be envisaged to most closely relate to brain maturation, but even this may depend on somatic factors as the external auditory meatus opens postnatally, at least in rats (A. Palmer, Huntingdon Research Centre, personal communication). Stress during pregnancy is, in fact, associated with decreased offspring somatic development. Neonatal and juvenile offspring from rats subjected to restraint (Herrenkohl and Whitney, 1976; Barlow, Knight and Sullivan, 1978; Politch and Herrenkohl, 1979) handling (Werboff, Anderson and Haggett, 1968) or electric shock (Sobrian, 1977) or from mice subjected to crowding (Chevins, 1981) during pregnancy, show decreased body weights compared with control offspring. Examination

of the female offspring from stressed rodents later in postnatal development have revealed other influences upon general development. Politch and Herrenkohl (1984a) report that female offspring from mice restrained during pregnancy show delayed vaginal opening, but again it is unknown as to what extent somatic under-development may influence this phenomenon.

The teratogenic activity of stress is suggested by the influences upon survival of embryos and foetuses. Restraint stress during early pregnancy in rats, reduces the number of conceptuses that implant, and during late pregnancy increases the incidence of abortions (Euker and Riegle, 1973). Similarly, avoidance conditioning of rats during pregnancy reduces the number of offspring born and surviving up to postnatal day 25 (Hockman, 1961; Morra, 1965) and restraining rats prior to pregnancy, reduces litter size and the proportion of male foetuses born (Lane and Hyde, 1973). It is worth noting that in mice, exposure to stressful conditions increases cannibalism of litters (Rohner and Werboff, 1979) and this, together with effects upon lactation, may be implicated in reducing postnatal survival of prenatally stressed rodents.

Other teratogenic effects of stress include: hydrocephaly in rats induced by restraint during pregnancy (Euker and Riegle, 1973) and cleft palate in mice also induced by restraint during pregnancy (Barlow, McElhatton, Morrison and Sullivan, 1974). Additionally, crowding mice (Allen and Haggett, 1977) and crowding or restraining rats (Dahlöf, Hard and Larsson, 1978; Chapman and Stern, 1978) during pregnancy has been shown to produce morphological abnormalities in the genital system of the male foetus and neonate: these animals show decreased ano-genital distances and this is another example of

a demasculinisation as defined earlier in this chapter.

It is apparent then that the effects of stress during pregnancy upon the offspring have been the subject of many studies. It is also clear from the literature that the effects of stress during pregnancy can be grouped according to the type of effect. These classes of effect of stress during pregnancy upon the offspring are: exploratory and fear-mediated behaviour; sexual, behaviour, reproductive endocrinology and development; growth and somatic, neurological and morphological development. With this framework, it is possible to examine other consequences of stress during pregnancy upon the offspring, and this was one purpose of this thesis. For example, if sexual behaviour is affected in offspring from rodents stressed during pregnancy, it is logical to suppose that other similarly controlled behaviour may also be influenced. One such behaviour is intermale aggression, and the effects of stress during pregnancy upon the expression of this behaviour in male offspring, has not been previously examined. It is also apparent from the existing literature that most studies have employed the rat as the experimental animal, and used both severe and artificial stressors. The purpose of this thesis was to systematically and thoroughly study the effects of stress during pregnancy, using the mouse as the experimental animal, and to use a more natural stressor. Chronic crowding with male mice was chosen as the stressful condition and this was modified from Keeley (1962). Another purpose of this study was to test the hypothesis that the effects of stress during pregnancy upon the offspring, are mediated by maternal adrenocortical products. This hypothesis has been developed from Ward (1972) and Dahlöf, Hard and Larsson (1977). It is necessary to point out that

adrenocorticotrophic hormone (ACTH) is thought not to cross the placenta as an intact molecule in physiological concentrations, at least in rats and rabbits (Milkovic and Milkovic, 1961; Genazanni, Fraoli, Fioretti and Felber, 1975). However, corticosterone can cross the placenta (Zarrow, Philpott and Denenberg, 1970) as can most steroids (Johnson and Everitt, 1980) and the suggestion that corticosterone or other adrenal products are involved in producing the effects of stress during pregnancy upon the offspring, is consistent with this hypothesis.

Stress during pregnancy can also exert delayed effects upon offspring development. For example, poor lactation or maternal care due to prior stress could affect postnatal development of offspring. The use of fostering procedures in this study allows some control over these factors and also allows the identification of the foetal life stage as the major period of risk.

In considering the underlying causes of the various abnormalities in the offspring, it is not easy to link a maternal mechanism of mediation with physiological mechanisms in the offspring, which ultimately produce all the described effects of stress during pregnancy. What physiological change in the mother, and consequently in the offspring, as a result of maternal stress, generates these developmental and behavioural effects? Increased pituitary-adrenocortical output is the primary endocrine stress response, and details of this, the placental passage of hormones and maternal factors influencing foetal endocrine development are given in chapter 2. Theoretical evidence exists to suggest that glucocorticoids are involved in the production of the "teratogenic" effects of stress, and this mechanism will now be reviewed with respect to the 3 major categories of effects upon the offspring.

The fear mediated and "emotional" types of behaviour shown to be affected in offspring from rodents stressed during pregnancy, are thought by some authors to be influenced by hormones of the pituitary-adrenocortical system (e.g. Ader and Grota, 1973). Maternal stress during pregnancy may influence these behaviour patterns in the offspring by direct action of maternal corticosterone on the foetal brain, or by a peripheral effect on the offspring's adrenals. Offspring from rats stressed during pregnancy show adrenal underdevelopment (Dahlöf, Hard and Larsson, 1977) and decreased amplitude of the corticosterone stress response (Politch, Herrenkohl and Gala, 1978) and these effects on the pituitary-adrenal system may also cause the effects of prenatal stress on aspects of behaviour and development controlled by this system. Corticosterone is known to have a catabolic action on protein (Chapter 2) and this may well cause most of the other effects of stress during pregnancy upon the offspring. Catabolism of protein in the foetus, at a time when body, brain and other organ systems are most rapidly growing, and in some cases still developing, can be expected to have serious consequences. This model can certainly be envisaged to cause the somatic underdevelopment evident in offspring from rodents stressed during pregnancy, and may also be involved in producing the described effects upon offspring morphological development. Further, it has been shown that offspring from rodents stressed during pregnancy show retarded neurological development. Glucocorticoids are known to differentially influence the development of organs (Beato and Doenecke, 1980). In rodents, the brain of the male foetus is masculinised by the interaction of testosterone-derived steroids upon receptors during a critical period. If the normally occurring

prenatal testosterone surge is early or late with respect to brain development, then masculinisation could be diminished. Ward and Weisz (1980) report that male foetuses from rats stressed during pregnancy show an acceleration of this prenatal testosterone surge, and suggest that mistimed development of the pituitary-gonadal system from that of the brain, may cause the demasculinisation of the sexual responses of these animals in adulthood. A general effect of stress during pregnancy upon the process of sexual differentiation of the offspring can also be seen to influence other aspects of reproduction. Less specific effects on the brain may have repercussions on the control of endocrine systems. The adrenocortical rhythm is probably brain-derived and alteration to this may be a cause of the retardation of puberty in female offspring. However, as puberty is dependent on body weight as well as endocrine factors, body underdevelopment may actually explain Politch and Herrenkohl's (1984a) results.

The mechanisms of the production of the effects of stress during pregnancy upon the offspring outlined above, are central to this thesis and are detailed in other chapters. The working hypothesis of this thesis is that the effects of stress during pregnancy upon offspring development are mediated by maternal pituitary-adrenocortical products. The strategy used to test the working hypothesis was that ACTH, corticosterone (or other adrenal steroids) if involved in producing the effects of stress during pregnancy upon the offspring, should have similar effects when administered during pregnancy. ACTH, corticosterone, progesterone and androstenedione were administered to pregnant mice to examine whether they reproduced the effects of crowding. A further test of the hypothesis of adrenal involvement is that adrenalectomy should abolish the effects

of crowding during pregnancy upon offspring development. Further details of this strategy together with the teratogenic effects of hormones are given in Chapter 2. A simpler sequence of mediation, namely that crowded animals may feed less or give birth earlier, was also examined. This hypothesis suggested itself part way through this project and stimulated the experiments reported in Chapter 4.

Finally, the organ systems of a mammal interact in complex ways both during development and in adult life, involving both hierarchical organisation and feedback loops. For example, higher brain centres influence the hypothalamus which in turn controls the pituitary, and through it peripheral endocrine glands and target tissues; steroid hormones feedback to the pituitary and hypothalamus, and may themselves directly influence behaviour through their action on receptors in other hypothalamic areas. Given this complexity, it would be naive to expect to uncover simple causal chains to explain the mechanism of action of stress during pregnancy, upon offspring development and behaviour.

LITERATURE SUMMARY 1

Summary of pathology reported in offspring from rodents exposed to stressors during pregnancy

Pathology in offspring	Stressor during pregnancy	Reference
a) Neurobehavioural		
i) Impaired masculine sexual responses in males (demasculinisation)	Restraint	Ward, 1972.
	"	Dunlap et al., 1978
	"	Rhees & Fleming, 1981
	Avoidance conditioning[1]	Masterpasqua et al., 1976
	Chronic crowding	Chapman et al., 1976[2]
	"	Allen & Haggett, 1977*
	"	Harvey & Chevins, 1984*
	Malnutrition[3]	Rhees & Fleming, 1981
Masculine sexual responses not improved by naloxone therapy	Restraint	Rhees et al., 1983
Masculine sexual responses improved by androgen therapy		
a) perinatal (effective)	Restraint	Dörner et al., 1983
b) adulthood (effective)	Chronic crowding	Harvey & Chevins, 1984*
c) adulthood (uneffective)	Restraint	Ward, 1977
ii) Augmented feminine sexual responses in males (feminisation)	Chronic crowding	Dahlöf et al., 1977
	Restraint	Chapman et al., 1976
	"	Dahlöf et al., 1977
	"	Herrenkohl & Whitney, 1976
	"	Rhees & Fleming, 1981
	"	Meisel et al., 1979
	"	Ward, 1972
	"	Chapman & Stern, 1978
	"	Politch & Herrenkohl, 1984a*
	"	Whitney & Herrenkohl, 1977
	Malnutrition	Rhees & Fleming, 1981
iii) Homosexual responses in males	Restraint	Götz & Dörner, 1981
	Social fear[4]	Dörner et al., 1980
	"	Dörner et al., 1983
iv) Impaired sexual responses in females (defeminisation)	Chronic crowding	Allen & Haggett, 1977*
	Restraint	Beckhardt & Ward, 1983[2]

Pathology in offspring	Stressor during pregnancy	Reference
v) Reduced maternal aggression (defeminisation)	Restraint	Politch & Herrenkohl, 1979
vi) Increased infanticide in males and females	Handling	Miley et al., 1981, 1982, 1983
	Light[5]	Vom Saal, 1983*
vii) Decreased arousal, exploration, activity and fear mediated responses	Chronic crowding	Keeley, 1962*
	"	Lieberman, 1963*
	"	Chapman et al., 1976
	Water submersion[3]	Defries, 1964*
	"	Defries et al., 1967*
	Handling	Ader & Conklin, 1963
	"	Ader & Plaut, 1968
	Avoidance conditioning[1]	Thompson, 1957
	"	Kaplan & Thompson, 1957
	"	Hockman, 1965
	Avoidance conditioning	Rohner & Werboff, 1979*
	"	Morra, 1965
	"	Thompson & Quinby, 1964
	Electric shock[3]	Thompson et al., 1962
	"	Ader & Belfer, 1962
	"	Hutchings & Gibbon, 1962
	"	Masterpasqua et al., 1976
	"	Joffe, 1977
	"	Smith et al., 1975
b) Endocrine		
i) Adrenal atrophy	Restraint/cold[3]	Dahlöf et al., 1978
	Formaldehyde[3]	Schnurer, 1963
	Inanition[3]	Barry, 1920
ii) Thyroid and thymi atrophy	Formaldehyde[3]	Schnurer, 1963
	Inanition[3]	Barry, 1920
iii) Testis atrophy	Restraint/cold[3]	Dahlöf et al., 1978
	Formaldehyde[3]	Schnurer, 1963
	Inanition[3]	Barry, 1920
iv) Altered stress secretions of corticosterone	Handling	Ader & Plaut, 1968
	Restraint	Politch et al., 1978
v) Altered secretion patterns of testosterone in males	Restraint	Stahl et al., 1978
	"	Dörner, 1980
	"	Ward & Weisz, 1980

Pathology in offspring	Stressor during pregnancy	Reference
vi) Altered secretion of prolactin	Restraint	Politch et al., 1978
	"	Herrenkohl & Gala, 1979
a) stress secretions (males)	"	"
b) basal secretions (females)	"	"
vii) Lengthened oestrous cycles	Restraint	Herrenkohl & Politch, 1978
	"	Politch & Herrenkohl, 1984a*
viii) Reduced fertility	"	Herrenkohl, 1979
c) Neurochemical and Neurological		
i) Decreased brain protein	Handling	Petropoulos et al., 1972
ii) Altered neuro-transmitter concentrations in brain regions	Restraint	Moyer et al., 1978
	Electric shock[3]	Sobrian, 1977[2]
	Avoidance conditioning	Rohner & Werboff, 1979*
iii) Decreased nucleic acid concentrations in brain regions	Undernutrition[3]	Hammer & van Marthens, 1981
	Handling	Petropoulos et al., 1972
iv) Retarded reflex development	Restraint	Barlow et al., 1978
	Chronic crowding	Chevins, 1981*
v) Accelerated motor development	Electric shock[3]	Sobrian, 1977
vi) Accentuated taste neophobia	Novel environment	Pfister et al., 1981
d) Morphological and Developmental		
i) Hydrocephaly	Restraint	Euker & Riegle, 1973
ii) Cleft palate	Restraint	Barlow et al., 1974*
iii) Decreased ano-genital distance in males	Chronic crowding	Allen & Haggett, 1977*
	"	Dahlöf et al., 1978
	Restraint	Dahlöf et al., 1978
	"	Chapman & Stern, 1978
iv) Altered litter sex ratio (increased females)	Restraint	Lane & Hyde, 1973

Pathology in offspring	Stressor during pregnancy	Reference
v) Decreased litter size and survival	Restraint	Euker & Riegle, 1973
	"	Lane & Hyde, 1973
	Avoidance conditioning	Morra, 1965
	Handling	Hockman, 1961
vi) Delayed ear pinna unfolding	Restraint	Barlow et al., 1978
vii) Decreased body weight	Restraint	Barlow et al., 1978
	"	Herrenkohl & Whitney, 1976
	Handling	Werboff et al., 1968
	Chronic crowding	Chevins, 1981*
	Electric shock[3]	Sobrian, 1977
viii) Gastric erosion	Handling	Ader & Plaut, 1968
ix) Delayed vaginal opening	Restraint	Politch & Herrenkohl, 1984a*
	"	Barlow et al., 1978[2]

Footnote:
1 Premating procedure
2 Limited or no effects
3 Effects may be mediated directly
4 Study in humans
5 Effects modified by prenatal stress
* Study in mice (all other studies employed rats)

CHAPTER 2

ENDOCRINOLOGY OF THE STRESS RESPONSE, HORMONAL PROFILE DURING PREGNANCY AND FOETAL ENDOCRINE DEVELOPMENT

The abnormalities evident in the offspring from rodents exposed to stressors during pregnancy have been reviewed in Chapter 1, and a hypothesis of their causation outlined. This chapter is addressed to the details of this model. To this end, the endocrinology of the stress response, the metabolic action of hormones, the hormonal profile during pregnancy and foetal endocrine development are examined. Particular attention is paid to crowding as a stressor and the physiology of the rat and mouse, because of their relevance to this study.

Rodents existing in dense populations or crowded conditions show marked changes in endocrine activity. In mice, grouping or crowding can disrupt the oestrous cycle in females (Bronson and Chapman, 1968; Nichols, 1980) and decrease testosterone secretion and reproductive organ growth in males (Jean-Faucher, Berger, De Turckheim, Veyessiere and Jean, 1981). In both rats and mice, crowded conditions activate the hypothalamo-pituitary-adrenocortical axis as evidenced by increased adrenocorticotrophic hormone (ACTH) secretion, increased adrenocortical output and adrenal hypertrophy (e.g. Christian, Lloyd and Davis, 1965; Nichols, 1980). *In vitro*, adrenal glands from rats crowded for three weeks to a floor area of 30 cm^2/animal show elevated rates of adrenal mitochondrial hydroxylation of deoxycorticosterone, resulting in increased corticosterone synthesis and secretion, compared with hydroxylation rates of adrenals from rats housed at a density of 110 cm^2/animal (McCarthy, Green and Sohal, 1976). Crowding is therefore a genuine

stressor and is capable of increasing adrenocortical output after chronic exposure. Some stressors (e.g. cold) fail to elicit a long term endocrine stress response due to adaptive conditioning of the animal (Burchfield, Woods and Elich, 1980).

Other experimental procedures commonly employed in studies of stress have been shown to increase adrenocortical output. Handling male rats results in increased plasma corticosterone concentrations, particularly during the "peak" phase of the 24 hour rhythm (Brown and Martin, 1974). Restraining female rats also results in increased plasma corticosterone concentrations (Smith and Gala, 1977). Both handling and restraint of rats increases prolactin (PRL) secretion (Brown and Martin, 1974; Smith and Gala, 1977). Undernutrition is also a stressor in rats and is reported to increase adrenocortical output (Adlard and Smart, 1972) and decrease thyroid stimulating hormone secretion (TSH; Hugues, Reinberg, Jordan, Selbaoun, Modigliani and Burger, 1982). Stressors including undernutrition have in fact been shown to alter the secretions of many other hormones including growth hormone (GH), vasopressin and adrenal-medullary hormones, pituitary-thyroid, pituitary-ovarian and pituitary-gonadal secretions in many mammalian species (Mason, 1968a, b, c; Bronson and Chapman, 1968; Rose, 1969; Brown and Martin, 1974; Terry, Willoughby, Brazeau and Martin, 1976; Tveit and Almlid, 1980; Makara, Palkovits and Sventogothai, 1980; Jean-Faucher, Berger, De Turckheim, Veyessiere and Jean, 1982). Whether these are primary stress responses, or a result of the effects of pituitary-adrenocortical hormones is unclear.

During pregnancy, the pituitary-adrenocortical axis shows reduced stress responsiveness: in mice, isolation results in an increase in adrenal weight in virgin females but the same procedure

does not influence adrenal weight in pregnant mice (Brain and Nowell, 1970a). This difference in pituitary-adrenal sensitivity was attributed to increased output of ovarian androgens during pregnancy at the expense of oestrogens. Oestrogens are known to stimulate ACTH secretion (Barrett, 1960) but are not required for the stress response. Pregnancy is also known to reduce sensitivity of the hypothalamo-pituitary-adrenal system to stress in guinea pigs (Hoet, Pagni, Ekka and Saba, 1965). However, it is possible that isolation is not perceived as a stressor by pregnant rodents in laboratory conditions because it relates to natural circumstances. Despite the phenomenon of reduced pituitary-adrenal sensitivity in pregnancy, stressors which are employed in studies reporting the effects of stress during pregnancy upon the offspring (see Chapter 1) have been empirically shown to increase adrenocortical output. Rats handled on day 16* of pregnancy show elevated plasma corticosterone concentrations, rising from 375 ng/ml to 550 ng/ml during the peak phase of the 24 hour rhythm, and rising from 120 ng/ml to 320 ng/ml during the trough phase of the 24 hour rhythm (Grota and Ader, 1970). In mice, restraint and food deprivation on days 14-15 of pregnancy results in an increase in plasma corticosterone concentrations from 790 ng/ml to 7000 ng/ml, and undisturbed mice had a plasma corticosterone concentration of 1200 ng/ml on day 15 of pregnancy (Barlow, McElhatton, Morrison and Sullivan, 1974). Premating avoidance conditioning does not influence corticosterone secretion during pregnancy in rats (Joffe, Mullick, Ley and Rawson, 1978).

The stress response primarily involves changes in the secretions from the hypothalamo-pituitary-adrenocortical axis, and control of this system will now be reviewed in detail. The hypothalamus

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*Where day of pregnancy is given, conception has been designated as day 0.

synthesises and secretes corticotrophin releasing factor (CRF) which has recently been sequenced as a 41 residue peptide (Vale, Speiss, Rivier and Rivier, 1981). CRF links the central nervous system with the pituitary-adrenal system, and its secretion is necessarily influenced by the input from different neural pathways (Redgate, Fahringer and Szechtman, 1973; Allen, Allen, Greer and Jacobs, 1973). CRF induces the secretion of ACTH from the anterior lobe the pituitary gland (e.g. Hodges, 1970; Knigge, Joseph and Nocton, 1981). Stress-induced secretion of CRF and ACTH is rapid and detectable increases in ACTH secretion have been reported within 60-120 seconds following exposure to ether or laparotomy in rats (Hodges, 1970; Vernikos-Danellis and Heybach, 1980). Degeneration of tryptaminergic, noradrenergic and dopaminergic neurones by intracerebroventricular administration of 5,6-hydroxytryptamine 6-hydroxydopamine and desmethylimipramine respectively, has shown that intact tryptaminergic and noradrenergic but not dopaminergic pathways are required for stress-induced ACTH secretion in the rat (Amar, Mandal and Sanyal, 1982).

ACTH stimulates certain cells of the adrenal cortex to secrete steroids. The adrenal gland in mammals consists of two tissues arranged in two distinct zones, an inner medulla surrounded by an outer cortex. The medulla is derived from neural tissue and secretes adrenaline and noradrenaline. These compounds are involved in tissue metabolism, glycogenolysis, mobilisation of fatty acids and the activity of smooth muscle. The adrenal cortex is mesodermal in origin and is composed of three types of cells which are located in three layers: an outer zona glomerulosa, an intermediate zona fasiculata and an inner zona reticularis. The zona glomerulosa secretes aldosterone, a compound involved in mineral

balance, and is not strongly influenced by ACTH. Under the influence of ACTH the zona fasiculata cells secrete glucocorticoids and the zona reticularis cells secrete androgenic steroids. In rats and mice, the dominant glucocorticoid is corticosterone.

The primary action of ACTH in rodents is to stimulate corticosterone release from zona fasiculata cells. ACTH also stimulates the secretion of adrenal androgens (Baird, Uno and Melby, 1969; Vinson, Bell and Whitehouse, 1976; Kime, Vinson, Major and Kilpatrick, 1980) adrenal oestrogens (Baird, Uno and Melby, 1969) and adrenal progestogens (Fajer, Holzbauer and Newport, 1971; Piva, Gagliano, Motta and Martini, 1973; Ogle and Kitay, 1977) and exerts a trophic effect, stimulating adrenal cellular growth and proliferation (e.g. Estivarez, Iturriza, McLean, Hope and Lowry, 1982). Additionally, ACTH via its action upon glucocorticoids is involved in the control of parturition (e.g. Liggins, 1979) and directly influences behaviour (e.g. Brain, Nowell and Wouters, 1971; Brain and Evans, 1972; Brain and Poole, 1974; Brain and Evans, 1977; Brain, 1978; DeWied, 1976, 1980; Bohus and DeWied, 1980).

Adrenal steroid secretion is independently influenced by hormones other than ACTH, but whether these hormones play any major physiological role in controlling adrenal output is unknown. Adrenal progesterone secretion is stimulated by PRL, luteinizing hormone (LH) and GH but is inhibited by follicle stimulating hormone (FSH) in rats (Piva, Gagliano, Motta and Martini, 1973). LH, FSH, PRL, PRL-synergistically with oestrogen, and alpha melanocyte stimulating hormone (αMSH) have been shown to stimulate corticosterone secretion in rats (Sugihara, Miyabara, Yun, Ohta and Yonemitsu, 1982; Vinson, Bell and Whitehouse, 1976; Vasquez and Kitay, 1978; Ogle and Kitay, 1979) and αMSH, βlipotrophic

hormone and human chorionic gonadotrophin stimulate dehydroepiandrosterone sulphate synthesis in human foetal adrenals (Brown, Ginz, Milne and Oakey, 1982). Foetal endocrine development and function is further discussed later.

Reciprocally, adrenal steroids are known to influence ACTH secretion. Oestrogen may stimulate ACTH secretion (Barrett, 1960) and alter adrenal sensitivity to ACTH and hence feedback control of ACTH during pregnancy (Brain and Nowell, 1970a). Progesterone suppresses corticosterone secretion but the site of inhibition is unclear (Rodier and Kitay, 1974; Phillips and Poolsanguan, 1978). Corticosterone is well known to suppress ACTH secretion (Dallman and Jones, 1963; Motta, Piva and Martini, 1970; Smelick, 1963, 1970). Corticosterone operates the negative feedback loop controlling pituitary ACTH release and adrenocortical output. Corticosterone has identified target sites of inhibition of ACTH, the ventral hypothalamus, regulating ACTH release by direct inhibition of CRF (Smelik and Vermes, 1980) and ACTH secreting cells in the pituitary.

The most important products liberated from the hypothalamo-pituitary-adrenocortical axis in the stress response are the glucocorticoids, and these compounds exert well established effects upon metabolism. Glucocorticoids mobilize fatty acids, inhibit glucose uptake into adipose cells, inhibit amino acid uptake into muscle, inhibit synthesis of collagen and mucopolysacharides, induce liver amino transferase activity allowing glucose synthesis from amino acids (gluconeogenesis), induce liver glycogen deposition, suppress lymphoid function and resistance to infection, and are involved in mineral balance (e.g. Shulster, Burstein and Cooke, 1976; Gower, 1979; Beato and Doenecke, 1980). Glucocorticoids (both natural and synthetic) are also known to decrease muscle mass, muscle

protein synthesis and muscle nucleic acid concentrations (Seene and Viru, 1982), suppress liver nucleic acid synthesis (Henderson, Fischel and Doenecke, 1971) reduce body weight and alter nucleic acid ratios in neural tissue (Howard, 1965) and decrease thymidine incorporation into non-lymphoid tissues (Loeb and Yeung, 1973).

It is apparent that the induction of the stress response, altering hormone secretion from the pituitary-adrenocortical system and other endocrine axes, will produce major deviations from the normal hormonal-profile during pregnancy. To appreciate this, it is necessary to outline the maternal hormonal profile during pregnancy.* The following hormone concentrations in the mouse have been extracted from the literature.

In the mouse, luteinizing hormone (LH) levels in plasma are variable throughout pregnancy, but three distinct peaks have been measured: prior to implantation, mid-pregnancy (40 ng/ml) and at parturition (Murr, Bradford and Geschwind, 1974). In the mouse follicle stimulating hormone (FSH) and PRL have also been studied (Murr, Bradford and Geschwind, 1974). Plasma FSH levels fluctuate throughout pregnancy (500-900 ng/ml) achieving maximal levels at day 0 (conception) and at parturition. PRL secretion follows the opposite pattern to FSH, with levels minimal at day 0, peaking at day 8 of pregnancy (400 ng/ml) and declining sharply at parturition.

In the rat, plasma PRL concentrations remain uniformly low until parturition (Amenomori, Chen and Meites, 1970; Morishige, Pepe and Rothchild, 1973; Klindt, Robertson and Friesen, 1981). Gonadotrophin secretion throughout pregnancy has also been studied in the rabbit (McNeilley and Friesen, 1978), and rhesus monkey

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*Pregnancy lengths differ between the various species mentioned. For information related to this see Shepard (1976).

(Weiss, Butler, Hotchkiss, Dierschke and Knobil, 1976) and many other species (Rosenblatt and Siegel, 1981).

Secretion of sex steroids during pregnancy has been studied in many species. In the mouse, plasma oestradiol-17β levels are variable throughout pregnancy, but a single peak (60 ng/ml) is distinguishable prior to parturition (McCormack and Greenwald, 1974; Barkley, Geschwind and Bradford, 1979). A similar pattern of oestradiol secretion throughout pregnancy has been shown in the rat (Shaikh, 1971; Taya and Greenwald, 1981), whilst in the rabbit oestrogens remain consistently low (Lau, Saksena and Salmonsen, 1982). Oestrogens are important during pregnancy for stimulating uterine progesterone receptors (Laure and Pasqualini, 1981) and are implicated in control of the birth process (Turnbull, Flint, Jeremy, Patten, Keirse and Anderson, 1974; Downing, Porter and Lincoln, 1981). The placenta is a substantial source of oestrogens (Tulchinsky, 1973; Evans and Wagner, 1981; Larsson, Wagner and Sachs, 1981; Walsh and McCarthy, 1981).

Progesterone is responsible in many species for maintenance of pregnancy, and a relatively high stable output of this steroid typically occurs during pregnancy. In the mouse there is an additional peak of plasma progesterone on day 15 of pregnancy (110 ng/ml) prior to a steady decline until parturition (McCormack and Greenwald, 1974; Barkely, Geschwind and Bradford, 1979). A similar pattern has been reported in rats (Morishige, Pepe and Rothchild, 1973; Pepe and Rothchild, 1973, 1974; Ogle and Kitay, 1977) and between days 5 and 12 of pregnancy adrenal progesterone secretion is curtailed at the expense of corticosterone output (Ogle and Kitay, 1977). The ovary seems to be the major source of progesterone in the pregnant rat (Sanyal, 1978).

Plasma testosterone levels change during pregnancy in the mouse. Maximal output of testosterone occurs in two peaks between days 8-13 (140 ng/ml) and days 14-17 (Barkley, Geschwind and Bradford, 1979). Similar testosterone secretion patterns have been reported in the rat (Taya and Greenwald, 1981) and rabbit (Lau, Saksena and Salmonsen, 1982). The importance of these androgen surges during pregnancy is not understood, however the late pregnancy surge may be of foetal origin.

Activity of the pituitary-adrenocortical axis during pregnancy has not been fully studied and ACTH levels can only be inferred from corticosterone output. Plasma corticosterone titres rise almost ten-fold throughout pregnancy in mice, achieving 1500-2000 ng/ml by day 18 (e.g. Barlow, Morrison and Sullivan, 1974; Barlow, Morrison, McElhatton and Sullivan, 1974; Solomon, Gift and Pratt, 1979). This increase is less pronounced in the rat (Ogle and Kitay, 1977). The pregnant rat shows a 24 hour rhythm of corticosterone (Cohen, 1976).

A substantial source of the corticosterone in maternal circulation may be of foetal origin. In the mouse, foetal plasma corticosterone levels are higher than maternal levels on day 14 of gestation (Michaud and Burton, 1977). Similarly, increased foetal, compared with maternal corticosterone concentrations, have been reported in the rat (Dupouy, Coffigny and Magre, 1975). In mice and rats, the foetal adrenals are capable of maintaining corticosterone levels in maternal circulation if maternal corticosterone secretion capability is inhibited late in pregnancy (e.g. Milkovic, Paunovic, Kniewald and Milkovic, 1973; Barlow, Morrison and Sullivan, 1974; Milkovic, Klepac and Milkovic, 1976). Further maternal-foetal endocrine interaction is reviewed later.

Placental transfer of hormones occurs largely via the physical process of diffusion, where molecular size is a major limiting factor. Large molecules such as protein and peptide hormones cannot diffuse across the placental barrier intact, at least in physiological concentrations. Smaller molecules or fragments may cross the placenta along concentration gradients. ACTH has been shown not to cross the placental barrier as an intact molecule, in physiological concentrations in the rat (Jones, Lloyd and Wyatt, 1976; Milkovic and Milkovic, 1961) and rabbit (Genazzani, Fraioli, Fioretti and Felber, 1975), however ACTH fragments may enter the foetus when maternal concentrations are very high. Similarly, Triiodothyronine and Tetraiodothyronine will cross the placenta to the foetus when maternal binding capacity is surpassed and concentrations in maternal circulation are elevated (Jost, 1979).

Radiolabel studies have shown that virtually all unbound steroids readily traverse the placental barrier from mother to foetus and vice versa (Johnson and Everitt, 1980). In rats, corticosterone (Zarrow, Philpott and Denenberg, 1970) 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone, 11β-hydroxyprogesterone, 20α-hydroxyprogesterone (Milkovic, Klepac and Milkovic, 1976), testosterone and other androgens (Vreeburg, Woutersen, Ooms and Van der Werff ten Bosch, 1981) and oestrogens (e.g. Stumpf and Sar, 1978; Kuhn and Bollen, 1982) have been shown to cross the placenta. Similar transplacental steroid transfer has been shown in humans (e.g. Migeon, Prystowsky, Grumbach and Byron, 1956). All steroids, precursors and metabolites probably cross the placenta, with the exception of aldosterone which has been empirically shown not to transfer between maternal and foetal circulations (Milkovic, Klepac and Milkovic, 1976).

As previously mentioned, foetal endocrine development is influenced by hormones of maternal origin. Development of the foetal hypothalamo-pituitary-adrenocortical axis is particularly susceptible to modification by maternal adrenocortical hormones. Elevation of ACTH or corticosterone levels in maternal circulation, inhibits the development of the foetal adrenal: a phenomenon produced by suppression of foetal pituitary ACTH secretion by the action of maternal corticosterone (Jones, Lloyd and Wyatt, 1953; Migeon, Prystowsky, Grumbach and Byron, 1956; Skebelskaya, 1968; Milkovic, Milkovic, Sencar and Paunovic, 1970; Milkovic, Milkovic and Paunovic, 1973; Milkovic, Joffe and Levine, 1976). Activation of the maternal pituitary-adrenocortical axis during pregnancy by stressors also inhibits foetal adrenal development (see Chapter 1). Removal of maternal corticosterone during pregnancy has the opposite effect and results in hypersecretion of foetal ACTH and foetal adrenal hypertrophy (Edward-Davis and Plotz, 1954; Angervall, 1962; Milkovic, Milkovic, Sencar and Paunovic, 1970; Milkovic, Milkovic and Paunovic, 1973; Milkovic, Paunovic, Kniewald and Milkovic, 1973; Milkovic, Joffe and Levine, 1976). Angervall (1962) also reports foetal thyroid hyperplasia following maternal adrenalectomy during pregnancy. The development of the foetal pituitary and adrenal glands are thus inextricably linked to maternal pituitary-adrenocortical output. The development of other endocrine glands in the foetus may also be influenced by maternal pituitary-adrenocortical activity. For example, foetal ACTH regulates the development of insulin secreting pancreatic β cells (Jack and Milner, 1975), such that suppression of the foetal pituitary by maternal corticosterone also inhibits foetal pancreatic development. Of interest here, is that stress during pregnancy produces somatic underdevelopment

in the offspring (see Chapter 1), an effect probably mediated by exposure of the foetus to maternal corticosterone, resulting in reduced thyroid and pancreas output and catabolism of body protein. The endocrine control of growth and development is further discussed in Chapters 5 and 6.

The state of development of foetal endocrine axes is typically assessed by detecting the synthesis and secretion of hormones during different days of gestation. The foetal rat pituitary is capable of synthesising ACTH by day 17 of gestation (e.g. Skebelskaya, 1968; Milkovic, Milkovic, Sencar and Paunovic, 1970; Milkovic, Milkovic and Paunovic, 1973; Jenkin, McMillen and Thorburn, 1979). Complete hypothalamic control of the pituitary-adrenocortical axis has been reported to occur by day 19 of gestation (Bugnon, Fellman, Gouget and Cardot, 1982) with both the hypothalamus and pituitary as sites of negative feedback inhibition of corticosterone (Dupouy, 1974). In humans, the foetal pituitary is capable of synthesising and secreting ACTH, PRL, αMSH and βendorphin (Brubaker, Baird, Bennett, Brown and Solomon, 1982; Furuhashi, Takahashi, Fukaya, Kono, Shinkawa, Tachibana and Suzuki, 1982; Puolakka, Kauppila, Tuimala and Pakarinen, 1982). The adrenals of foetal mice (Barlow, Morrison and Sullivan, 1974), rats (e.g. Dupouy, Coffigny and Magre, 1975; Milkovic, Klepac and Milkovic, 1976), pigs (Lohse and First, 1981), sheep (Durand, Cathiard, Locatelli, Dazord and Saez, 1981; Challis, Manchester, Mitchell and Patrick, 1982), rhesus monkeys (MacNulty, Novy and Walsh, 1981) and humans (e.g. Brown, Ginz, Milne and Oakey, 1981; Tiniacos, 1982) are functional, and produce a variety of steroids when challenged with ACTH or other hormones. In the lamb foetus, sensitivity to ACTH increases as the adrenal matures (Rose, Meis, Urban and Greiss, 1982).

Pituitary-gonadal activity is initiated during intrauterine life. Luteinizing hormone releasing hormone (LHRH, GnRH) can be detected in the hypothalamus of the rat foetus on day 17 of gestation (Nemeskeri and Kurcz, 1981) and in the guinea pig foetus on day 28 of gestation (Schwanzel-Fukuda, Robinson and Silverman, 1981). LH can be detected immunoreactively in rat foetus pituitaries by day 18 of gestation (Jenkin, McMillen and Thorburn, 1979). The rat foetus exhibits sexually-differentiated patterns of LHRH and LH release (Gogan, Slama, Bizzini-Koutznetzova, Dray and Kordon, 1981) and both foetal rats (Daikoku, Adachi, Kowano and Wakabayashi, 1981) and foetal mice (Pointis and Mahoudeau, 1976) respond to LHRH challenge by LH secretion during the final days of gestation. In the rabbit foetus, LH secretion from the pituitary occurs from day 20 onwards which is relatively earlier than in rats and mice (Veyessiere, Berger, Jean-Faucher, De Turckheim and Jean, 1982a) and in humans from week 11 of gestation (e.g. Jenkin, McMillen and Thorburn, 1979).

FSH synthesis is initiated later in development of the foetus than LH. In the rat foetus, FSH can be detected immunoreactively on day 20 of gestation (e.g. Jenkin, McMillen and Thorburn, 1979). In rabbits FSH is present in foetal pituitary by day 25 and is secreted into circulation by day 27 of gestation (Younglai, Pan and Bhavani, 1981; Veyessiere, Berger, Jean-Faucher, De Turckheim and Jean, 1982a). PRL synthesis and release is also initiated prenatally (e.g. Fang and Kim, 1975; Yaginuma, 1981).

The secretion of gonadotrophins in the foetus, as in the adult, stimulates sex steroid production from the gonads. Testicular and ovarian steroid biosynthesis in the gonads of foetal rabbits is initiated on day 18 of gestation, and sex steroids can be detected

in glandular tissue from days 18 or 19 (George and Wilson, 1979; Veyessiere, Berger, Jean-Faucher, De Turckheim and Jean, 1975; Veyessiere, Berger, Jean-Faucher, De Turckheim and Jean, 1982b). In the rat, testosterone can be detected by immunoassay in the plasma of male foetuses on day 18 of gestation (Ward and Weisz, 1980; Weisz and Ward, 1980). Feedback regulation of testosterone is functional by day 20 of gestation in the rat (Naessany and Picon, 1982). Androgen and oestrogen receptors are present in foetal rat brain (hypothalamus, amygdala and preoptic area) from day 15 and day 21 of gestation respectively (Maclusky, Lieberburg and McEwen, 1979; Vito and Fox, 1981). The appearance of androgen and oestrogen receptors in neural tissue is an essential pre-requisite for sexual differentiation of the brain. This process is reviewed in detail later. Androgens in foetal circulation also differentiate the genitals (Bloch, 1979; Ratzan and Weldon, 1979; Neumann, 1979).

Other hormones known to be produced by the foetus include: insulin, thyroid hormones, αMSH, vasopressin, somatostatin, prostaglandins, oxytocin, βlipotropin and βendorphin (from studies on mice, rats, rabbits, sheep, monkeys and humans - Jack and Milner, 1975; McIntosh, Pictet, Kaplan and Grumbach, 1977; Karaplis and Powell, 1981; Nemeskeri and Kurcz, 1981; Stark, Daniele, Husain, Milliez, Morishima, James and van der Wiele, 1981; Burford and Robinson, 1982; Leisti, Miller and Johnson, 1982; Furuhashi, Takahashi, Fukaya, Kono, Shinkawa, Tachibana and Suzuki, 1982). In humans, the foetus is capable of de novo cholesterol synthesis (Carr, Ohashi and Simpson, 1982) from which the foeto-placental unit can manufacture most steroids (Cekan, 1972).

Sexual differentiation of the brain is dependent upon the interaction of sex steroids and receptors in neural tissue. The

male foetal brain is masculinised by the aromatised products of androgens. According to the aromatisation hypothesis, androgens e.g. testosterone and androstenedione are enzymically converted by aromatase to oestradiol and oestrone respectively, which interact with brain receptors to masculinise neural and neuroendocrine systems (e.g. Naftolin, Ryan and Petro, 1972; Naftolin and Ryan, 1975; Westley and Salaman, 1976; Martini, 1978; Döhler and Hancke, 1978; Dorner, 1980; Goy and McEwen, 1980; Gogan, Slama, Bizzini-Koutznetzova, Dray and Kordon, 1981; Bardin and Catterall, 1981; Maclusky and Naftolin, 1981; McEwen, 1981a, b). Exposure of male and female rodents perinatally to androgens masculinises their adult behaviour patterns, and disruption of oestrogen biosynthesis perinatally disrupts masculine behaviour in males in adulthood (see literature summary 2).

In the male rat foetus, testicular testosterone is released in a surge during day 18 of gestation and the correct timing of this surge is important for masculinisation of adult brain (and therefore behaviour - Ward and Weisz, 1980). This testosterone surge may exert its effects upon sexual differentiation of the normal male rat by masculinising hypothalamic gonadotrophin regulating structures, resulting in masculine (non-cyclic) patterns of LH secretion (Gogan, Slama, Bizzini-Koutznezova, Dray and Kordon, 1981). LHRH secreting structures are identified as specific sites of masculinisation, influencing sexually differentiated patterns of gonadotrophin secretion (empirical study on guinea pigs with reference to rats and mice - Schwanzel-Fukuda, Robinson and Silverman, 1981). As aromatase and steroid receptors are both present in foetal rat brain prior to the testosterone surge (both enzyme and receptors detected from day 15 of gestation

onwards - Reddy, Naftolin and Ryan, 1974; Maclusky, Lieberburg and McEwen, 1979; Vito and Fox, 1981) one limiting factor to neural and neuroendocrine differentiation in this species would seem to be the amplitude and timing of the androgen surge, which persists into the early postnatal period (Pang, Caggiula, Gay, Goodman and Pang, 1979). A second general limiting factor to sexual differentiation of the brain is the relative concentration of both enzyme and receptors. There are sex differences in sex steroid receptor concentrations in rat brain at least postnatally (Rainbow, Parsons and McEwen, 1982). If this difference is present prenatally, masculinisation will be accordingly affected. It is not known whether sex differences in steroid receptor concentrations are present prior to hormone action; it is a possibility that sex differences in receptor concentrations only develop after exposure to specific hormones, as steroid receptor concentrations are regulated by specific steroid concentrations (e.g. Kolata, 1977). Additionally, brain and somatic masculinisation is also limited by alpha-foetoprotein (αFP), which avidly binds to oestrogens preventing their interaction with receptors (e.g. Attaradi and Ruoslahti, 1976). In foetal rat brain, maximal concentrations of αFP occurs on day 18 of gestation (coincidental with the male foetal testosterone surge), and distribution of relative concentrations are olfactory lobe > cerebrum > cerebellum > remaining portion of brain (Massarat, Kaul and Sahib, 1981). αFP, although present in neural tissue and intraneuronally, is manufactured elsewhere, in foetal liver, yolk sac and gastrointestinal tract, and molecules with different properties are synthesised e.g. oestrophilic αFP and non-oestrophilic αFP (Massarat, Kaul and Sahib, 1981; Schachter and Toran-Allerand, 1982). Oestrophilic αFP is thought to protect

the female foetus from the masculinising properties of oestradiol.

In the light of recent evidence, the concept that sexual differentiation of the brain is completed by the masculinising action of androgen derived oestrogens upon neural structures, must be reconsidered. Vom Saal, Grant, McMullen and Laves (1983) have shown that male foetuses exposed to high concentrations of oestradiol, show increased sexual vigour but reduced aggression in adulthood, compared with litter mates exposed to lower concentrations of oestradiol prenatally. These findings suggest that the aromatisation hypothesis at best only applies to the differentiation of some neural systems controlling some aspects of behaviour. Aggression, like copulation, requires the action of testosterone for its expression in adulthood. However, Vom Saal's study shows that masculinisation of the neural structures controlling aggression is blocked by oestradiol. The aromatisation hypothesis as it is understood only applies to the masculinisation of neural systems influencing sexual behaviour. Further to this, oestradiol which was previously thought to masculinise all sensitive neural structures, may actually act as an anti-androgen in some areas of the brain.

The discussion turns now to how the aberrations in development and behaviour evident in offspring from rodents exposed to stressors during pregnancy may be caused. It has been shown that the hormonal profile during pregnancy is complex: as well as maternal hormones maintaining pregnancy and initiating lactation, they are also known to influence endocrine development. There is thus the potential for disruption of foetal endocrine development by alteration of the maternal hormonal profile during pregnancy. Induction of the stress response during pregnancy can be intuitively seen to affect the foetus. Corticosterone, the major compound released in the

stress response in rats and mice readily crosses the placenta. In the mouse, the foetus is protected from the potent metabolic action of corticosterone by placental conversion of this compound to 11-dehydrocorticosterone (Michaud and Burton, 1977). In conditions of extreme stress, where the pregnant rodent can secrete corticosterone up to ten fold above normal secretion rates (e.g. Barlow, McElhatton, Morrison and Sullivan, 1974) it is unlikely that the foetus can be protected from the metabolic action of this hormone.

The theoretical effects of foetal glucocorticoid exposure (based upon the known metabolic activity of these compounds) are inhibited body and organ development. The development of brain structures in the mouse and other altricial mammalian species, is incomplete until postnatal life (Rodier, 1980) these structures are therefore potentially vulnerable to disruption by perinatal exposure to glucocorticoids, which are recognised to present a neurological hazard (Weichsel, 1977). Similarly, foetal adrenal development has been shown to be inhibited following corticosterone exposure and this is due to a different mechanism, that of suppression of foetal ACTH secretion (e.g. Milkovic, Milkovic, Sencar and Paunovic, 1970). Some of the hypothetical and known effects of foetal corticosterone exposure are similar to those evident in offspring from rodents exposed to stressful conditions during pregnancy e.g. decreased body growth, adrenal atrophy. The implication of this is that stress-induced activation of the maternal pituitary-adrenocortical axis may mediate the effects of stress during pregnancy upon offspring body and adrenal development. This hypothesis has been suggested as the mediating mechanism causing the adrenal and testis atrophy and disturbances in masculine copulatory behaviour

in offspring from rats restrained or crowded during pregnancy (Ward, 1972; Dahlöf, Hard and Larsson, 1977). However, the literature has failed to identify teratogenic adrenocortical products: Ward suggested androstenedione as the maternal adrenocortical product responsible for altering sexual behaviour in the adult male offspring from rats restrained during pregnancy. However, androstenedione has now been shown not to be the maternal adrenal product responsible for producing the deficits in copulatory behaviour typical of prenatally stressed male rats: Gilroy and Ward (1978) found no deficits in the copulatory responses of male offspring from rats treated with androstenedione during pregnancy.

The rationale employed in this thesis to test the hypothesis that the reported consequences of stress during pregnancy are mediated by maternal pituitary-adrenocortical products, was to administer hormones known to be produced by this system to pregnant mice. If this hypothesis is correct, then administration of ACTH or adrenal steroids to pregnant mice will have similar effects upon offspring development to those produced by stress. Additionally, if the maternal adrenal is required for the production of the effects of stress during pregnancy upon the offspring, maternal adrenalectomy should prevent the harmful effects of stress, and this possibility was also examined.

There is already a large literature reporting the teratogenic effects of hormones and this has been summarised in tabulated form in literature summary 2. Few studies are relevant to this thesis because they do not describe effects reported to result from stress during pregnancy, or because they have used synthetic compounds in excessively large doses. However, some progress has been made

in examining the role of the maternal pituitary-adrenocortical axis in the production of the abnormal copulatory responses reported in male offspring from rodents exposed to stressors during pregnancy. Chapman and Stern (1978) administered ACTH to pregnant rats and found that the male offspring showed augmented feminine sexual responses, but did not show impaired masculine sexual responses. Thus activation of the maternal adrenocortical axis during pregnancy was shown to "feminise" but not to "demasculinise" sexual behaviour in males. This result was surprising because ACTH administration to mice during pregnancy, has been shown by Simon and Gandelman (1977) to impair the expression of another sexually differentiated pattern of behaviour, that of aggression in male offspring. For this reason this study re-examined the effects of ACTH administration to pregnant mice upon male offspring masculine sexual responses. Since this study was commenced, ACTH administration during pregnancy has also been shown to demasculinise the sexual behaviour of male offspring in both rats and mice (Rhees and Fleming, 1981; Stylianopoulou, 1983; Politch and Herrenkohl, 1984b). Additionally, some work has attempted to identify specific maternal adrenocortical steroids, secreted in conditions of stress or ACTH challenge, as mediating agents of the reported effects upon male offspring sexual behaviour. Perinatal progesterone exposure impairs masculine sexual responses in male rats (Hull, Franz, Snyder and Nishita, 1980) whilst perinatal androstenedione actually increases copulatory vigour in male rats (Gilroy and Ward, 1978). Corticosterone acetate administration during pregnancy impairs copulation in male offspring in mice (Politch and Herrenkohl, 1984b).

In reporting previously unknown effects of exposure to stressors

during pregnancy, upon offspring development e.g. puberty (assessed by determining both the day of vaginal opening and first oestrus and body weights at these events - Chapter 7) and intermale aggression (Chapter 8) this study examines the hypothesis that these effects are mediated by maternal pituitary-adrenocortical activation and exposure of the foetus to hormones of this axis. Additionally, this thesis examines the effects of maternal stress or pituitary-adrenocortical manipulation during pregnancy, upon foetal and neonatal somatic development and survival (Chapter 5) and neurological development (Chapter 6). As administration of hormones may influence food intake or pregnancy length, which may in turn affect foetal development, these factors are examined in Chapter 4.

LITERATURE SUMMARY 2

Summary of pathology reported in offspring from rodents subjected to endocrine manipulation and administration of hormones and related compounds during pregnancy

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
a) ACTH		
20 i.u./day (2-6)	Delayed implantation, inhibited implantation disrupted pregnancy.	Chatterjee & Harper, 1970
12 i.u./day (1-3)		Yang et al., 1969
4 i.u./day (1-8,1-18, 8-18)		Kittinger et al., 1980
5 mg/day (14-18)[1]	Foetal death and resorbtion, still births and reduced litter size	Robson & Sharaf, 1952
5 mg/day (11-17)[2]		Robson & Sharaf, 1952
12 i.u./day (1-3)		Yang et al., 1969
4 i.u./day (1-8,1-18, 8-18)		Kittinger et al., 1980
2-4 mg/day (1-11)	Decreased foetal and neonatal body development	Velardo, 1957
4 i.u./day (1-8, 1-18,8-18)		Kittinger et al., 1980
4 i.u./day (perinatal)[2,3]		Monder et al., 1981
4 i.u./day (perinatal)[2,3]	Delayed eye opening	Monder et al., 1981
4 i.u./day (perinatal)[2,3]	Delayed vaginal opening	Monder et al., 1981
1 i.u. and 8 i.u./day (12-17)[2]		Harvey & Chevins, 1981
ACTH tumour (1)	Adrenal atrophy	Milkovic et al., 1970, 1973 and 1976
5 i.u./day (14-20)	Testis atrophy, reduced foetal androstenedione secretion	Wilke et al., 1982
1 i.u. and 8 i.u./day (12-17)[2]	Decreased aggression in males	Simon & Gandelman, 1977
20 i.u./day (14-21)	Impaired masculine sexual responses in males	Rhees & Fleming, 1981
8 i.u./day (14-21)		Stylianopoulou, 1983
1 i.u. and 8 i.u./day (12-17)[2]		Harvey & Chevins, 1984
4 i.u./day (14-21)[2]		Politch & Herrenkohl, 1984[b]
1 i.u. and 8 i.u./day (12-17)[2]	Copulation improved by androgen therapy in adulthood	Harvey & Chevins, 1984

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
1 i.u. and 8 i.u./ day (14-21)	Augmented feminine sexual responses in males	Chapman & Stern, 1978
8 i.u./day (14-21)		Stylianopoulou, 1983
8 i.u./day (14-21)	Increased ano-genital distance in females and decreased ano-genital distance in males	Chapman & Stern, 1978
1 i.u. and 8 i.u./ day (14-21)		Stylianopoulou, 1983
12 i.u./day (10-20)	Decreased protein and nucleic acids in cerebellum, hypo-thalamus and cerebral cortex	Petropoulos et al., 1972
0.16 i.u.- 1.60 i.u./hr. (21)	Depletion of foetal adrenal absorbic acid and cholesterol	Jones et al., 1953
b) Glucocorticoids		
i) Dexamethasone		
1 mg and 100 mg/ Kg (peri)[4]	Body and brain weights reduced, impaired learning	Dekosky et al., 1982
10 µg/ml in water (15-22)	Decreased nucleic acids in placenta, foetal kidney, heart, lung, liver, brain, pituitary, testis and adrenal	Klepac, 1982
0.2 mg/Kg/day (19-20)	Inhibitory effect on organ growth, decreases survival	Frank & Roberts, 1979
ii) Cortisone acetate		
3 mg/day (4-21)	Adrenal atrophy, increased mortality reduced birth weights	Edward-Davis & Plotz, 1954
10-20 mg/day (13-19)[1]	Foetal death and reabsorption	Robson & Sharaf, 1952
iii) Cortisone		
2.5 mg/day (1-18)[2]	Cleft palate, shortened head, Spina bifida, reduced birth weight and increased mortality	Fraser & Fainstat, 1951

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
10-15 mg/hr (21)	Increased foetal cholesterol	Jones et al., 1953
iv) Hydrocortisone		
2.5 µmole/day (8,10,12,14)[2]	Reduced activity	Lieberman, 1963
4 mg/day (19)	Foetal pituitary ACTH depletion and adrenal atrophy	Skebelskaya, 1968
v) Prednisolone		
100-400 mg/day (12-18)[2]	Reduced birth weight, reduced weaning weight, retarded eye and ear opening, retarded reflex development	Gandelman & Rosenthal, 1981 Gandelman & Guerriero, 1982
vi) Corticosterone		
0.6 mg/day from day 2 postnatally	Reduced brain weight and altered nucleic acid ratios in brain	Howard, 1965
vii) Corticosterone acetate		
500 µg/day (14-21)[2]	Impaired masculine sexual responses in males	Politch & Herrenkohl, 1984b
c) Hypophysectomy		
Surgery day (14)[1]	Abortion unless steroid therapy given	Robson & Sharaf, 1952
d) Adrenalectomy		
Surgery day (4,6, 14,16)	Abortions and still-births	Edward-Davis & Plotz, 1954
(14)	Adrenal hypertrophy	Milkovic et al., 1973, 1976
(12,13)		Angervall, 1962
(19)		Skebelskaya, 1968 Havlena & Werboff, 1963
(12,13)	Thyroid and adrenal hyperplasia	Angervall, 1962
pre-pregnancy	Increased adrenal output	Thoman et al., 1970
(12,13) pre-pregnancy	Reduced birth weights and litter size	Angervall, 1962 Thoman et al., 1970

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
(1)	Implantation unaffected	White et al., 1980
(7)	Abolishes deleterious effects of ACTH treatment during pregnancy upon foetus	Velardo, 1957
(5)		Yang et al., 1969
e) Progestogens		
i) Medroxyprogesterone	Embryolethal	Eibs et al., 1982
ii) Progesterone	High titres in mummified foetuses	Kephart et al., 1981
——	Risk of hypospadias in pregnancies maintained with progesterone[5]	Mau, 1981
1.5 mg/Kg/day (8-21)	Decreased brain nucleic acids and reduced body weight	Coyle et al., 1976
2 mg/day (23)	Lengthened pregnancy, inhibited maternal lactation, increased offspring mortality and reduced body weights	Herrenkohl, 1974
2.5 mg/day (20-22)	Reduced plasma corticosterone	Roudier et al., 1982
3.3 mg/Kg/day (7-22)	Stimulates brain monoamine oxidase activity and reduced birth weight	Snyder et al., 1979
50 mg/Kg (6-10)[6]	Reduced placental and foetal growth	Knoll-Kohler et al., 1982
3.3 mg/Kg (8-18)	Impaired learning and copulation in males	Snyder & Hull, 1980
4-12 mg implant (6-21)		Hull et al., 1980
——[5]	Lower sex drive and assessed aggression in males	Kester et al., 1981 Ehrardt et al., 1981 Meyer-Bahlburg et al., 1982
——	Progesterone defends foetus from action of oestradiol and testosterone	Dorfman, 1967

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
f) Oestrogens		
i) Diethylstilbestrol		
——— [5]	Masculinises males	Kester et al., 1981
200 mg/Kg/day (7-18)	No effect on course of pregnancy or foetal body weight but caused hyper-prolactinaemia and mammary carcinomas	Huseby & Thurlow, 1982
ii) Oestriol	Correlated with birth weight, anencephaly and adrenal hyperplasia	Hardy et al., 1981
iii) Oestradiol		
Antibodies against oestradiol	Trophic effect on foetal and placental growth	Csapo et al., 1974
2.5 µg/day (10-15) [2]	Inhibits foetal and placental growth	Miller, 1978
3 µg/day (1-parturition) [7]	Depresses both maternal and foetal body weight	Czaja, 1983
5 µg/day (18-20)	Advances parturition	Downing et al., 1981
10 µg/day (14-21)	Inhibits foetal growth and suppresses foetal thyroid activity	Kuhn et al., 1981, 1982
10 µg-50 µg/day (12-18) [2]	Aggression in males unaffected	Gandelman et al., 1982
5 mg/day (14) [2]	cryptorchid males	Jean et al., 1975
g) Ovariectomy		
Surgery day (15)	Increased foetal mortality	Legrande et al., 1979
(13)	Increased foetal and placental growth	Crosskerry et al., 1981
h) Androgens		
i) Norethindrone		
50 µg-100 µg/day (14-18) [2]	Increased ano-genital distance in females	Gandelman et al., 1981

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
ii) Testosterone		
1 mg/day (13-18)[2] 1-2 µg/day (12,14,16)[2]	Increased aggression in females	Gandelman et al., 1980 Mann & Svare, 1983
iii) Testosterone Propionate		
2 mg/day (12-15)[8] 1.5 mg/day (12-16)[2]	Increased ano-genital distance in females	Landauer et al., 1981 vom Saal, 1979
2 mg/day (12-15)[8]	Irregular oestrous cycles	Landauer et al., 1981
5 mg/day (28-38) and 1 mg/day (38-58)[9]	Increased masculine sexual responses in males and decreased feminine sexual responses in females	Goldfoot et al., 1975
1 mg/day (16,19)[2]	Abnormal vaginas and feminine sexual responses in females	Huffman & Hendricks, 1981
iv) Androstenedione		
1 mg/day (14-21)	No effects upon masculine sexual responses in males	Gilroy & Ward, 1978
v) Androgen based progestins		
——[5]	Increased violence in males	Reinisch, 1981
vi) <u>In utero</u> proximity		
Females located between two males	Increased ano-genital distance[2]	vom Saal & Bronson, 1978 Clemens et al., 1978
	Increased aggression[2]	vom Saal & Bronson, 1978
	Lengthened oestrous[2] cycles	vom Saal & Bronson, 1980
	Androgen induced sterility	Tobet et al., 1982
	Impaired active avoidance[2]	Hauser & Gandelman, 1983
Males located between two females	Increased infanticide	vom Saal, 1983
	Increased masculine sexual responses and aggression	vom Saal et al., 1983

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
i) Androgen and aromatisation inhibitors		
i) 1,4,6-Androstratriene-3,17-dione		
1 mg/day (10-22)	Reduced masculine sexual responses in males	Gladue & Clemens, 1980
1 mg/day (10-22) 5 mg/day (10-21)	Increased feminine sexual responses in males	Clemens & Gladue, 1978 Whalen & Olsen, 1981
ii) Flutamide		
5 mg/day (14,15,16, 17,18)	Reduced masculine sexual responses, reduced testosterone, reduced seminal vesicle and prostate weights and elevated LH and FSH in males	Marschall et al., 1981
iii) Cyproterone acetate		
500 mg/Kg/day (2-18)[2]	Embryolethal and inhibits blastocyst development	Eibs et al., 1982
10 mg/day (9-21)	Males show shorter (feminine type) avoidance acquisition	Scouten et al., 1975
j) Neurotransmitters and related compounds		
i) Noradrenaline		
0.25 µmole/day (8,10,12,14)	Decreased activity	Lieberman, 1963
ii) Adrenaline		
0.25 µmole/day (8,10,12,14)	Increased activity	Lieberman, 1963
iii) 5-Hydroxytryptamine		
5-20 mg/Kg/day (1-5,6,10-11)	Implantation sites deprived of epithelium, increased resorbtions, increased ophthalmic and cardiovascular malformations	Aliverti et al., 1982

Hormone treatment or endocrine manipulation	Pathology in offspring	Reference
iv) Naloxone		
20-60 µg/day (peri)[2,3]	Heat hyperalgesia	Monder et al., 1979, 1981
v) Haloperidol		
1 mg/Kg/day (16-23)	Delayed vaginal opening	Bhanot & Wilkinson, 1982
vi) 6-Hydroxydopamine		
40 µg/day (16)	Destruction of para- and pre-vertebral ganglia	Aloe et al., 1981
vii) Nerve growth factor		
40 µg antibodies against nerve growth factor (16)	Reduced body weight, reduced size of ganglia, general neuro-endocrine damage and unresponsiveness to noxious stimuli	Aloe et al., 1981
k) Thyroid manipulation		
i) Thiouracil 0.25% in food (1-22) continued exposure until postnatal day 35	Reduced thyroid and adrenocortical output	Meserve & Leathem, 1981
l) Growth hormone		
0.1 mg-3 mg/day	Increased foetal-placental growth, increased brain cell number	Ganalska-Malinowska et al., 1981 Crosskerry & Smith, 1979

Footnote: Parentheses indicate days of pregnancy during which the treatment was administered. All studies used rats unless otherwise stated.

1 Study in rabbits
2 Study in mice
3 Administered 5 days prior to birth and up to postnatal day 5
4 Administered from birth to postnatal day 4
5 Study in humans
6 Confounded by administration of protein free diet and 50 µg/day oestrone
7 Study on guinea pigs
8 Study on hamsters with administration of 200 µg testosterone propionate on postnatal day 1
9 Study on guinea pigs with similar results after administration of androstanediol propionate and Dihydrotestosterone propionate

CHAPTER 3

MATERIALS AND METHODS

ANIMALS AND HUSBANDRY

Females used were virgin 'TO' strain outbred albino mice, obtained from A. Tuck and Son Ltd., Battlesbridge, Essex 2-5 weeks prior to mating, or were virgin stock females. Prior to mating, females were housed in groups of 10 in large plastic cages (42 x 25 x 11 cm), allowed *ad libitum* supply of food (Labsure animal diet, Christopher Hill Ltd., Dorset) and water, and maintained on a reverse lighting regime (red lights on 1200-2200 hrs). Several animal rooms were used, in which lighting regimes were synchronised. Temperature variation within and between rooms was in the range 18-23^{o}C. All animal cages contained sawdust bedding which was changed weekly or when necessary.

At 10-12 weeks of age, females were placed individually into small plastic cages (30 x 13 x 11 cm) which had wire mesh floors, with a sexually experienced 'TO' male. The females were observed daily up to a period of 7 days, for the appearance of a vaginal plug. The finding of a vaginal plug was deemed to indicate day 0 of pregnancy. Males were then removed and females were left singly housed in small cages with sawdust bedding until day 12 of pregnancy when treatments were administered. Births occurred between days 17-19 of pregnancy. Routinely, females were inspected twice daily for birth of litters. On discovery of a litter, individual pups were weighed on an electronic balance (Mettler PL1200). The number of still born pups were recorded. Litters were culled to 8 pups at random and fostered to an untreated female that had given birth within the previous 24 hours. Foster mothers were

primiparous females assigned to this group at random. Litters were then left undisturbed until weaning on postnatal day 21, birth day being designated as day 0. Postnatal deaths were recorded. At weaning, offspring were weighed again and housed in groups of 8-10 (except females used in experiments examining the onset of puberty, see Chapter 7) according to sex and treatment such that there were representatives from each litter in each group.

TREATMENTS DURING PREGNANCY

All treatments were administered during days 12-17 inclusive of pregnancy. Untreated controls from the same batch of mice were always provided.

(i) CHRONIC CROWDING. The crowding procedure was modified from the methodologies of Keeley (1962) and Dahlöf, Hard and Larsson (1977). Pregnant females were assigned at random to 1 of 2 treatment groups - chronic crowding or control. Females assigned to the crowding group were removed from individual housing on day 12 of pregnancy (between 0900 and 1200 hours) and placed in a large cage containing male mice. Crowding cages contained 25-28 adult male mice and 2-5 pregnant females, in each case so that the total housing density was 30 animals per cage (35 cm^2 floor space/animal). Persistent aggression was observed between males, and females were repeatedly pursued and mounted. Several males were moved daily between cages to ensure social instability and continued fighting within crowded groups. Pregnant females were removed from crowding cages on day 17 of pregnancy and individually housed in small cages containing clean sawdust. Females assigned to the control group remained individually housed (390 cm^2 floor space/animal) and were undisturbed throughout pregnancy.

ii) HORMONE ADMINISTRATION. Injections were given subcutaneously, sometimes with the use of a restraint tube, daily (on days 12-17 inclusive of pregnancy) between 1500 and 1600 hours, at a time when endogenous corticosterone levels in plasma of non-pregnant female mice are known to be falling (Nichols and Chevins, 1981a). All animals receiving injections remained individually housed.

a) ACTH ADMINISTRATION. Dosages of ACTH used were based on those employed by Simon and Gandelman (1977). Special care was taken in the use of ACTH: all glassware and saline was sterilized by autoclaving at 117°C to prevent microbial digestion of the peptide, and ACTH (obtained in 1000 i.u. pellets) was made into aliquots (100 i.u.s ACTH in 0.2 ml saline) and immediately frozen at -20°C until use.

Pregnant females were assigned at random to 1 of 4 treatment groups: low dose ACTH, high dose ACTH, saline gelatine vehicle-injection control and untreated control. The low dose ACTH group received daily injections of 1 i.u. ACTH in 0.4 ml vehicle; the high dose ACTH group received daily injections of 8 i.u. ACTH in 0.4 ml of vehicle, and the saline gelatine vehicle group received daily injections of 0.4 ml vehicle only. The vehicle was 40 mg ml^{-1} gelatine in 0.9% saline. Solutions were stored at 0-4°C, and gently warmed until liquid in a current of warm air prior to injection.

b) PROGESTERONE ADMINISTRATION. Dosages of progesterone used were based on those employed by Hull, Franz, Snyder and Nishita (1980). Pregnant females were assigned at random to 1 of 4 treatment groups: low dose progesterone, high dose progesterone, olive oil vehicle-injection control, and untreated control. The low dose progesterone group received daily injections of 250 µg progesterone in 0.1 ml

vehicle; the high dose progesterone group received daily injections of 500 µg progesterone in 0.1 ml vehicle and the olive oil vehicle-injection control group received daily injections of 0.1 ml olive oil only.

c) ANDROSTENEDIONE AND CORTICOSTERONE ADMINISTRATION. The dose of androstenedione used was based on Gilroy and Ward (1978). There has been no previous study of the effects of corticosterone administration during pregnancy upon offspring development, but related compounds are known to have teratogenic effects (e.g. Skebelskaya, 1968). Two different corticosterone treatment regimes were used: acute administration via daily injections and chronic administration via surgically implanted osmotically driven minipumps.

In the androstenedione and acute corticosterone administration experiments, pregnant females were assigned at random to 1 of 4 treatment groups: androstenedione, corticosterone, peanut oil vehicle-injection control and untreated control. The androstenedione group received daily injections of 100 µg androstenedione in 0.1 ml vehicle; the corticosterone group received daily injections of 100 µg corticosterone in 0.1 ml vehicle, and the peanut oil vehicle-injection control group received daily injections of 0.1 ml peanut oil only.

In experiments investigating the effects of chronic corticosterone administration, pregnant females were assigned at random to 1 of 3 treatment groups: chronic corticosterone, propylene glycol vehicle-surgery control and untreated (unoperated) control. The minipumps (Alzet) were small capsules with an inner reservoir into which the test solution was injected. An outer jacket surrounds the reservoir and this contains a substance that creates an osmotic potential with body fluid. The outer case is water permeable, and as water flows into the outer jacket the inner reservoir is progressively collapsed.

Propylene glycol was used as the solvent because it readily dissolves corticosterone, is compatible with the materials of the minipump and is relatively non-teratogenic according to Shepard (1976). The delivery rate of the minipump was 1 µl hr^{-1}. The dosage of corticosterone in vehicle was 10 µg ul^{-1}. The procedure for minipump implantation was as follows: minipumps were filled with the test solutions by syringe, flow moderators were fitted and minipumps were placed overnight in 5 ml volume specimen tubes containing sterile saline to establish a steady delivery rate. Surgery was performed under ether anaesthesia. Pregnant mice were removed from home cages on day 12 of pregnancy (between 0900 and 1200 hours) and anaesthetised. Fur was shaved from an area of the animals back and the shaved area was swabbed with an alcohol solution. A mid-dorsal incision was made into the skin with scalpel and scissors. Connective tissue was cleared and a subcutaneous channel opened in an antero-posterior direction. The minipump was inserted into this channel with flow moderator nearest the animal's head. The channel was irrigated with sterile saline and the incision closed with 3 or 4 wound clips.

iii) BILATERAL ADRENALECTOMY. On day 9 of pregnancy females were assigned at random to 1 of 4 treatment groups: adrenalectomy-crowding; adrenalectomy-individual housing; Sham surgery-crowding; and Sham surgery-control. The procedure for removal of the maternal adrenals was identical to that for implantation of minipumps except that secondary incisions were made with irridectomy scissors into the body cavity. The kidney was located, and by gentle pressure exerted on the underside of the animal, the kidney was partially or wholly exteriorised. The adrenal fat capsule was clamped with 2 pairs of small curved forceps, and the capsule with adrenal was

teased clear of the kidney. This method of exteriorisation of the kidney allows success in total removal of the adrenal with decreased incidence of rupture of this gland. The kidney was re-placed inside the body cavity with use of sterile swabs dampened with sterile saline. Care was taken not to disturb the uterus unnecessarily. The body cavity was closed with monofilament fibre sutures using 2 or 3 stitches. The procedure was repeated for the remaining adrenal gland. The primary incision was closed with 3 or 4 wound clips and cicatrine antibiotic spray was applied to the wound surface. Adrenalectomised animals received 0.9% saline drinking solution instead of water. Sham adrenalectomised females received primary and secondary incisions and kidneys were exteriorised. Post-operatively animals were placed into home cages which were warmed on a hotplate until recovery from the anaesthetic.

All animals were left undisturbed until day 12 of pregnancy when females assigned to the crowding stress condition were rehoused according to the procedure previously outlined. Crowding cages containing adrenalectomised females were supplied with both water and saline drinking solutions. Adrenalectomised and Sham-operated females assigned to the control housing condition remained individually housed throughout the final third of pregnancy. Crowded females were rehoused individually on day 17 of pregnancy to ensure undisturbed births.

SUMMARY: Pregnant females were assigned at random to treatment groups. Treatments were given during the final third of pregnancy. With the exception of crowded females, all other animals remained individually housed during pregnancy. Litters were weighed and

culled to 8. Some litters were assessed for sex ratio and body length. All litters were fostered to untreated undisturbed dams that had recently given birth. At weaning offspring were usually group housed in large cages, such that a representative animal from each litter was contained in each group. More detailed specific methods are presented in Chapters 4, 5, 6, 7 and 8.

DATA PRESENTATION AND STATISTICAL ANALYSES

Parametric statistical techniques were applied to data that met criteria for their use - derived from a random sample, approximating a normal distribution, equal variance within groups, interval or ratio scales. Parametric tests used were one-way analysis of variance (1WANOVA) and t-tests. Non-parametric statistical techniques were applied to data that did not meet all the above criteria: data treated by non-parametric techniques was usually either of a nominal or ordinal scale or not assumed to be normally distributed. Non-parametric tests used were Kurskal-Wallis analysis of variance (KWANOVA) and Mann-Whitney U test (MWU). Proportional data was analysed with use of Fisher's Exact Probability test. Means with standard errors were calculated for data approximating a normal distribution. Medians with 95% confidence limits were calculated for data assumed to be non-normally distributed. Usually, parametric procedures compare means whilst non-parametric procedures compare medians. Non-parametric techniques can be applied to most data without assumptions. Useful statistical references were Siegel (1956), Campbell (1979), Parker (1976) and Murdock and Barnes (1970).

The following abbreviations have been used in graphical presentation of results: undisturbed control (CON), chronically

crowded with aggressive males (CROWDING), saline-gelatine vehicle injected (GEL VEHICLE, GEL), low dose ACTH injected (1 i.u. ACTH, LACTH), high dose ACTH injected (8 i.u. ACTH, HACTH), propylene glycol vehicle minipump implanted (PROP. GLY, VEH), chronic corticosterone minipump implanted (CH.CORT), olive oil vehicle injected (OL.OIL), low dose progesterone injected (LPROG), high dose progesterone injected (HPROG), peanut oil vehicle injected (PE.OIL), acute corticosterone-injected (AC.CORT), Sham adrenalectomy individually housed controls (SHAM.CON), Sham adrenalectomy chronically crowded (SHAM.CROWD), adrenalectomised individually housed controls (ADX. CON), adrenalectomised chronically crowded (ADX . CROWD).

MATERIALS

ACTH (procine, 65 i.u. mg^{-1} obtained in 1000 i.u. vials)
Androstenedione (Δ^4-androstene-3,17-dione) corticosterone (Δ^4-pregnen-11 ,21-diol-3,20-dione) progesterone (Δ^4-pregnene-3,20-dione) gelatine (swine skin, 175 bloom) and peanut oil were all supplied by Sigma.

Sodium chloride (analar, for saline) olive oil, propylene glycol (Propane-1,2-diol) diethyl ether, and Hibitane (Chlorohexidine gluconate solution) were all supplied by BDH.

A Whites torsion balance (range 0.01-5.00 mg) was used to measure quantities of steroid hormone.

1 ml syringes and needles (25G x $\frac{5}{8}$) were supplied by Becton-Dickinson and Gillette surgical.

Osmotic minipump drug delivery system model 2001 were supplied by Alzet.

Betadine (iodine antiseptic) was obtained from Napp laboratories.

Cicatrine (antibiotic) was obtained from Wellcome laboratories.

Fur clippers were obtained from Clukes.

Swann-Morton scalpel and blades (size 3 and 4) springbow straight scissors, dissecting scissors, small curved forceps, treves rat tooth forceps, Michel 5" inserting forceps, 7" clip forceps (Childe) and 8 mm wound clips were all supplied by Arnold R. Horwell.

Sutures (20 mm round body, half circle needle with sterile mono-filament fibre) were supplied by St. Thomas' Hospital.

CHAPTER 4

EFFECTS UPON MATERNAL FOOD INTAKE AND POSTPARTAL BODY WEIGHT, THE LENGTH OF PREGNANCY AND PLASMA CORTICOSTERONE CONCENTRATIONS

INTRODUCTION

The central hypothesis of this thesis was that adrenal steroids of maternal origin, driven by ACTH, cause the developmental changes in the offspring of rodents stressed during pregnancy. However, this "hormonal hypothesis" is not the only possibility, and other causal mechanisms may be involved in producing the described developmental changes in the offspring from stressed rodents. Two alternative hypotheses have been identified here as possible factors influencing the development of offspring from mice crowded during pregnancy: stress may inhibit feeding (or indeed in the case of crowding, the physical presence of many animals may interrupt feeding thereby reducing food intake) or may induce premature birth of offspring. The effects of stress during pregnancy upon offspring body development (lowered birth weight is a well reported phenomenon, see Chapter 1) may be due to undernutrition or premature birth, rather than a maternal endocrine change. Additionally, it has been suggested that some laboratory stressing procedures e.g. electric shock or temperature extremes, may be directly injurious to the foetus, irrespective of any maternal physiological response (Chapman and Stern, 1978). Clearly, these alternative mediating factors must be examined to assess the limitations of the results of this and related studies. The hypotheses of mediation of the effects of stressing procedures administered during pregnancy will now be reviewed.

Ward (1972) and Dahlöf, Hard and Larsson (1977) suggested that

the abnormalities in sexual behaviour and atrophy of adrenal and testis evident in male offspring from rats restrained or crowded during pregnancy, are a result of exposure of the offspring to maternal adrenocortical products during the perinatal period. As neither study employed fostering techniques, the prenatal period could not be exclusively identified as the period of risk. The hormone exposure hypothesis is supported by results from studies of the endocrine response of rodents to restraint and crowding. Restraint has been shown to increase plasma corticosterone concentrations in the pregnant rat (Barlow, McElhatton, Morrison and Sullivan, 1974) and crowding increases 11ß-hydroxylation in adrenals *in vitro* (a measure of corticosteroid secretion) in non-pregnant rats (McCarthy, Green and Sohal, 1976). There is limited evidence to date of the effects of crowding upon the endocrine system of pregnant rodents. Despite the effects of such stressors on pituitary-adrenocortical activity, the reported consequences of such procedures applied during pregnancy upon the offspring, may not be mediated by an endocrine mechanism. Crowding and restraint procedures may physically disrupt patterns of feeding. In a recent study of growing male rats, crowding depressed food and water consumption and weight gain, but whether this was due to physical inhibition of feeding or to endocrine or psychological influences on feeding is unclear (Armario, Ortiz and Balasch, 1984). In fact, hormones are known to influence feeding and body weight, and as such the effects of stress during pregnancy may be due to hormonal alteration of maternal food intake, rather than the action of hormones on the foetus. In rodents, oestrogens decrease both food intake and body weight (e.g. Kuchar, Mozes, Boda and Koppel, 1982; Donohoe and Stevens, 1981; Sandberg, David and Stewart, 1982; Czaja, 1983) whilst androgens exert the reverse effect (Nunez and Grundman, 1982;

Kuchar, Mozes, Boda and Koppel, 1982; Slusser and Wade, 1981).

The reported consequences of undernutrition during pregnancy upon the offspring are strikingly similar to those resulting from stress. Barry (1920) severely undernourished pregnant rats and reported still births, reduced birth weights and underdevelopment of adrenals, testis, thyroids and thymi as the most important of the consequences. Barry reported that the foetal brain was spared the harmful reduction of growth and development observed in other organs following maternal undernutrition, and this finding is now known to be due to the foetal brain receiving a more favourable distribution of nutrients compared with other organs (Zamenhof and van Marthens, 1982). Rhees and Fleming (1981) however, report impaired sexual responses in the male offspring from rats undernourished during pregnancy, and this may indicate an effect on the brain, or alternatively represent a further expression of the effects of undernutrition on foetal testis development. The loss of certain dietary constituents seem to be responsible for causing the effects of undernutrition during pregnancy upon the offspring. Notably, protein is important for normal foetal development: protein restriction during pregnancy results in loss of maternal body weight, reduction of placental and foetal growth and increased offspring mortality rates during postnatal life (Beck, Dollet, Max and Debry, 1982; Knoll-Kohler, Klan, Wehner and Handke, 1982) whereas restriction of fats during pregnancy does not influence the course of pregnancy or foetal development (Dresser, Russell and Ludwick, 1982).

The strategy employed in this study to examine whether the effects of crowding or pituitary-adrenocortical manipulation during pregnancy may influence offspring development by exerting an effect

on the nutritional status of the mother, was simply to investigate food intake in pregnant mice during the period of treatment. Quantity of food consumed by pregnant mice daily during the final third of pregnancy was recorded. After giving birth to litters, maternal body weight was recorded, both because this gives a further indication of maternal nutritional status and because maternal weight gain during pregnancy in itself is important for offspring development, at least in humans (Tavris and Read, 1982). A substantial reduction in food intake by pregnant mice following any treatment during pregnancy, would suggest that undernutrition may be involved in producing the effects of such treatment upon offspring development. It is interesting to note that undernutrition is itself stressful, resulting in pituitary-adrenocortical hyperfunction and thyroid hypofunction in a variety of species (Adlard and Smart, 1972; Smart and Adlard, 1974; Tveit and Almlid, 1980; Oei, Sample, Taylor, Nordschow, Lemons, Jansen and Schreiner, 1982; Hugues, Reinberg, Jordan, Sebaoun, Modigliani and Burger, 1982) and the implication of this in interpreting results from studies of the consequences of undernutrition during pregnancy upon offspring development is clear.

In addition to the possibility of stressing procedures causing maternal undernutrition, they may also alter the length of pregnancy. Offspring that are born prematurely can be expected to be generally underdeveloped in comparison to offspring born at full term. Other than a mechanical component associated with foetal size, parturition is under hormonal control (Findlay, 1975) and as such, conditions which alter the maternal hormonal profile during pregnancy may well influence pregnancy length and the birth process. Progesterone, derived largely from the corpora lutea in species with short

pregnancies, and also from the placenta, maintains pregnancy in most mammalian species by preventing myometrial contractions and expulsion of uterine contents (Heap and Flint, 1979). Falling progesterone levels allow the onset of parturition, although the action of many hormones are required for the completion of the birth process and these will not be reviewed in detail here. Most relevant to this study is the involvement of pituitary-adrenal hormones in controlling parturition. Adrenal oestrogens and glucocorticoids, derived largely from the foetus, are instrumental in triggering parturition (e.g. Liggins, 1979). These compounds increase maternal circulatory levels of prostaglandin F2 alpha (PGF2α). This in turn induces myometrial contractions, ripening and dilation of the cervix (e.g. Challis, 1979) and stimulates secretion of relaxin (Gordon and Sherwood, 1983). Relaxin is secreted from the ovary and causes relaxation of ligaments of the pubic symphysis to allow passage of foetuses through the birth canal. LH may also stimulate relaxin secretion (Gordon and Sherwood, 1982) and as there is evidence to suggest that ACTH and glucocorticoids inhibit LH secretion (Barb, Kraeling, Rampacek, Fonda and Kiser, 1982) so stress or pituitary-adrenocortical manipulation can be theoretically seen to both advance the initiation of parturition (c.f. Liggins) and yet to block completion of normal birth. In order to establish whether crowding or other endocrine manipulations during pregnancy altered the timing of births, pregnancy lengths were recorded. If abortions and still births can be considered to reflect abnormalities of pregnancy or the birth process, this additional interpretation can be placed on the results presented in Chapter 5.

Finally, if crowding during pregnancy mediates its effects

upon offspring development via maternal pituitary-adrenocortical hyperfunction, this should be reflected in maternal plasma corticosterone concentrations. Since ACTH does not normally cross the placenta (see Chapter 2) and is likely to mediate its teratogenic effects via adrenocortical secretions, the effectiveness of the dosages of ACTH used here, in increasing maternal plasma corticosterone concentrations, was examined. Similarly, in order to choose effective doses of corticosterone to administer in the minipump experiments, pilot studies examining maternal plasma corticosterone concentrations following hourly injections of varying doses of this hormone were performed, and are reported here, together with actual concentrations of corticosterone in maternal circulation during the chronic administration regime.

METHODS

Animal husbandry and all treatments during pregnancy followed the procedures outlined in Chapter 3. The effects of crowding and endocrine manipulation upon food intake was studied as follows. In the crowding condition from day 12 of pregnancy onward, a weighed quantity of food pellets were placed into food hoppers. Twenty four hours later (at 1100-1200 hours) the remaining food was weighed and amount eaten in 24 hrs was calculated. Food pellets were weighed on a Mettler PL1200 electronic balance. This procedure was repeated until day 17 of pregnancy when females were removed. The quantities of food eaten relate to a group of 30 animals, with a small proportion of this group being pregnant females. Upon removal of females, continued measurement of food intake in the remaining group of males was made for a further 48 hrs, and mean daily food intake of the group of males calculated. This value of

food intake (crowded males) was subtracted from values of food intake previously obtained (crowded males + females) to give estimated food consumption by the pregnant females. The overall mean quantity of food eaten/animal/day was calculated for pregnant mice in each crowding cage - females in same cage had same value of food consumed and the unit of variance was number of cages.

Food intake in other treatment groups could be more accurately measured, because pregnant females were individually housed. A weighed quantity of food pellets were placed in food hoppers. Remaining food was weighed every 24 hrs and quantity of food eaten per day was calculated. The mean quantity of food eaten/animal/day (from days 12-17 of pregnancy) formed the basis of statistical analysis. Daily food intake was found not to vary between days 12-17 of pregnancy. As an additional measure of maternal nutritional status, maternal postpartal body weight, was recorded. Within 13 hrs of birth of a litter, females were sacrificed and weighed.

Maternal plasma corticosterone concentrations were measured, following crowding, ACTH or corticosterone administration. Pregnant females were anaesthetised with diethyl ether and approximately 300 µl of blood was collected, from the retro-orbital sinus as used by Nichols (1980). The sample was heparinised, centrifuged to separate cellular components from plasma and stored at -20^{o}C. Plasma corticosterone concentrations were determined by radioimmuno assay which has been fully described elsewhere (Nichols, 1980; Nichols and Chevins, 1981a, b, c). Mr. M. Bentley did the radioimmunoassays reported in this chapter. Results from the corticosterone assay are presented in nanogrammes per millilitre (ng/ml).

The following procedure was observed when blood sampling. Females to be blood sampled were undisturbed for 1-4 hours prior to

blood sampling. Blood sampling was performed in adjacent rooms to where animals were housed. The animal room was entered no more than 3 times in a sampling session and no female was sampled more than once in 24 hours.

In study of the adrenocortical response to crowding blood was taken at 10.00 hours on the day of crowding (day 12 of pregnancy) and subsequently at 11.00 hours, 14.00 hours and 18.00 hours. Further blood samples were taken at 10.00 hours on day 13 and 14. Data from a separate experiment in which blood samples were taken at 17.00 hours on day 15 of pregnancy are also included in results. In the studies of circulatory corticosterone concentrations following injections of ACTH (1 i.u. or 8 i.u. in 0.4 ml saline-gelatine vehicle) or corticosterone (10 µg or 100 µg in 0.1 ml propylene glycol) blood was taken on day 15 of pregnancy, 4 hours after a single injection of ACTH (at 19.00 hours) and 1 hour after the last of 3 hourly injections of corticosterone (at 19.00 hours). In a study of circulatory corticosterone concentrations following implantation of minipumps delivering corticosterone (10 µg/hr) or propylene glycol vehicle only (10 µl/hr) blood was taken on day 15 of pregnancy at 19.00 hours.

Pregnancy length was determined as the number of days between the appearance of a vaginal plug, and the birth of a litter. The appearance of a vaginal plug was deemed to indicate day 0 of pregnancy. Birth of litters was recorded with an accuracy of 0.5 days. As pregnant females were assigned at random to treatment groups, a random distribution of early and late conceptions can be assumed.

EXPERIMENT 4:1. The effects of chronic crowding during pregnancy upon maternal food intake and postpartal body weight. Two crowding cages were used in the study of food intake and each of these contained 3 or 4 pregnant mice.

RESULTS. The effects of chronic crowding during late pregnancy upon maternal food intake and postpartal body weight are shown in table 4:1. Chronic crowding did not decrease food intake. No significant effect of chronic crowding on maternal postpartal body weight was detected.

Table 4:1 The effects of chronic crowding during the final third of pregnancy upon maternal food intake and postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Daily food intake (g)	Postpartal body weight (g)
Undisturbed controls	11.88 ± 0.64 (6)	39.38 ± 0.90 (14)
Chronically crowded	12.39 ± 2.76 (7)	38.63 ± 0.76 (16)
	t-test	N.S.

( ) indicate number of animals. In the crowding condition the overall mean quantity of food consumed by the males was 8.09 g.

EXPERIMENT 4:2. The effects of ACTH administration during pregnancy upon maternal food intake and postpartal body weight.

RESULTS. The effects of ACTH administration during late pregnancy upon maternal food intake and postpartal body weight are shown in table 4:2. No significant effects of ACTH administration upon maternal food intake or postpartal body weight were detected.

Table 4:2 The effects of ACTH administration during the final third of pregnancy upon maternal food intake and postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Daily food intake (g)	Postpartal body weight (g)
Undisturbed controls	11.32 ± 0.57 (9)	40.28 ± 0.87 (12)
Saline-gel vehicle	10.79 ± 0.14 (9)	39.68 ± 0.45 (11)
Low dose ACTH	10.49 ± 0.52 (9)	37.80 ± 0.69 (11)
High dose ACTH	10.32 ± 0.38 (11)	39.35 ± 0.70 (12)
KWANOVA	N.S.	lWANOVA N.S.

( ) indicate number of animals

EXPERIMENT 4:3. The effects of chronic corticosterone administration during pregnancy upon postpartal body weight.

RESULTS. The effects of chronic corticosterone administration during late pregnancy upon maternal postpartal body weight are shown in table 4:3. No significant effects of chronic corticosterone administration upon maternal postpartal body weight were detected.

Table 4:3 The effects of chronic corticosterone administration during the final third of pregnancy upon maternal postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Postpartal body weight (g)
Undisturbed controls	38.39 ± 1.01 (9)
Propylene glycol vehicle	37.34 ± 0.90 (10)
Chronic corticosterone	36.86 ± 0.62 (11)
1WANOVA	N.S.

( ) indicate number of animals

EXPERIMENT 4:4. The effects of progesterone administration during pregnancy upon maternal food intake and postpartal body weight.

RESULTS. The effects of acute progesterone administration during late pregnancy upon maternal food intake and postpartal body weight are shown in table 4:4. No significant effects of progesterone administration upon maternal food intake or postpartal body weight were detected.

Table 4:4 The effects of acute progesterone administration during the final third of pregnancy upon maternal food intake and postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Daily food intake (g)	Postpartal body weight (g)
Undisturbed controls	10.57 ± 0.48 (7)	37.75 ± 1.04 (13)
Olive oil vehicle	10.34 ± 0.49 (7)	37.89 ± 0.90 (12)
Low dose progesterone	11.55 ± 0.60 (7)	39.20 ± 0.83 (11)
High dose progesterone	11.79 ± 0.44 (8)	37.48 ± 1.12 (16)
KWANOVA	N.S.	lWANOVA N.S.

( ) indicate number of animals

EXPERIMENT 4:5. The effects of androstenedione or corticosterone administration during pregnancy upon maternal food intake and postpartal body weight.

RESULTS. The effects of acute corticosterone or androstenedione administration during late pregnancy upon maternal food intake and postpartal body weight are shown in table 4:5. No significant effects of either treatment upon maternal food intake or postpartal body weight were detected.

Table 4:5 The effects of acute corticosterone or acute androstenedione administration during the final third of pregnancy upon maternal food intake and postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Daily food intake (g)	Postpartal body weight (g)
Undisturbed control	11.81 ± 0.39 (8)	41.14 ± 0.63 (8)
Peanut oil vehicle	11.15 ± 0.70 (8)	41.02 ± 1.23 (7)
Androstenedione	10.70 ± 0.18 (7)	39.91 ± 1.30 (6)
Corticosterone	11.36 ± 0.61 (8)	40.24 ± 0.78 (8)
KWANOVA	N.S.	lWANOVA N.S.

( ) indicate number of animals

EXPERIMENT 4:6. The effects of adrenalectomy during pregnancy upon maternal food intake and postpartal body weight.

RESULTS. The effects of adrenalectomy during pregnancy upon maternal food intake, and the effects of adrenalectomy and crowding during pregnancy upon maternal postpartal body weight are shown in table 4:6. No significant effects of adrenalectomy upon maternal food intake or postpartal body weight were found.

Table 4:6 The effects of adrenalectomy and crowding during the final third of pregnancy upon maternal food intake and postpartal body weight. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Daily food intake (g)	Postpartal body weight (g)
Sham-surgery control	11.06 ± 0.59 (6)	37.77 ± 1.80 (5)
Sham-surgery crowded	——[1]	38.78 ± 1.34 (5)
Adrenalectomy controls	12.97 ± 0.96 (5)	42.32 ± 1.62 (5)
Adrenalectomy crowded	——[1]	38.79 ± 1.54 (4)
MWU	N.S.	1WANOVA N.S.

1 Food intake was not measured in these groups
( ) indicate number of animals

DISCUSSION

The purpose of these experiments was to examine whether crowding, the various hormone administration regimes or adrenalectomy influenced maternal food intake. Direct measurement of food intake revealed that no treatment severely depressed feeding. That food consumption was not severely depressed by any treatment is further suggested by no differences being found in maternal postpartal body weights. However, some results must be regarded with caution especially the adrenalectomy experiments because of low numbers of test animals. Data on food intake in crowded pregnant mice could only be obtained by calculation indirectly and was not statistically analysed. It is probable that this data is inaccurate, especially because of the problem of separating food consumed by pregnant females alone from that eaten by the males. It is noted that removing the pregnant females from crowding cages may well have changed eating patterns in the group of males. In a recent study of growing male rats, animals in high density housing conditions ate and drank less, and showed reduced weight gain compared with rats in a low density housing condition (Armario, Ortiz and Balasch, 1984). Even if in this study a reduction of food intake by pregnant crowded mice went undetected, a moderate (approximately 10%) depression of food intake following stress or endocrine manipulation is unlikely to affect foetal development, particularly as severe starvation procedures often have no detectable effects on aspects of offspring development and behaviour (personal communication from Dr. J.L. Smart, Dept. Child Health and Development, Manchester). In addition to no treatment having any detectable effect upon food intake, no treatment was found to significantly reduce maternal postpartal body weights. Although there was a

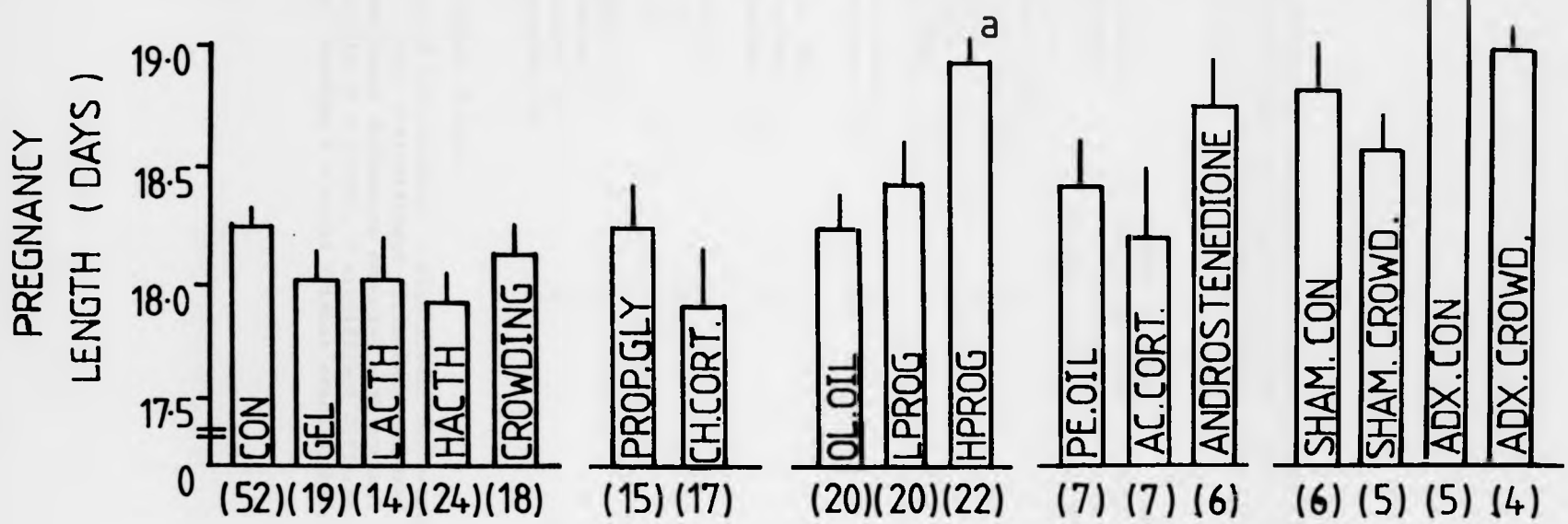
variability in maternal postpartal body weight between experiments, it should be noted that this is probably due to differences in initial starting weights between experiments, but that experimental and control animals are matched within experiments. No evidence has been found of undernutrition associated with stress or pituitary-adrenocortical manipulation during pregnancy, and as such attention can be returned to the endocrine hypothesis, or hypothesis of premature births reported in the following section, as causing the reported abnormalities in offspring from mice crowded or hormone treated during pregnancy.

STUDY OF THE LENGTH OF PREGNANCY. The effects of chronic crowding, hormone administration or adrenalectomy during pregnancy upon the length of pregnancy.

RESULTS. The effects of crowding, hormone administration and adrenalectomy upon the mean length of pregnancy are shown in Fig. 1. There were no differences in mean pregnancy lengths between crowded and control mice ($P > 0.1$ t-test). One way analysis of variance revealed that there were no significant differences between the following experimental groups: control, saline-gelatine vehicle, low dose ACTH treated and high dose ACTH treated mice ($P = 0.78$) control, propylene glycol vehicle and chronic corticosterone treated mice ($P = 0.12$) control, peanut oil vehicle, androstenedione treated or corticosterone treated mice ($P = 0.33$) and Sham-surgery control, Sham-surgery crowded, adrenalectomised control and adrenalectomised crowded mice ($P = 0.07$). However, one way analysis of variance revealed a significant difference in mean pregnancy lengths between the following experimental groups: control, olive oil vehicle, low dose progesterone treated and high dose progesterone treated mice ($P = 0.004$). Further analysis revealed that only the high dose progesterone treatment significantly delayed parturition (see Fig. 1).

The effects of crowding, hormone administration and adrenalectomy upon the day of parturition are shown in table 4:7. Proportional analysis revealed that more mice treated with the high dose of ACTH gave birth earlier than day 18 of pregnancy compared with control mice. However, as the same result was observed in the saline-gelatine vehicle group, ACTH has no more effect in inducing early parturition than the injection of vehicle. Proportional analysis also showed that more chronic corticosterone treated mice had pregnancy lengths shorter than 18 days, compared with controls.

FIG. 1 PITUITARY ADRENO-CORTICAL MANIPULATION (STRESS, HORMONE ADMINISTRATION AND ADRENALECTOMY) AND THE EFFECTS UPON THE COURSE OF PREGNANCY. MEANS ± S.E.M. ARE PRESENTED.



( ) = NUMBER OF FEMALES

a DIFFERS FROM CON AND OL.OIL TTESTS $P < 0{\cdot}0005$

Table 4:7 The effects of pituitary-adrenocortical manipulation (stress, hormone administration and adrenalectomy) during the final third of pregnancy, upon the day of parturition. Data presented are as numbers of mice

Treatment during late pregnancy	Number of mice with pregnancies of less than 18 days duration	Number of mice with pregnancies of 19 days or more duration
Undisturbed control (52)[a]	5	12
Saline-gelatine vehicle (19)	5[b]	2
Low dose ACTH (14)	3	2
High dose ACTH (24)	6[b]	1
Chronic crowding (18)	2	2
Propylene glycol vehicle (15)	2	5
Chronic corticosterone (17)	6[c]	4
Olive oil vehicle (20)	3	5
Low dose progesterone (20)	3	8
High dose progesterone (22)	0	15[e]
Peanut oil vehicle (7)	1	2
Androstenedione (6)	0	4[d]
Corticosterone (7)	1	2
Sham-surgery control (6)	0	3
Sham-surgery crowding (5)	0	1
Adrenalectomy control (5)	0	5[f]
Adrenalectomy crowding (4)	0	3

( )[a] Indicates number of mice.

b P = 0.06 compared with control, c significant difference compared with control P = 0.017, d significant difference compared with control P = 0.038, e significant difference compared with control (P = 0.0002) and olive oil vehicle (P = 0.005), f significant difference compared with Sham-surgery crowding P = 0.024 (Fisher exact probability)

Additionally, more high dose progesterone treated mice showed pregnancy lengths longer than 19 days, compared with both control and olive oil vehicle treated mice, and this effect was also found in androstenedione treated mice compared with controls, and adrenalectomised control mice compared with Sham-surgery crowded mice.

DISCUSSION

The purpose of these experiments was to examine whether crowding, the various hormone administration regimes or adrenalectomy influenced the timing of parturition. Measurement of the length of pregnancy, revealed that only the chronic corticosterone treatment was associated with early parturition, whilst treatment with the higher of two doses of progesterone delayed births. This result is consistent with the known effects of these hormones in controlling parturition: in the rat the process of parturition is initiated by changing ratios of corticosterone and progesterone (Martin, Cake, Hartmann and Cook, 1977). In conditions of crowding or after ACTH treatment, corticosterone secretion is elevated (see next section) but it is also probable that other adrenal steroids e.g. progesterone will be secreted, and this may account for the absence of any effect of these treatments upon the length of pregnancy. An additional important factor in explanation of why crowded animals did not show early birth is that undisturbed isolation is required in many species prior to birth of offspring (Findlay, 1975). The known action of adrenal glucocorticoids and oestrogens in triggering births (Liggins, 1979) may have been antagonised by environmental conditions. The finding that more saline-gelatine vehicle treated mice had shorter pregnancies than control mice could be a random effect: the stress of injection can be ignored as the cause of this result as none of the other vehicle groups showed early parturition. The low number of animals in the androstenedione and adrenalectomy groups, make comment on the validity of results difficult.

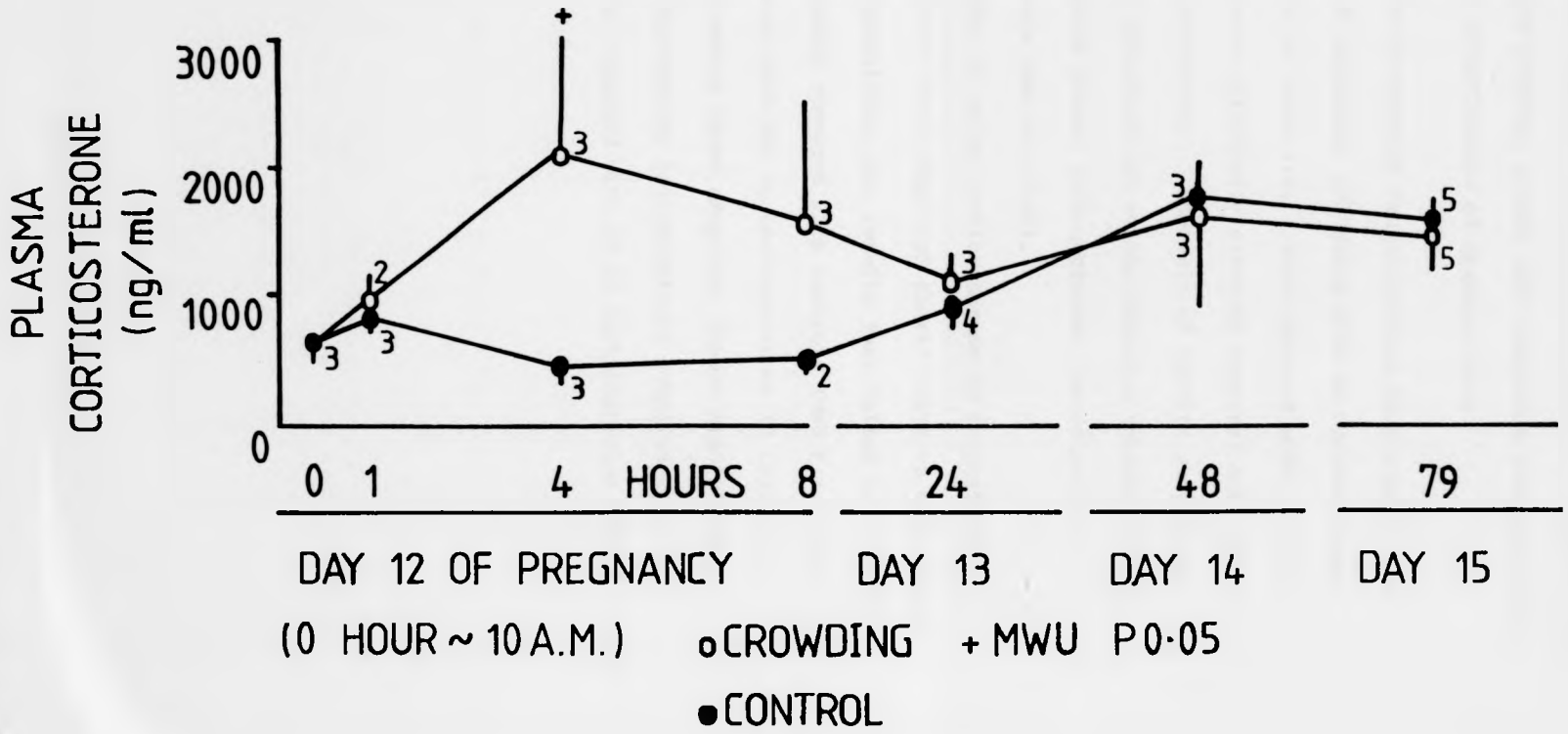
This study was designed to examine whether variation in length of time foetuses were retained in the uterus could be a factor mediating the effects of stress or hormone administration during

pregnancy upon offspring development. Premature birth of foetuses could produce underdevelopment and affect maturation of the brain, which occurs throughout the perinatal period in mice (Rodier, 1981). There is little evidence to suggest that premature births mediate the effects upon offspring development of crowding during pregnancy. The same applies for most of the endocrine manipulations during pregnancy with the exception of chronic corticosterone administration, where premature births may at least aggravate the effects of this compound upon offspring development.

EXPERIMENT 4:7. The effects of chronic crowding during pregnancy upon maternal plasma corticosterone concentrations: verification that crowding activates the stress response.

RESULTS. The effects of crowding during pregnancy upon maternal plasma corticosterone concentrations are shown in Fig. 2. Crowding increased plasma corticosterone concentrations. Corticosterone concentrations were elevated, throughout the first 24 hrs of crowding, and plasma corticosterone concentrations were significantly higher in crowded animals 4 hrs after crowding, compared with plasma corticosterone concentrations of individually housed, undisturbed mice (see Fig. 2). Crowding did not influence plasma corticosterone concentrations later in pregnancy. Plasma corticosterone concentrations increased in both crowding and control groups, as pregnancy progressed.

FIG. 2 THE EFFECT OF CHRONIC CROWDING WITH MALES DURING PREGNANCY UPON PLASMA CORTICOSTERONE. MEANS ± S.E.M. ARE PRESENTED.



EXPERIMENT 4:8. The effects of ACTH or corticosterone administration during pregnancy upon maternal plasma corticosterone concentrations: verification of the effectiveness of hormone doses.

RESULTS. Plasma corticosterone concentrations in pregnant mice (7 p.m. on day 15 of pregnancy) following ACTH or corticosterone injections are shown in table 4:8. Both doses of ACTH and both doses of corticosterone effectively elevated maternal mean plasma corticosterone concentrations. Analysis of results showed that handling, injection procedure and saline-gelatine vehicle did not significantly influence plasma corticosterone concentrations compared with controls (MWU $P = 0.46$).

Mice injected with 10 µg of corticosterone (3 injections at hourly intervals before blood sampling) showed elevated mean plasma corticosterone concentrations, but results just failed to achieve statistical significance compared with controls (MWU $P = 0.15$). However, mice injected with 100 µg corticosterone (3 injections at hourly intervals before blood sampling) showed significantly elevated plasma corticosterone concentrations compared with controls but not mice injected with 10 µg corticosterone (MWU $P < 0.24$).

Table 4:8 Plasma corticosterone concentrations in pregnant mice following ACTH or corticosterone injections. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Plasma corticosterone concentration (ng/ml)
Undisturbed control	1739 ± 366 (6)
Saline-gel vehicle injected	1451 ± 331 (5)
1 i.u. ACTH injected	6647 ± 1247 (4)**
8 i.u. ACTH injected	10143 ± 964 (5)**
10 µg corticosterone injected	4334 ± 1172 (6)
100 µg corticosterone injected	5713 ± 464 (6)*

( ) indicate number of animals
* significant difference compared with control mice $P < 0.001$ (MWU)
** significant difference compared with control and saline-gelatine vehicle injected mice, $P < 0.005 - P < 0.002$ (MWU)

EXPERIMENT 4:9. The effects upon maternal plasma corticosterone concentrations of implantation of osmotically driven minipumps delivering corticosterone solution: verification of the effectiveness of minipumps.

RESULTS. Plasma corticosterone concentrations in pregnant mice (7 p.m. on day 15 of pregnancy) following implantation of osmotically driven minipumps delivering a solution of corticosterone (10 µg/ul/hr) are shown in table 4:9. The minipump delivery system was effective. Mice implanted with minipumps delivering the corticosterone solution showed significantly elevated mean plasma corticosterone concentrations, compared with control mice and mice implanted with minipumps delivering vehicle only. Although mice implanted with minipumps delivering vehicle only showed elevated mean plasma corticosterone concentrations compared with control mice, differences were not significantly different (MWU $P > 0.10$). This result suggests that stress resulting from initial surgery or carrying the minipumps, is not long term or severe.

Table 4:9 Plasma corticosterone concentrations in pregnant mice following implantation of osmotically driven minipumps delivering a corticosterone solution. Data presented are as means ± S.E.M.

Treatment during late pregnancy	Plasma corticosterone concentration (ng/ml)
Undisturbed control	1322 ± 220 (7)
Surgery-propylene glycol vehicle control	1649 ± 147 (14)
Chronic corticosterone	3117 ± 417 (15)*
KWANOVA	P = 0.0004

( ) indicates number of animals
* significant difference compared with control ($P < 0.01$) and surgery-vehicle control ($P < 0.001$) mice (MWU)

DISCUSSION

The procedure used throughout this study to induce a stress response, chronic crowding with male mice, does result in elevated plasma corticosterone concentrations in pregnant mice. This result indicates that this stressor is effective and the finding that crowding does activate the pituitary-adrenal system is in agreement with previous studies (e.g. McCarthy, Green and Sohal, 1976). However, McCarthy, Green and Sohal (1976) report that in rats, crowding has a prolonged effect on adrenal steroid synthesis and secretion. In this study, plasma corticosterone concentrations in pregnant mice were only elevated transiently, during the first 24 hours of crowding. There are several possible explanations for this finding. The crowded female mice may habituate to the crowded conditions: conditioned suppression of the adrenocortical stress response following chronic stressor exposure, has been reported in rats (Burchfield, Woods and Elich, 1980). It is known that the pituitary-adrenocortical axis of pregnant rodents is less stress responsive (Hoet, Pagni, Ekka and Saba, 1965; Brain and Nowell, 1970). However, it is probable that stress peaks of corticosterone, in response to bouts of fighting or pursuit within the crowding cages, went undetected in the infrequent sampling regime. Corticosterone concentrations of non-stressed pregnant mice in this study, agree well with those obtained throughout pregnancy in previous studies on mice (Barlow, Morrison and Sullivan, 1974).

It was also demonstrated that both doses of ACTH effectively elevated maternal plasma corticosterone concentrations. Whilst the higher dose of corticosterone injected into mice effectively raised plasma corticosterone concentrations, the lower dose was

actually chosen for use in the minipumps. The higher dose of corticosterone was judged to be excessive in a chronic administration regime, and the lower dose is shown to be adequate in raising plasma corticosterone concentrations when released from minipumps.

Although crowding and the ACTH and corticosterone treatment regimes were found to effectively elevate maternal plasma corticosterone concentrations, which supports the working hypothesis of this thesis, no information could be obtained on the effects of these treatments on the circulatory levels of other hormones, and consequently, only limited use was made of the radioimmunoassay technique. Caution must be exercised in interpreting all the assay results as corticosterone recovery was relatively low (84% at best) compared with the original assay design (96% Nichols, 1980).

GENERAL DISCUSSION

The hypothesis that the reported effects of stress during pregnancy upon behavioural development in offspring are mediated by activation of the maternal pituitary-adrenal axis (Ward, 1972; Dahlöf, Hard and Larrson, 1977) has been adopted as the working hypothesis of this study. However, it has been noted that there are alternative hypotheses. Laboratory procedures designed to induce the stress response may affect offspring development by suppressing food intake, shortening pregnancy length or by directly acting on the foetus.

In this study, crowding with males was used as the stressing procedure. It has been shown that crowding does not detectably decrease food intake in pregnant mice or shorten the length of pregnancy. It has been shown that crowding, like other laboratory stressors (e.g. restraint in rats: Barlow, McElhatton, Morrison and Sullivan, 1974) does activate the maternal pituitary-adrenal system, although only transiently. The transient nature of the elevation of corticosterone in crowded, pregnant mice may be useful in identifying periods of risk to foetal development. Although no information was obtained to indicate levels of other adrenal steroids following crowding during pregnancy, corticosterone is the primary product of the stress response in the mouse (e.g. Nichols, 1980) and is therefore the prime candidate mediating the effects of stress during pregnancy upon offspring development. In the context of results from this chapter, the working hypothesis, of an endocrine mechanism mediating the effects of stress during pregnancy, remains valid. The rationale of experiments in this study has been explained in chapters 1 and 2, and it has also been shown that the endocrine manipulations used to test the working

hypothesis, do not severely affect maternal food intake or (with the exception of chronic corticosterone) shorten the length of pregnancy. It remains a possibility that the reported effects of stress during pregnancy upon offspring development are not solely due to *in utero* steroid exposure, but are aggravated by mild undernourishment and advancing of time of birth.

CHAPTER 5

EFFECTS UPON SOMATIC DEVELOPMENT AND MORTALITY RATES IN NEONATAL OFFSPRING

INTRODUCTION

Embryonic and foetal growth and somatic development are dependent upon many factors. Maternal nutritional status can influence foetal somatic development: severe undernutrition or malnutrition during pregnancy has been shown to reduce foetal body and organ weights in rats (Barry, 1920; Crosskerry, Smart and Charnock, 1981) both via inhibition of placental growth (Knoll-Kohler, Klan, Wehner and Handke, 1982) and directly by reduction of foetal nutrition. Successful implantation prior to placental development adequate placental function, and sufficient maternal body weight, are also necessary for normal intrauterine body growth and development (Howie, 1982; Tanner, 1978; van Assche and Robertson, 1983).

Normal foetal-placental growth is regulated by hormonal rather than nutritional or physical factors (Crosskerry and Dobbing, 1978). The hormonal mechanisms controlling prenatal growth are complex, and products from both maternal and foetal endocrine systems regulate this process. Maternal oestradiol is reported to limit maternal-foetal weight gain in guinea pigs (Czaja, 1983). Oestradiol is an identified inhibitor of placental growth (Csapo, Dray and Erdos, 1974; Miller, 1978). Administration of oestradiol to pregnant rodents also suppresses foetal thyroid output, insulin and PRL secretion (Kuhn and Bollen, 1981; Kuhn, Bollen and Darras, 1982) and these hormones are required for normal foetal growth and body development. Thus maternal oestrogens may naturally act to limit foetal growth, as may glucocorticoids, the action of which are

reviewed later.

The foetus secretes a variety of hormones that stimulate growth and somatic development. Amongst these are PRL and insulin, and foetal endocrine factors controlling somatic development will now be reviewed. Dwarfism in mice is associated with lack of PRL-containing cells in pituitary (Barkley, Bartke, Gross and Sinha, 1982). Insulin deficiency results in reduced foetal growth in many species (Jost, 1979). Insulin probably has a direct effect upon somatic development, via its action upon glucose metabolism, whereas PRL may influence body development indirectly. PRL stimulates testosterone secretion in rats (Baranao, Legnani, Chiauzzi, Bertini, Suescun, Calvo, Charreau and Calandra, 1981; Waeber, Reymond, Reymond and Lemarchand-Beraud, 1983) and in humans (Slonim, Glick, Island and Kasselberg, 1982) and testosterone is correlated with foetal body length at least in human males (Reyes, Boroditsky, Winter and Faiman, 1974). Additionally, growth hormone (GH) is a major stimulant of postnatal body growth (Kaplan, 1982) and it is probable that this polypeptide, released from the foetal pituitary, also controls intrauterine growth rate. Certainly, administration of GH to pregnant rodents increases offspring body and brain weight (Ganalska-Malinowska and Romer, 1981; Crosskerry and Smith, 1979). The relative contribution of both maternal and foetal secretions of PRL, insulin and GH to foetal growth has yet to be determined, and it is likely that complex interactions exist between many hormones contributing to somatic development of the foetus.

However, it is apparent that the maternal hormonal profile during pregnancy is important for normal foetal somatic development. Stressors administered during pregnancy have been shown to reduce offspring body weight in both rats and mice (Barlow, Knight and

Sullivan, 1978; Herrenkohl and Whitney, 1976; Werboff, Anderson and Haggett, 1968; Chevins, 1981). These results are caused presumably by induction of a maternal stress response, however the precise mechanism mediating these effects are unclear. As stress is associated with increased adrenocortical and decreased GH output (Terry, Willoughby, Brazeau and Martin, 1976; Brown and Martin, 1974; see Chapter 2) as well as influencing many other hormones, the reductions in body weight in offspring from rodents stressed during pregnancy may be due to altered secretion of any of these hormones. Further, there are several ways by which intrauterine development can be affected. The stressor may influence foetal somatic development by affecting litter size. There is an inverse relationship between litter size and foetal weight in rodents; surgical removal of foetuses increases the weight of remaining foetuses (Crosskerry and Dobbing, 1978) and litter sizes are reported to be larger from rats handled in pregnancy where the individual weight of offspring was decreased (Werboff, Anderson and Haggett, 1968). Maternal nutritional status and length of time foetuses are retained *in utero* are additional factors known to influence perinatal somatic development. Maternal endocrine status is particularly important for foetal somatic development, and evidence exists to suggest that the action of stressors during pregnancy upon offspring body development, may be mediated by activation of the maternal pituitary-adrenocortical axis. Administration of ACTH during pregnancy has been shown to limit foetal body weight gain in rodents (Velardo, 1957; Kittinger, Guittiemez-Cernosek, Cernosek and Pasley, 1980; Monder, Yasukawa and Christian, 1980). ACTH probably exerts its effects through maternal glucocorticoids, since administration of artificial or synthetic glucocorticoids during pregnancy also reduces offspring body weight

(Fraser and Fainstat, 1951; Gandelman and Rosenthal, 1981; Gandelman and Guerriero, 1982; Edward-Davis and Plotz, 1954). The effects of corticosterone administered during pregnancy upon offspring somatic development has not been studied, however neonatal corticosterone treatment does retard body development (Howard, 1965).

The effects of stress during pregnancy upon offspring somatic development are further assessed in this chapter. The effects of chronic crowding during pregnancy upon parameters of offspring somatic development and offspring mortality rates were studied. The hypothesis that the effects of chronic crowding during pregnancy, upon offspring somatic development, can be reproduced by exposure of the foetus to maternal adrenocortical products was tested. The effects of maternal pituitary-adrenocortical manipulation during pregnancy upon offspring somatic development was also examined. Body weight at birth (day 0) was used as the primary index of body development, although day 0 body length was also studied to assess *in utero* growth. Litter size and litter sex ratios were recorded as these are factors which may influence foetal somatic development, and also to assess mortality rates of either sex of foetus. The number of abortions, small litters, litters with prenatal deaths and litters with postnatal deaths was recorded and body weight at weaning (day 21) was also measured.

METHODS

Animal husbandry and treatments followed the procedure outlined in Chapter 3. At birth individual pups were weighed on an electronic balance (Mettler PL1200) to an accuracy of 0.01 g, and a mean pup body weight for each litter was calculated. At birth snout-rump lengths were measured with calipers (Camlab Scientific

Instruments) to an accuracy of 0.05 mm and a mean pup body length for each litter was calculated. The percentage of male pups within each litter and number of litters with stillbirths was recorded. Litters with less than 8 pups were classified as abnormally small. Other litters were culled to 8 pups and fostered as described in Chapter 3. On day 21 individual offspring were weighed and again a mean body weight for each litter was calculated. The number of litters with postnatal offspring deaths was recorded.

All results are thus based on the litter as the unit of variance to avoid spurious results due to litter effects (Abbey and Howard, 1973). Data from the various treatments were pooled wherever possible, for clarity of results, presentation. No significant differences in birth weights or letter mortalities were found in control mice in the various experiments. Methods of data presentation and analysis are explained in Chapter 3.

STUDY 5:1. The effects of chronic crowding during pregnancy upon parameters of litter development.

STUDY 5:2. The effects of chronic crowding during pregnancy upon mortality rates within litters.

RESULTS. The effects of chronic crowding from days 12-17 of pregnancy upon litter development, and foetal somatic development are shown in Table 5:1. Litters from crowded mice showed significant reductions in the numbers of pups, compared with litters from control mice. There was no difference between litters from crowded or control mice in the sex ratio of pups. The body length of offspring from litters of crowded mice was marginally reduced compared with offspring from litters of control mice, but differences did not achieve statistical significance. Offspring in litters from crowded mice showed significantly reduced birth weights compared with offspring in litters from control mice. This difference was not evident on postnatal day 21. Plate I shows the relative somatic underdevelopment of neonates from crowded mice, compared with offspring from control mice.

The effects of chronic crowding during pregnancy upon offspring mortality rates are shown in Table 5:2. There was a significant increase in the incidence of abortions, small litters and stillbirths and increased total mortality rate, associated with crowding. Litters from crowded mice also showed an increased incidence of postnatal deaths compared with litters from control mice, but results failed to achieve statistical significance. An overall analysis of maternal reproductive abnormalities and offspring mortality rates associated with crowding during pregnancy is shown in Fig. 3.

Table 5:1 The effects of chronic crowding during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no.)	Litter sex ratio (%males)	Day 0 body length (mm)	Day 0 body weight (g)	Day 21 body weight (g)
Undisturbed controls	11.27 ±0.21	54.43 ±2.36	31.71 ±0.31	1.58 ±0.01	11.13 ±0.17
N (litters)	(115)	(31)	(21)	(117)	(95)
Chronically crowded	9.62* ±0.60	53.18 ±2.95	30.96 ±0.21	1.45* ±0.03	11.31 ±1.11
N (litters)	(26)	(14)	(7)	(26)	(27)

*significant difference compared with control $P < 0.0005$ (t-test)

Table 5:2 The effects of chronic crowding during the final third of pregnancy upon prenatal and postnatal litter mortality rates. Data given are as numbers of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Undisturbed controls	121	107	4	3	7	10	0
Chronically crowded	35	20	6*	4*	3	7*	3*

*significant difference compared with control P = 0.017 - P = 0.004
(Fisher Exact Probability)

PLATE I The effects of chronic crowding or ACTH treatment during pregnancy upon body development. Female pups are shown. Pups were sacrificed at birth (day 0) by cranial rupture and had been frozen at -20°C. Scale bar is in centimetre divisions, A = control, B = saline-gelatine vehicle, C = low dose ACTH, D = high dose ACTH, E = chronic crowding

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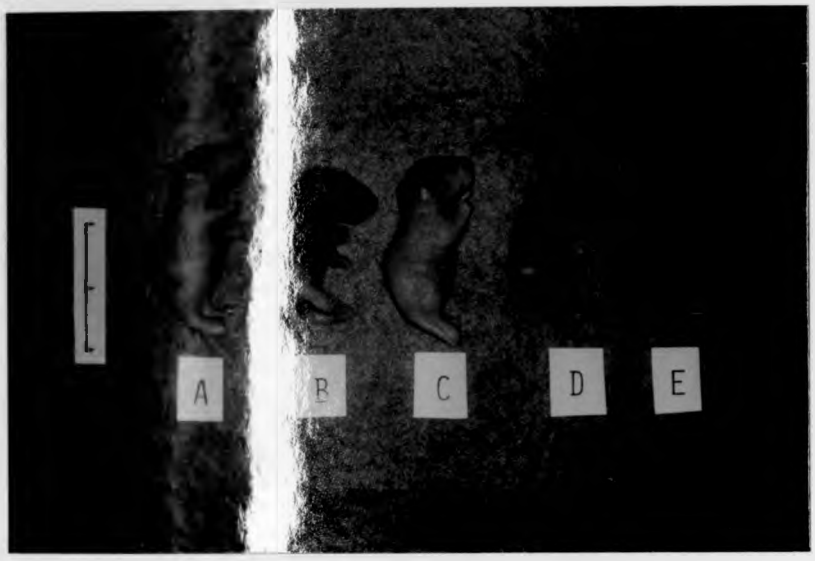

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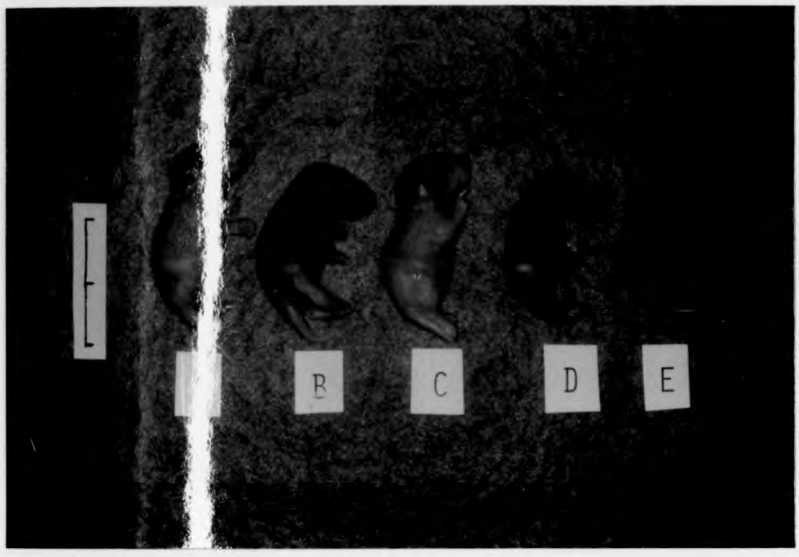

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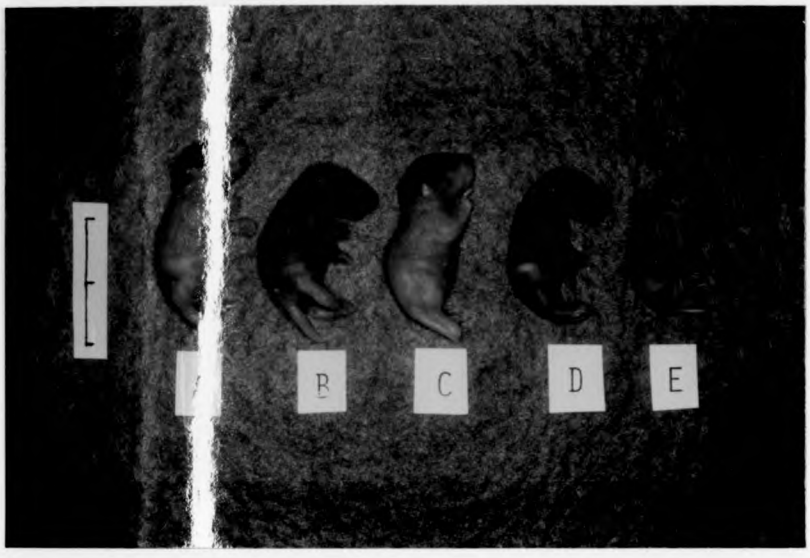
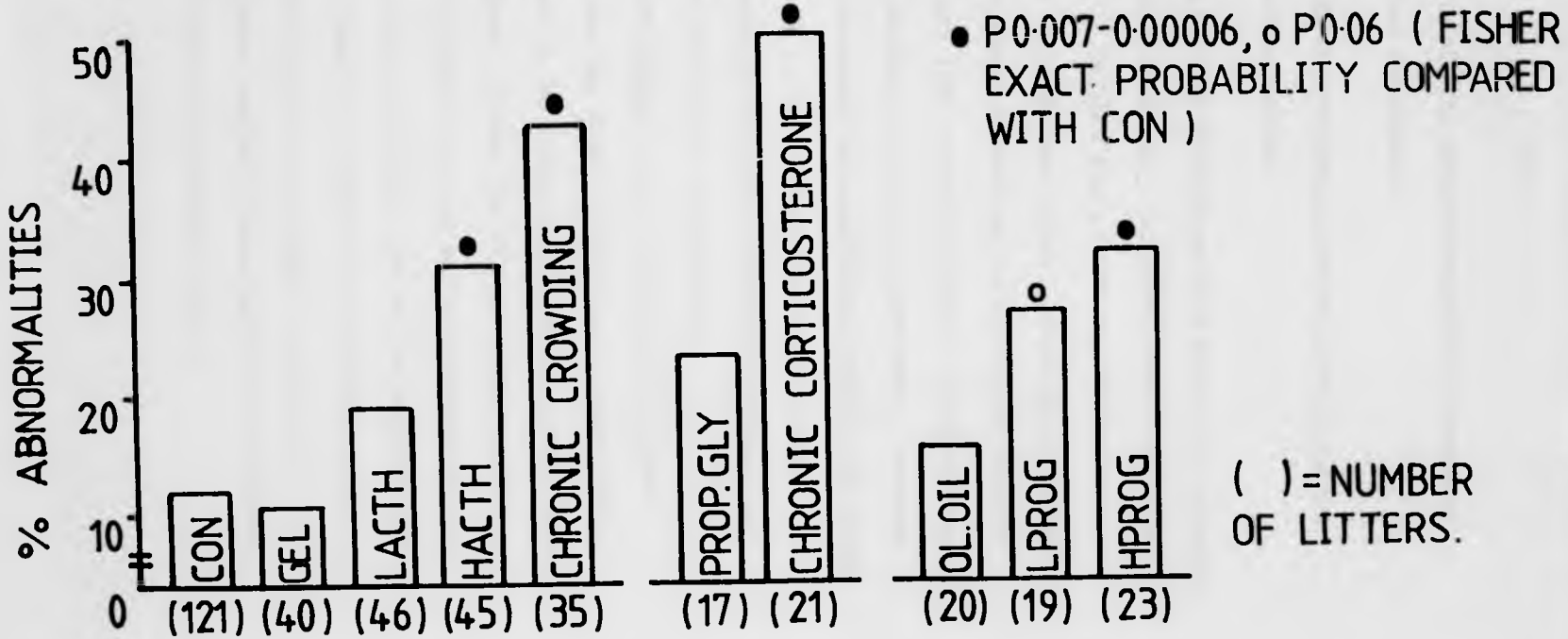


FIG. 3 TOTAL PERCENTAGE OF MATERNAL REPRODUCTIVE ABNORMALITIES (ABORTIONS AND SMALL LITTERS) AND LITTER MORTALITIES (PRENATAL AND POSTNATAL DEATHS) ASSOCIATED WITH CHRONIC STRESS AND CHRONIC AND ACUTE HORMONE ADMINISTRATION DURING LATE PREGNANCY..



STUDY 5:3. The effects of ACTH administration during pregnancy upon parameters of litter development.

STUDY 5:4. The effects of ACTH administration during pregnancy upon mortality rates within litters.

RESULTS. The effects of ACTH administered from days 12-17 inclusive of pregnancy, upon litter development and foetal somatic development are shown in Table 5:3. Neither dose of ACTH significantly influenced litter size or litter sex ratio. Offspring in litters from ACTH treated mice showed reduced day 0 body lengths compared with offspring in litters from control and vehicle injected mice, but results failed to achieve statistical significance. However ACTH treatment during pregnancy did significantly reduce body weight at both birth and day 21. Plate I shows the relative somatic underdevelopment of neonates from ACTH treated mice compared with offspring from control and vehicle injected mice.

The effects of ACTH administration during pregnancy upon offspring mortality rates are shown in Table 5:4. There were no significant differences in the incidence of abortions, small litters or stillbirths. However, litters from high dose ACTH treated mice showed significantly increased postnatal and total mortality rates compared with litters from both control and vehicle injected mice. The low dose ACTH treatment was ineffective in producing litter mortalities. An overall analysis of maternal reproductive abnormalities and offspring mortality rates associated with ACTH administration during pregnancy is shown in Fig. 3.

Table 5:3 The effects of ACTH administration during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no)	Litter sex ratio (% males)	Day 0 body length (mm)	Day 0 body weight (g)	Day 21 body weight (g)
Undisturbed Controls	11.27 ±0.21	54.43 ±2.36	31.71 ±0.31	1.58 ±0.01	11.13 ±0.17
N (litters)	(115)	(31)	(21)	(117)	(95)
Saline-gel vehicle	11.05 ±0.28	49.43 ±4.69	31.62 ±0.25	1.58 ±0.02	10.18 ±0.26
N (litters)	(40)	(13)	(6)	(39)	(32)
Low dose ACTH	10.76 ±0.33	54.82 ±5.06	31.22 ±0.66	1.52** ±0.02	9.31** ±0.37
N (litters)	(47)	(10)	(4)	(43)	(28)
High dose ACTH	11.23 ±0.39	54.88 ±2.54	30.48 ±0.27	1.44** ±0.02	9.77* ±0.32
N (litters)	(43)	(14)	(6)	(42)	(29)
lWANOVA	NS	NS	NS	P < 0.0001	P < 0.0001

*significant difference compared with control P < 0.001 (t-test)
**significant difference compared with control and saline-gel vehicle P < 0.05 - P < 0.0005 (t-test)

Table 5:4 The effects of ACTH administration during the final third of pregnancy upon prenatal and postnatal litter mortality rates. Data given are as numbers of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Undisturbed controls	121	107	4	3	7	10	0
Saline-gel vehicle	40	36	1	2	1	3	0
Low dose ACTH	46	37	3	2	4	6	0
High dose ACTH	45	31	1	4	7*	11*	2

*significant difference compared with control and saline-gel vehicle P = 0.027 - P = 0.004 (Fisher Exact Probability)

STUDY 5:5. The effects of chronic corticosterone administration during pregnancy upon parameters of litter development.

STUDY 5:6. The effects of chronic corticosterone administration during pregnancy upon mortality rates within litters.

RESULTS. The effects of chronic corticosterone administration from day 12 of pregnancy until parturition, upon litter development and foetal development are shown in Table 5:5. Litter size was not influenced by corticosterone administration. Litter sex ratio and day 0 body length were not studied in this experiment. Offspring from litters of corticosterone-treated mice showed reduced birth weight compared with offspring from litters of both control and propylene glycol vehicle treated mice. This difference was not evident on postnatal day 21: offspring from litters of both propylene glycol vehicle treated and corticosterone treated mice showed significantly increased mean day 21 body weights compared with control litters.

The effects of chronic corticosterone administration upon offspring mortality rates are shown in Table 5:6. There were no significant differences in the incidence of abortions or small litters from corticosterone treated mice compared with control or vehicle-treated mice. However litters from corticosterone treated mice showed significantly increased incidence of stillbirths and postnatal deaths compared with litters from both control and vehicle treated mice. An overall analysis of maternal reproductive abnormalities and offspring mortality rates associated with chronic corticosterone administration during pregnancy is shown in Fig. 3.

Table 5:5 The effects of chronic corticosterone administration during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no)	Day 0 body weight (g)	Day 21 body weight (g)
Undisturbed controls N (litters)	11.27 ±0.21 (115)	1.58 ±0.01 (117)	11.13 ±0.17 (95)
Propylene glycol vehicle N (litters)	10.44 ±0.41 (16)	1.61 ±0.03 (15)	12.37* ±0.44 (14)
Chronic corticosterone N (litters)	11.60 ±0.58 (20)	1.45** ±0.04 (19)	11.99* ±0.33 (17)
1WANOVA	NS	P < 0.0001	P < 0.009

*significant difference compared with control P < 0.05 (t-test)
**significant difference compared with control and propylene glycol vehicle P < 0.005 (t-test)

Table 5:6 The effects of chronic corticosterone administration during the final third of pregnancy upon prenatal and postnatal mortality rates. Data given are as numbers of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Undisturbed controls	121	107	4	3	7	10	0
Propylene glycol vehicle	17	13	2	1	1	2	0
Chronic corticosterone	21	10	1	5*	5*	10**	0

*significant difference compared with control P = 0.004 - P = 0.0005 (Fisher Exact Probability)
**significant difference compared with control and propylene glycol vehicle P = 0.046 - P = 0.0003 (Fisher Exact Probability)

STUDY 5:7. The effects of progesterone administration during pregnancy upon parameters of litter development.

STUDY 5:8. The effects of progesterone administration during pregnancy upon mortality rates within litters.

RESULTS. The effects of acute progesterone administered from days 12-17 inclusive of pregnancy upon litter development and foetal somatic development, are shown in Table 5:7. Neither dose of progesterone significantly influenced litter size, litter sex ratio, day 0 body length, birth weight or day 21 body weight.

The effects of progesterone administration during pregnancy upon offspring mortality rates are shown in Table 5:8. There were no significant differences in the incidence of abortions, small litters or postnatal deaths, in litters from mice treated with either dose of progesterone, compared with those from control or vehicle injected mice. Litters from low dose progesterone treated mice showed a significantly increased incidence of stillbirths, compared with litters from control mice, and litters from high dose progesterone treated mice showed a significantly increased incidence of stillbirths compared with those from both control and vehicle injected mice. An overall analysis of maternal reproductive abnormalities and offspring mortality rates associated with progesterone administration during pregnancy is shown in Fig. 3.

Table 5:7 The effects of acute progesterone administration during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no)	Litter sex ratio (% males)	Day 0 body length (mm)	Day 0 body weight (g)	Day 21 body weight (g)
Undisturbed controls	11.27 ±0.21	54.43 ±2.36	31.71 ±0.31	1.58 ±0.01	11.13 ±0.17
N (litters)	(115)	(31)	(21)	(117)	(95)
Olive oil vehicle	11.55 ±0.55	54.14 ±5.44	31.67 ±0.38	1.55 ±0.04	10.51 ±0.34
N (litters)	(20)	(7)	(7)	(18)	(13)
Low dose progesterone	12.16 ±0.58	59.71 ±4.91	31.15 ±0.35	1.59 ±0.04	10.51 ±0.38
N (litters)	(19)	(7)	(7)	(19)	(11)
High dose progesterone	11.45 ±0.40	52.42 ±2.58	31.57 ±0.26	1.63 ±0.03	11.21 ±0.64
N (litters)	(22)	(7)	(7)	(22)	(13)
1WANOVA	NS	NS	NS	NS	NS

Table 5:8 The effects of acute progesterone administration during the final third of pregnancy upon prenatal and postnatal mortality rates. Data given are as numbers of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Undisturbed controls	121	107	4	3	7	10	0
Olive oil vehicle	20	17	2	0	1	1	0
Low dose progesterone	19	14	0	3*	2	5*	0
High dose progesterone	23	15	0	5**	3	8**	0

*significant difference compared with control P = 0.05 - P = 0.028 (Fisher Exact Probability)
**significant difference compared with control and olive oil vehicle P = 0.03 - P = 0.002 (Fisher Exact Probability)

STUDY 5:9. The effects of androstenedione or corticosterone administration during pregnancy upon parameters of litter development.

STUDY 5:10. The effects of androstenedione or corticosterone administration during pregnancy upon mortality rates within litters.

RESULTS. The effects of acute androstenedione or acute corticosterone administered from days 12-17 inclusive of pregnancy upon litter development and foetal somatic development are shown in Table 5:9. Neither androstenedione nor corticosterone significantly influenced mean litter size, litter sex ratio, day 0 body length or birth weight. There were significant differences between treatments in mean day 21 body weights; offspring from litters of androstenedione treated mice showed significantly increased day 21 body weights compared with offspring from litters of vehicle-injected mice and offspring from litters of corticosterone treated mice showed significantly reduced day 21 body weights compared with offspring from litters of control mice. However, results are complicated by the finding that offspring from litters of peanut oil injected mice showed reduced day 21 body weights compared with offspring from litters of control mice.

The effects of androstenedione and corticosterone administration during pregnancy upon offspring mortality rates are shown in Table 5:10. There were no significant differences in the incidence of abortions, small litters, prenatal deaths or postnatal deaths in litters from androstenedione or corticosterone treated mice compared with litters from either control or vehicle injected mice. Litters from vehicle injected mice showed significantly increased total mortality rate compared with control litters.

Table 5:9 The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no)	Litter sex ratio (% males)	Day 0 body length (mm)	Day 0 body weight (g)	Day 21 body weight (g)
Undisturbed controls	11.27 ±0.21	54.43 ±2.36	31.71 ±0.31	1.58 ±0.01	11.13 ±0.17
N (litters)	(115)	(31)	(21)	(117)	(95)
Peanut oil vehicle	11.13 ±0.40	49.74 ±5.39	31.33 ±0.29	1.60 ±0.06	9.33* ±0.46
N (litters)	(8)	(8)	(8)	(8)	(7)
Androstenedione	11.50 ±0.76	52.30 ±7.66	31.79 ±0.50	1.64 ±0.05	11.23** ±0.46
N (litters)	(6)	(6)	(6)	(6)	(6)
Corticosterone	12.37 ±0.38	53.50 ±4.46	30.59 ±0.27	1.49 ±0.05	9.04* ±0.82
N (litters)	(8)	(8)	(6)	(8)	(8)
lWANOVA	NS	NS	NS	NS	P 0.001

*significant difference compared with control P < 0.005 (t-test)
**significant difference compared with peanut oil vehicle P < 0.025 (t-test)

Table 5:10 The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon prenatal and postnatal mortality rates. Data given are as number of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Undisturbed controls	121	107	4	3	7	10	0
Peanut oil vehicle	8	5	0	2	1	3*	0
Androstenedione	8	6	0	0	2	2	0
Corticosterone	6	5	0	1	0	1	0

*significant difference compared with control P = 0.03 (Fisher Exact Probability)

STUDY 5:11. The effects of adrenalectomy coupled with chronic crowding during pregnancy upon parameters of litter development.

STUDY 5:12. The effects of adrenalectomy coupled with chronic crowding during pregnancy upon mortality rates within litters.

RESULTS. The effects of adrenalectomy on day 9 of pregnancy with and without chronic crowding from days 12-17 of pregnancy upon litter development and foetal somatic development are shown in Table 5:11. Neither adrenalectomy nor crowding regimes significantly influenced litter size, litter sex ratio, day 0 body length or mean birth weight. There were significant differences between treatments in mean day 21 body weights; offspring from litters of adrenalectomised control mice showed significantly lower day 21 body weights compared with offspring from litters of Sham-surgery crowded mice.

The effects of adrenalectomy and chronic crowding during pregnancy upon offspring mortality rates are shown in Table 5:12. Neither adrenalectomy nor crowding regimes significantly influenced the incidence of abortions, small litters, prenatal deaths, postnatal deaths or the total mortality rate.

Table 5:11 The effects of adrenalectomy during the final third of pregnancy upon litter development. Data given are as means ± S.E.M.

Treatment during late pregnancy	Litter size (no)	Litter sex ratio (% males)	Day 0 body length (mm)	Day 0 body weight (g)	Day 21 body weight (g)
Sham surgery control	11.83 ±0.31	47.86 ±2.34	30.90 ±0.50	1.51 ±0.10	10.53 ±0.48
N (litters)	(6)	(6)	(6)	(6)	(4)
Sham surgery crowded	12.80 ±0.58	60.30 ±6.11	30.21 ±0.25	1.42 ±0.06	11.20 ±0.50
N (litters)	(5)	(5)	(5)	(5)	(5)
Adrenalectomy control	11.60 ±0.68	52.00 ±3.40	29.96 ±0.37	1.49 ±0.06	8.89* ±0.75
N (litters)	(5)	(5)	(5)	(5)	(5)
Adrenalectomy crowded	12.25 ±0.85	48.48 ±5.34	30.56 ±0.20	1.44 ±0.04	10.89 ±0.62
N (litters)	(4)	(4)	(4)	(4)	(3)
1WANOVA	NS	NS	NS	NS	P = 0.06

*significant difference compared with Sham surgery crowded $P < 0.025$ (t-test)

Table 5:12 The effects of adrenalectomy during the final third of pregnancy upon prenatal and postnatal mortality rates. Data given are as numbers of litters

Treatment during late pregnancy	Total number of litters	Number of normal litters	Number of small litters	Number of litters with prenatal deaths	Number of litters with postnatal deaths	Total mortality rate	Number of abortions
Sham surgery control	6	3	0	1	2	3	0
Sham surgery crowded	5	1	0	1	3	4	0
Adrenalectomy control	5	3	0	1	1	2	0
Adrenalectomy crowded	6	0	0	1	3	4	2

DISCUSSION

Crowding during pregnancy adversely influences somatic development of the offspring. Offspring in litters from crowded mice showed reduced birth weights compared with offspring in litters from individually housed control mice, and this finding agrees with the results of previous studies reporting somatic underdevelopment in offspring from rodents stressed during pregnancy (Barlow, Knight and Sullivan, 1978; Herrenkohl and Whitney, 1976; Werboff, Andersson and Haggett, 1968; Chevins, 1981) but contrasts those of Allen and Haggett (1977), who report that crowding during pregnancy does not influence any parameter of somatic development in mice. The complimentary study of body length at birth showed that this parameter of somatic development was not altered in offspring from crowded mice. Body length was measured to ascertain whether stress during pregnancy retards foetal growth, and although no evidence was obtained to affirm this, further study of this parameter of development is required because of the difficulty of accurately measuring body length. Brook (1983) has reported that intrauterine growth retardation results in proportional reductions of body growth and weight gain in postnatal life. In this study, no deficits in body weight were detected at day 21 in litters from crowded mice. This finding contrasts with the results of Christian and Lemunyan (1958) who reported that crowding mice during pregnancy reduced offspring body weight postnatally. However, Christian and Lemunyan (1958) showed that this was largely due to inhibited lactation; an effect of stress controlled for in this study by fostering all litters at birth.

Crowded mice gave birth to numerically smaller litters. This result

reflects an effect of crowding on either the ability to support a pregnancy, or the viability of foetuses, or both. Werboff, Anderson and Haggett (1968) handled mice during pregnancy and reported increased litter sizes and foetal viability as a consequence. The results of Werboff, Anderson and Haggett's (1968) study not only disagree with those reported here, but also with other studies of the effects of stress during pregnancy in rodents which show the opposite effect (Hockman, 1961; Euker and Riegle, 1973; Lane and Hyde, 1973; Morra, 1965). Further, Euker and Riegle (1973) found that the frequency of foetal deaths in litters from rats restrained from days 12-20 of pregnancy conforms to an "all or none" pattern; either the whole litter is lost (abortion) or the whole litter survives during intrauterine life. The consequences of crowding during pregnancy reported here agree with Euker and Riegle's (1973) results, to the extent that an increased incidence of abortions was detected. However, as evidence was also obtained that crowding during pregnancy reduces litter size, suggesting that partial litter loss does occur following stress during pregnancy, the results reported here extend from Euker and Riegle's (1973) reported "all or none" pattern of foetal mortalities. Failure of implantation cannot account for the reduction of litter size following crowding, because stress was induced only after the period of conceptus implantation. The finding that more crowded litters contained stillborn pups than control litters agrees with the observations of Christian and Lemunyan (1958) and suggests that partial litter loss is a consequence of stress during pregnancy and also that the individual foetus is vulnerable to selective mortality. As an approximate inverse relationship exists between the numerical size of a litter, and the body weight of foetuses, reduced litter size strengthens the significance of the birth weight data.

No evidence was obtained to suggest that crowding during pregnancy alters litter sex ratio or postnatal mortality rates. Altered litter sex ratio and increased postnatal mortality rates are previously reported consequences of stress during pregnancy: Lane and Hyde (1973) have shown that restraint during pregnancy in the rat reduces the proportion of male pups born, and Morra (1965) found decreased postnatal survival rats in offspring from rats avoidance conditioned and exposed to heat during pregnancy. Differences in results from these studies with those reported here can be explained by differences in experimental procedure and species used.

Both doses of ACTH administered to pregnant mice were found to reduce offspring birth weights and body weights on postnatal day 21. Although there were no differences between the ACTH treatment and control groups in litter size, incidence of abortions, small litters or stillbirths, the high dose of ACTH administered during pregnancy did increase offspring postnatal and total mortality rates. These results agree quite well with those from other studies which show that in various rodent species, ACTH administration during pregnancy inhibits implantation, disrupts pregnancy, increases foetal deaths, resorbtion rates and stillbirths, and reduces litter sizes and offspring birth weights (Robson and Sharaf, 1952; Yang, Yang and Lin, 1969; Velardo, 1957; Chatterjee and Harper, 1970; Kittinger, Guittiemez-Cernosek, Cernosek and Pasley, 1980). However, the results of ACTH administration during pregnancy upon offspring somatic development and mortality rates extend from these previous reports, and from the effects of crowding during pregnancy, in that the observed deleterious consequences of such treatment persist into postnatal life. The difference in the effects of crowding from the effects of ACTH administration during pregnancy can be explained by

the ACTH treatment regime only crudely mimicking the hormonal milieau of a stressed mouse. Crowding pregnant mice has already been shown to produce an unexpected pattern of adrenocortical activity (Chapter 4) and there probably exist many differences in the endocrine response of pregnant mice to the various experimental treatments, accounting for the variability of the developmental consequences of these treatments as reported in this, and other chapters. Despite this, the general effects of ACTH administration during pregnancy upon offspring somatic development and perinatal mortality rates do reproduce the general effects of crowding during pregnancy, upon these parameters of development in the offspring. This supports the central hypothesis of this thesis, suggesting that the effects of stress during pregnancy upon offspring somatic development are mediated by activation of the maternal pituitary-adrenocortical system.

Several lines of evidence suggest that the teratogenic effects of ACTH are not direct, but in turn mediated by adrenocortical products. ACTH does not cross the placenta as an intact molecule (Chapter 2). Velardo (1957) and Yang, Yang and Lin (1969) have shown that the harmful effects of ACTH administration during pregnancy, upon foetal somatic development can be prevented if the maternal adrenals are removed. Further, administration of synthetic or artificial glucocorticoids during pregnancy, has been shown to produce effects upon offspring somatic development and survival similar to those resulting from ACTH treatment (Fraser and Fainstat, 1951; Edward-Davis and Plotz, 1954; Robson and Sharaf, 1952; Gandelman and Rosenthal, 1981). Although these studies supply evidence that synthetic or artificial glucocorticoids have teratogenic effects, there has been no previous study in any species of the consequences for offspring somatic development and survival

of corticosterone administration during pregnancy.

In this study, corticosterone was administered to pregnant mice in one of two treatment regimes: chronically, by implantation of an osmotically driven drug delivery system and acutely by daily injections. Offspring in litters from mice treated chronically with corticosterone, like those from crowded or ACTH-treated mice, showed reduced birth weights. This treatment also increased both the incidence of stillbirths and postnatal deaths, a result extending from both the effects of crowding and ACTH administration during pregnancy upon offspring perinatal mortality rates. Chronic corticosterone administration during pregnancy did not affect litter size, or the incidence of small litters or abortions. Propylene glycol, used in the chronic corticosterone experiments as a vehicle because of its properties as a solvent, compatible with the minipumps, and its lack of teratogenic activity as listed in Shepard (1976) had some effect upon later postnatal development which cannot be explained. This action of propylene glycol was detected in other experiments (see Chapter 7). Acute corticosterone administration during pregnancy also affected offspring somatic development. Offspring in litters from mice treated acutely with corticosterone during pregnancy showed reduced body weights on postnatal day 21 compared with controls. However, no significant differences were detected in birth weights or perinatal mortality rates between corticosterone injected mice and controls, and this is attributed to the limited number of experimental animals used in this study. That chronic corticosterone (and to a limited extent acute corticosterone) administration during pregnancy, reproduced the effects of crowding or ACTH administration during pregnancy upon offspring somatic development and perinatal mortality rates, supports the working hypothesis.

Other endocrine manipulations, administered during pregnancy to test the central hypothesis, also had some effects upon offspring somatic development or survival, and these will now be discussed. Progesterone administration during pregnancy did not influence any parameter of somatic development in the offspring. This finding contrasts with the results of Coyle, Anker and Cragg (1976) and Herrenkohl (1974) who report reduced postnatal body weight in rats following prenatal and perinatal progesterone exposure respectively. Herrenkohl (1974) suggested that this result is partially due to inhibition of lactation and consequent undernutrition of offspring, as litters were raised by natural mothers. In the study of mortality rates, progesterone administration during pregnancy was found to increase the incidence of stillbirths and this may have been caused by post-maturity of foetuses since progesterone lengthened pregnancies (Chapter 4). A delay in parturition may also have masked any effects of progesterone administration during pregnancy in retarding foetal body development. On the basis of these results, progesterone cannot be considered as the adrenal product causing the underdevelopment of the body detected in offspring from mice crowded or treated with ACTH during pregnancy. However, progesterone may be the maternal adrenal product causing the increased perinatal mortality rates detected in offspring from crowded or ACTH-treated mice.

Androstenedione administration during pregnancy neither influenced parameters of somatic development, nor mortality rates in offspring. On the basis of these results, androstenedione would seem not to be the maternal adrenal product producing the effects of crowding during pregnancy upon offspring somatic development and survival. Only limited confidence can be placed in the results of the androstenedione

experiments reported here, because of the limited number of animals used, and it is possible that further study may reveal a teratogenic effect of this compound.

The central hypothesis does not exclude the possibility that the described syndrome evident in offspring from mice crowded or treated with ACTH during pregnancy, is caused by foetal exposure to several adrenal products. Oestrogens which can be secreted from the adrenal in conditions of stress, are known to inhibit foetal growth and development (Csapo, Dray and Erdos, 1974; Kuhn and Bollen, 1981; Kuhn, Bollen and Darras, 1982; Miller, 1978). It is therefore possible that oestrogens, which were not tested in this study, may be involved in the production of some of the harmful effects of crowding during pregnancy upon somatic development of the offspring.

The importance of the intact maternal adrenal gland for producing the effects of crowding during pregnancy upon offspring somatic development, was not clearly established. An experiment was conducted to investigate whether adrenalectomy could prevent the previously described effects of crowding during pregnancy. The results were not wholly consistent with this prediction of the general working hypothesis. There were no differences between experimental groups in offspring birth weights or mortality rates. Whilst this result supports the working hypothesis, in that no harmful effects of crowding were detected after adrenalectomy, it is also suspicious in that crowding following Sham surgery had no detectable influence upon offspring somatic development. One possible reason why the previously described deleterious effects of crowding during pregnancy, upon offspring somatic development and survival, were not detected in the adrenalectomy experiment, is that very few experimental animals were used in this study. A very high

mortality rate amongst adrenalectomised mice reduced the number of animals in experimental groups. This experiment was conducted late into this project and it was not possible to repeat this experiment to obtain additional data. The results of this study were further complicated by the finding that adrenalectomy during pregnancy adversely affected offspring development: offspring in litters from adrenalectomised individually housed mice showed reduced postnatal day 21 body weights compared with offspring in litters from Sham-operated crowded mice. This result agrees with previous reports of the deleterious effects of adrenalectomy during pregnancy upon general development of the offspring (Angervall, 1962; Thoman, Sproul, Seeler and Levine, 1970) and may have been caused by foetal adrenal hypertrophy and hypersecretion of foetal corticosterone. It remains debatable whether adrenalectomy during pregnancy generates any results useful in identifying teratogenic hormones, since this procedure alters the circulatory levels of ACTH, mineralocorticoids, glucocorticoids, progestagens, androgens and oestrogens. Metyrapone administration was not used as this compound does not inhibit the synthesis of all glucocorticoids.

Although there are limitations to this study, the results show that corticosterone, especially the chronic administration regime, most closely reproduces the general consequences of both crowding and ACTH-treatment during pregnancy, upon offspring body development and perinatal survival. It is now necessary to examine how crowding, administration of ACTH or corticosterone during pregnancy influences foetal somatic development. Corticosterone was found to result in premature birth of litters (Chapter 4) and as such some of the effects of this compound upon development may be due to this. Probably, however, corticosterone has a direct effect on the foetus,

over and above this phenomenon and these possible direct actions will now be discussed.

Corticosterone in circulation is normally bound to corticosteroid binding globulin (CBG). The concentration of plasma corticosterone occurring in response to stressful conditions under the influence of ACTH, or the dosage of ACTH or corticosterone administered in the experiments reported here, is likely to surpass the binding capacity of CBG, resulting in a substantial fraction of corticosterone in maternal circulation which is not bound to CBG, biochemically active and capable of crossing the placenta (see Chapter 2). In the normal mouse pregnancy, the foetus is protected from the actions of corticosterone (which have been reviewed in Chapter 2) by the placental conversion of a physiologically significant proportion of this potent compound to the less potent 11-dehydrocorticosterone (Michaud and Burton, 1977). The capacity of this system is unknown and could probably be surpassed in conditions of elevated corticosterone secretion. In humans, the placenta similarly converts cortisol to the less potent cortisone (Giannopoulos, Jackson and Tulchinsky, 1982).

The consequences of *in utero* corticosterone exposure upon somatic development as reported in this study, are similar to those produced when this compound is administered postnatally. Administration of corticosterone to neonatal rats is reported to reduce weight gain and retard body growth, largely through the metabolic activity of this compound (Howard, 1965). Glucocorticoids including corticosterone are also known to decrease muscle mass at least in rats (Seene and Viru, 1982). The catabolic action of glucocorticoids on body protein is not the only mechanism by which somatic development can be retarded following early life exposure

to these compounds. Klepac (1982) chronically administered dexamethasone to rats during the final third of pregnancy and found decreased nucleic acid concentrations and protein in placenta, foetal adrenals, testes, pituitary, brain, liver, kidney, heart and lung. The loss of nucleic acids and protein in placenta would limit the growth and function of this organ, which in turn would limit foetal nutrition, growth and development. Similarly, the loss of nucleic acids and protein in endocrine glands and other organs would also limit development throughout the animal's life span. Inhibited development and function of kidney, liver, heart and lung following prenatal glucocorticoid exposure, could well contribute to inducing the mortalities during perinatal life, shown in this study to occur with increased frequency in litters from mice crowded, or treated with ACTH or corticosterone during late pregnancy.

As well as early life glucocorticoid exposure reducing structural proteins in endocrine glands (c.f. Klepac, 1982) such treatment may also affect their later function. Somatic development is well recognised to be controlled by hormonal factors (e.g. Kaplan, 1982) therefore a change in an animals' endocrine development can be expected to change body development. Most relevant to this discussion are the interactions of corticosterone with other endocrine systems. Corticosterone is known to suppress secretion of thyrotropin (Pamenter and Hedge, 1980) insulin (Billaudel and Sutter, 1982; Jack and Milner, 1975) and PRL (Gala, Kothari and Haisenleder, 1981; Bratusch-Marrain, Vierhapper and Waldhausl, 1982). Exposure of the foetus to corticosterone may not only suppress the secretion of these hormones during prenatal life, but the secretion of these hormones during postnatal life may also be affected. Adequate secretions of insulin, thyroid hormones

(Jost, 1979) and PRL (Barkley, Bartke, Gross and Sinha, 1982; Sinha and van der Laan, 1982) are necessary for normal body growth and somatic development during perinatal life, presumably because of their direct actions, or in the case of PRL indirect actions via androgens, upon cellular metabolism. The relative contribution of the direct metabolic or indirect endocrinological mechanisms, to the retardation of somatic development evident in offspring from mice crowded, treated with ACTH or corticosterone during pregnancy has yet to be determined.

In conclusion, the harmful effects of stress during pregnancy upon somatic development and survival of the offspring has been previously reported (Chevins, 1981; Barlow, Knight and Sullivan, 1978; Herrenkohl and Whitney, 1976; Werboff, Anderson and Haggett, 1968; Hockman, 1961; Euker and Riegle, 1973; Lane and Hyde, 1973; Morra, 1965). However, results from studies investigating the effects of crowding during pregnancy upon offspring somatic development in the mouse have been inconsistent: whilst some studies report a general deleterious effect of crowding during pregnancy upon somatic development of the neonatal offspring (Chevins, 1981; Werboff, Anderson and Haggett, 1968) other studies have detected no adverse effect of such treatment upon the neonatal offspring (e.g. Allan and Haggett, 1977; Christian and Lemunyan, 1958). This study shows that crowding during pregnancy retards foetal somatic development and that this is not due to alteration of other parameters of litter development such as litter size or litter sex ratio. Also shown is that these results can be reproduced at least to some extent by manipulation of the maternal pituitary-adrenal system during pregnancy. Both administration of ACTH and corticosterone during pregnancy resulted in reduced birth weights and increased mortality rates in the offspring.

Corticosterone has therefore been identified as the teratogenic adrenal product most probably producing the effects of stress during pregnancy upon offspring somatic development. However, as progesterone administration during pregnancy increased offspring mortality rates, this steroid may also be involved in producing this phenomenon in offspring from stressed mothers. Of more general interest is that adrenal steroids have been shown to influence foetal growth and somatic development, and this is not well documented in reviews of factors which influence foetal body development (e.g. Tanner, 1978; van Assche and Robertson, 1983).

CHAPTER 6

EFFECTS OF ACTH AND CORTICOSTERONE ADMINISTRATION DURING PREGNANCY UPON REFLEX AND NEUROMUSCULAR DEVELOPMENT IN NEONATAL OFFSPRING

INTRODUCTION

The factors contributing to normal foetal body development outlined in Chapter 5, necessarily influence the development of constituent foetal organ systems. The development of the whole foetal organism is dependent upon adequate placental function, nutritional factors and a favourable maternal-foetal endocrine status; however individual organ systems are variably influenced by these factors and mature perinatally at differential rates. For example, undernutrition during pregnancy reduces foetal body weight and the weight of thymus, adrenals, testes and thyroid, whereas body length, ovaries and brain are unaffected in rats (Barry, 1920). This disparity of the consequences of foetal undernutrition upon the development of different organ systems may be resolved by the differences in critical periods of growth rates in organ ontogeny. Both rats and mice are altricial rodent species and maturation of the central nervous system is incomplete until late into postnatal life.

Hormones are also known to differentially influence organ development. Glucocorticoids in particular exert well recognised influences in regulating organ growth; these compounds accelerate maturation of the foetal lung and neural retina (Beato and Doenecke, 1980) but are reported to inhibit perinatal somatic development (see Chapter 5) detrimentally affect motor function, and suppress brain growth and central nervous system development (e.g. Weichsel,

1977; Devenport and Devenport, 1983a, b; Klepac, 1982). Manipulation of maternal and consequently foetal glucocorticoid profiles, may therefore be expected to affect foetal neurological development, particularly as these compounds are inextricably linked to general maturational processes.

The induction of a stress response during pregnancy and its consequences for offspring neurological development have been investigated: crowding mice (Chevins, 1981) and restraining rats (Barlow, Knight and Sullivan, 1978) during late pregnancy delays neurological development of neonatal and juvenile offspring. Chevins' study chronically crowded mice to a floor area of approximately 32.4 cm^2/mouse during the final third of pregnancy, and offspring reflex ontogeny (development of limb grasp and righting reflexes etc) was assessed. Pups from stressed females displayed retardation of reflex ontogeny compared with offspring from individually housed mice. This study employed fostering procedures and assessed pregnancy length, and results seem to be due to the experimental manipulation during pregnancy, rather than other confounding factors. Barlow, Knight and Sullivan (1978) neither assessed the course of pregnancy nor fostered litters, and their results could be due to prematurity of births, or postnatal influences upon development, such as poor mothering or inhibited lactation. However the causation of the retardation of neurological development has yet to be identified.

This study was undertaken to examine the hypothesis that the retardation of neurological development evident in offspring from rodents stressed during pregnancy, is mediated by foetal exposure to maternal pituitary-adrenocortical secretions. The potential neurological hazard of perinatal glucocorticoid administration has been recognised (Howard, 1965; Takahashi, Goto, Sudo and Suzuki,

1982; Dekosky, Nonneman and Scheff, 1982; Weichsel, 1977) stress-induced glucocorticoid secretions may therefore have similar actions. Conclusions regarding the consequences of perinatal glucocorticoid exposure are limited in most studies by the use of postnatal administration regimes, and large doses of potent synthetic compounds (Weischel, 1977). More recent studies have concerned impaired neurological development following prenatal exposure to prednisolone (Gandelman and Rosenthal, 1981; Gandelman and Guerriero, 1982) but to date there is no evidence to suggest that *in utero* exposure to elevated concentrations of natural glucocorticoids may be detrimental to neurological development.

This chapter describes the effects of manipulation of the maternal pituitary-adrenocortical system, during late pregnancy upon offspring neuromuscular and neurological development. The effects of ACTH or corticosterone administration during pregnancy, upon the development of reflexes in neonatal offspring and upon aspects of somatic development in juvenile offspring was studied. The hypothesis that the effects of restraint (Barlow, Morrison and Sullivan, 1978) or chronic crowding (Chevins, 1981) upon offspring neuromuscular and neurological development, can be reproduced by exposure of the foetus to maternal adrenocortical products was tested. The effects of corticosterone administration during pregnancy was studied in particular, because of the known harmful effects of glucocorticoids upon neurological development and somatic development as shown in Chapter 5. The righting reflex, forelimb and hindlimb grasp reflexes, negative geotaxis reflex and auditory startle response were studied as indices of neuromuscular and neurological development. The day of unfolding of ear pinnae and day of eye opening were also recorded.

METHODS

Animal husbandry and treatment of pregnant mice with hormones followed the procedure outlined in Chapter 2. Litters were culled to 8 pups and fostered, and reflex ontogeny studied from the day of birth (day 0). Some of the reflexes described by Fox (1965) were monitored. As different reflexes appear during different periods of development, the assessment of specific reflexes was made during different periods. Reflexes studied from days 0-8 postnatally were the body righting response, the forelimb grasp reflex and the negative geotaxis response. The hindlimb grasp reflex was assessed from day 2-8 postnatally, during which time the litter was observed for unfolding of ear pinnae. Assessment of the auditory startle response was made from day 12 of postnatal life. From postnatal day 12 the litter was also observed for evidence of eye opening.

PROCEDURE

On each day of testing, a random sample of 4 pups from each litter was assessed for development of reflexes. The whole litter (i.e. all 8 pups) were observed for ear unfolding and eye opening. The pups were briefly removed from the home cage and placed on an insulating layer of cotton wool prior to examination.

The righting reflex was examined by placing the neonate on its dorsal surface, and timing the period taken for the pup to turn to rest on its ventral surface. A cut off time of 10 seconds was used. This procedure was repeated 3 times. In the case of this reflex, the time taken to complete the response is inversely related to neuromuscular development. The value used for analysis is the total time for all pups tested in a litter (i.e. 4 pups x 3 trials).

The negative geotaxis response was assessed by placing the pup

on a 25° slope, with its head pointing down the gradient. The time was taken, up to a maximum of 20 seconds, for the pup to turn 180° by crawling, to face up the slope. As in the case of the righting reflex, the time taken to complete the response is inversely related to neuromuscular development.

The limb grasp reflexes were assessed by holding the pup by the loose skin around the neck, and placing a 38 x 0.8 mm needle against the ventrum of the paw. The degree of paw flexion was recorded using a points system adapted from Fox (1965). A score of 0 was given for no flexion, a score of 1 for an unsustained muscular flinch, a score of 2 for maintained flexion approximating 45°, a score of 3 for maintained flexion approximating 90°, and a score of 4 for a maximal grasp of the needle. In the case of the hindlimb grasp, the reflex appears later in development, and the maximal grasp is somewhat less than in the forelimb, but the same scoring system was used. The degree of limb flexion is directly related to neurological development. The value used for analysis is the mean pup flexion score per litter sample (total flexion score divided by number of pups tested from litter).

The day of unfolding of ear pinnae and day of eye opening was determined for each litter by examining all pups in the litter, and recording the first day on which all pups showed both pinnae detached or both eyes open. Thus one value was obtained for each litter, and this was the basis of analysis. Similarly, the first day on which the pups in a litter sample showed the auditory startle response was recorded. One of a sample of 4 pups was placed in a cage containing clean sawdust. After approximately 10 seconds, a distinctly audible sound was generated from a sprung steel clip located 5 cm above the animal's head. Any flinch-startle response

was noted.

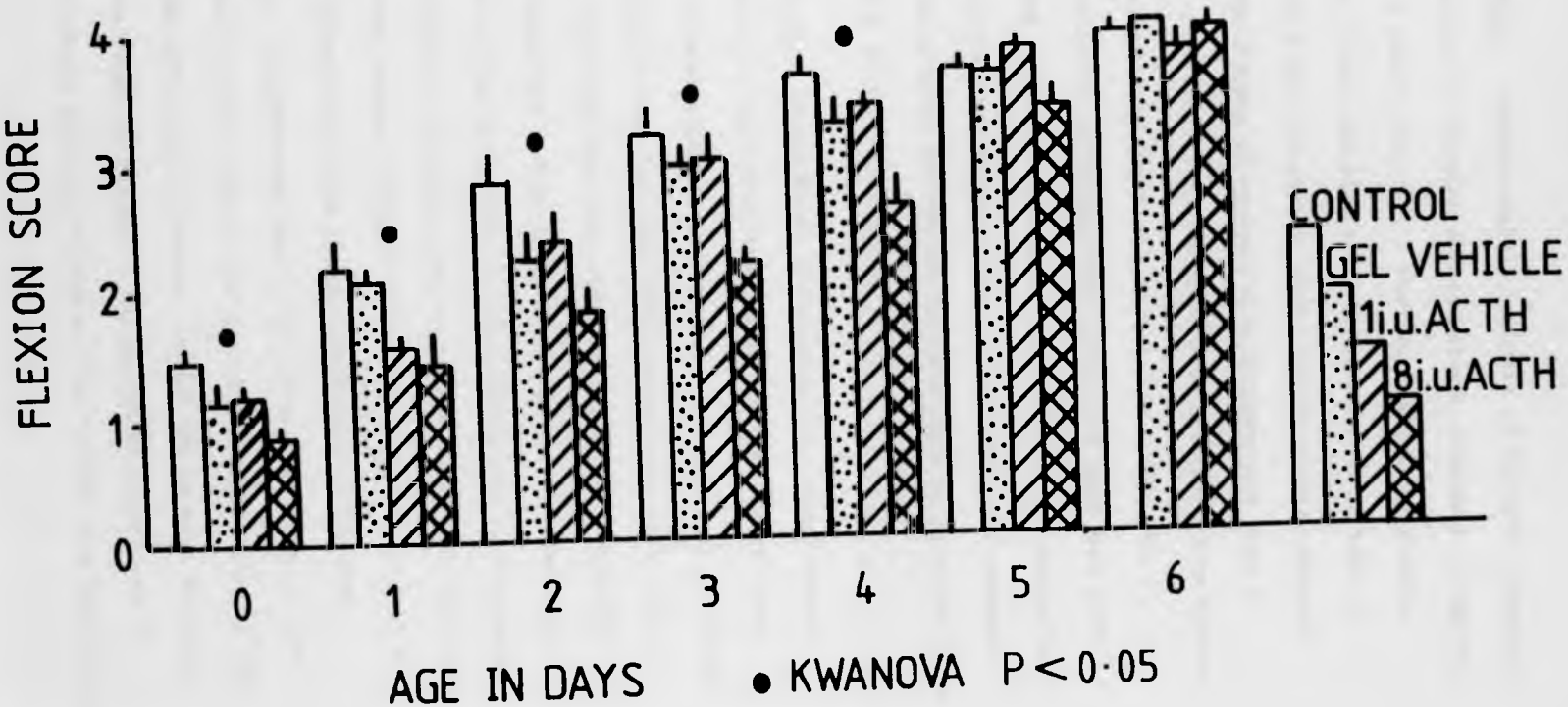
As in Chapter 5, all results are based on the litter as the unit of variance to avoid spurious results due to litter effects (Abbey and Howard, 1973). Methods of data presentation and analysis are explained in Chapter 3. The experiments reported in this chapter were conducted "blind" and at no stage was it known which litters belonged to which treatment group.

EXPERIMENT 6:1. The effects of ACTH administration during pregnancy upon early neurological and neuromuscular development of neonatal offspring. Litters were derived from 6 undisturbed control mice, 6 saline-gelatine vehicle treated mice, 8 low dose ACTH treated mice and 6 high dose ACTH treated mice. On postnatal day 3, one high dose ACTH treated litter suffered mortalities and was excluded from the experiment.

RESULTS. The effects of ACTH administered in two doses from days 12-17 inclusive of pregnancy, upon development of the forelimb grasp, hindlimb grasp, body righting and negative geotaxis reflexes in neonatal offspring are shown in Figs. 4 and 5 and tables 6:1 and 6:2 respectively.

FORELIMB GRASP REFLEX. Kruskal-Wallis analysis of variance revealed significant differences in flexion scores between treatment groups on postnatal days 0-4 inclusive (Fig. 4). Further analysis of results using Mann-Whitney U test showed that offspring from litters of low dose ACTH-treated mice achieved lower flexion scores, compared with offspring from litters of control mice on postnatal days 0 ($P = 0.054$) and 1 ($P = 0.030$) and compared with offspring from litters of saline-gelatine vehicle injected mice on postnatal day 1 ($P = 0.0001$). Similarly, offspring from litters of high dose ACTH treated mice showed lower flexion scores compared with offspring from litters of control mice, on postnatal days 0-4 inclusive ($P = 0.047$ - $P = 0.001$) and compared with offspring from litters of vehicle injected mice, on postnatal days 1, 3 and 4 ($P = 0.032$ - $P = 0.004$). Offspring from vehicle injected mice showed lower flexion scores compared with offspring from control mice, on postnatal days 0 ($P = 0.021$) and 2 ($P = 0.013$).

FIG. 4 ACUTE ACTH ADMINISTRATION DURING PREGNANCY AND DEVELOPMENT OF THE FORELIMB GRASP REFLEX IN NEONATAL OFFSPRING. MEANS ± S.E.M. ARE PRESENTED.



HINDLIMB GRASP REFLEX. Kruskal-Wallis analysis of variance revealed significant differences in flexion scores between treatment groups on postnatal days 3, 6 and 8 (Fig. 5). Further analysis of results using Mann-Whitney U test revealed that offspring from litters of low dose ACTH treated mice displayed lower flexion scores compared with offspring from litters of control mice on postnatal days 6 ($P = 0.021$) and 8 ($P = 0.015$) and compared with offspring from litters of vehicle injected mice on postnatal days 3 ($P = 0.021$) and 8 ($P = 0.001$). Similarly, offspring from litters of high dose ACTH treated mice showed lower flexion scores compared with offspring from litters of control mice on postnatal day 6 ($P = 0.021$) and compared with offspring from litters of vehicle injected mice on postnatal days 3 ($P = 0.004$) and 6 ($P = 0.047$).

BODY RIGHTING REFLEX. Significant differences were found between experimental groups in cumulative time taken to complete the body righting reflex on postnatal days 5-8 inclusive (table 6:1). During this period, offspring from low dose ACTH treated mice only, showed slower times in completing the body righting reflex compared with offspring in litters from either control or vehicle injected mice. However, analysis of the proportion of pups within the litter sample that failed to complete the body righting reflex within the criterion time in 58% or more trials, revealed that offspring from litters of high dose ACTH treated mice also showed impairment of the body righting reflex. On postnatal day 2, 50% of control litters, 33% of vehicle treated litters, 75% of low dose ACTH treated litters and 100% of high dose ACTH treated litters, showed 58% or more failures in completing the righting reflex. The increased proportion of high dose ACTH litters failing to complete the reflex, was significant

FIG.5 ACUTE ACTH ADMINISTRATION DURING PREGNANCY AND DEVELOPMENT OF THE HINDLIMB GRASP REFLEX IN NEONATAL OFFSPRING. MEANS ± S.E.M. ARE PRESENTED.

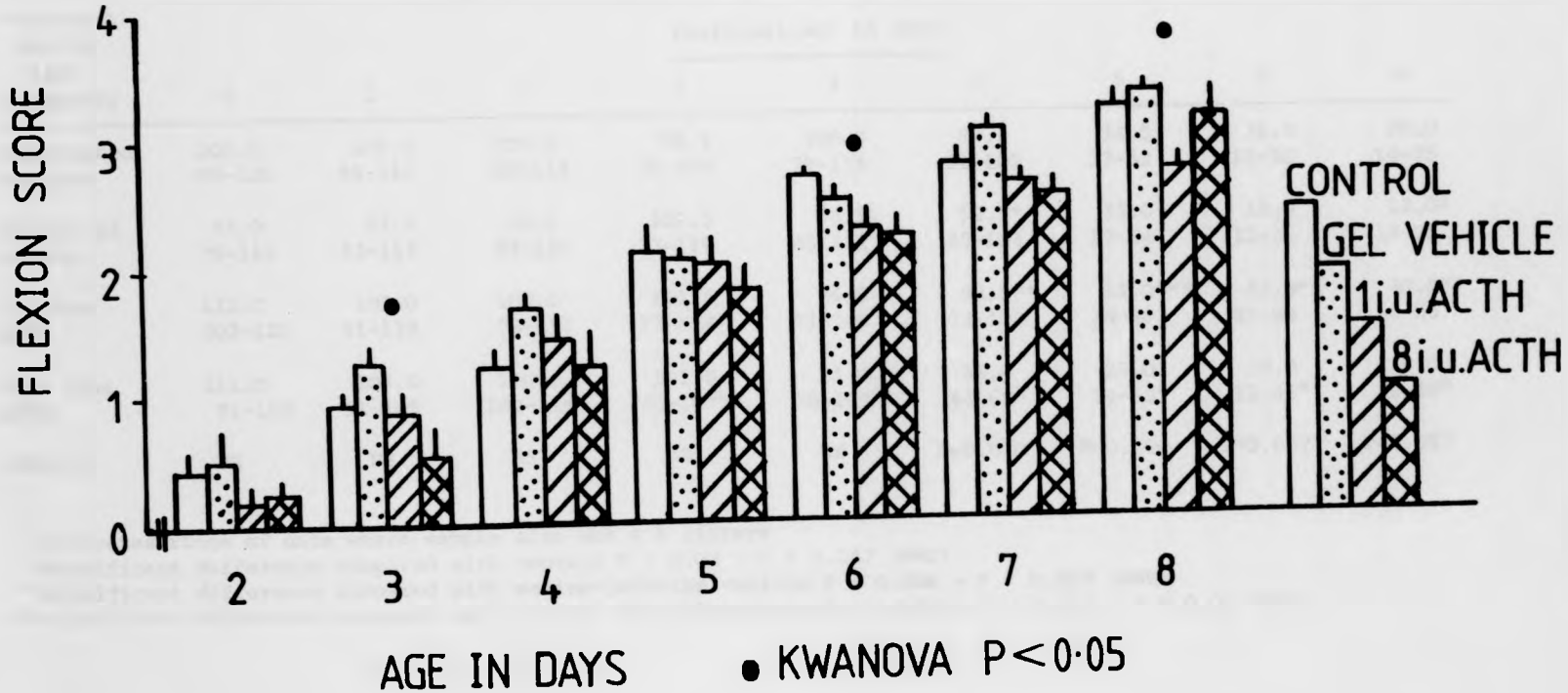


Table 6:1 The effects of ACTH administration during the final third of pregnancy upon offspring body righting reflex. Data presented are as median body righting time (seconds) and 95% confidence limits

Treatment during late pregnancy	Postnatal age in days								
	0	1	2	3	4	5	6	7	8
Undisturbed controls	108.5 99-120	105.0 95-110	100.0 88-114	98.5 78-109	100.0 79-114	82.5 52-102	34.5 27-52	36.0 15-50	20.0 14-35
Saline-gel vehicle	94.0 75-114	92.5 82-114	96.0 87-120	102.5 73-119	74.5 45-112	52.5* 45-104	33.0 17-54	19.5 12-36	12.0* 12-25
Low dose ACTH	112.0 102-120	105.0 91-118	103.0 78-114	101.5 77-114	86.5 71-109	82.5** 72-103	63.0*** 28-96	42.0** 25-98	30.5** 14-53
High dose ACTH	111.0 91-118	105.0 91-109	110.5 100-119	101.0 86-107[a]	81.0 36-103[a]	56.0 44-68[a]	25.0 14-60[a]	28.0 12-46[a]	18.0 12-29[a]
KWANOVA	NS	NS	NS	NS	NS	P=0.020	P=0.060	P=0.057	P=0.057

[a]indicates range of data where sample size was < 6 litters
*significant difference compared with control P < 0.03 - P < 0.047 (MWU)
**significant difference compared with saline-gelatine vehicle P < 0.006 - P < 0.015 (MWU)
***significant difference compared with control and saline-gelatine vehicle P < 0.015 - P < 0.02 (MWU)

compared with vehicle treated litters (P = 0.03, Fishers Exact Probability).

NEGATIVE GEOTAXIS REFLEX. There were no consistent effects of ACTH administration during pregnancy upon the time taken by pups in litters to complete the negative geotaxis reflex; results have not been presented. However, analysis of the proportion of animals within the litter sample that failed to complete this reflex within the criterion time, showed that more control litters had at least 1 pup from the sample of 4 failing to complete the reflex on postnatal day 4 compared with both vehicle treated litters and high dose ACTH treated litters (table 6:2).

Table 6:2 The effects of ACTH administration during the final third of pregnancy upon development of the negative geotaxis reflex in neonatal offspring. Data presented are as number of litters

	Postnatal age in days		
	Day 2	Day 3	Day 4
Treatment during late pregnancy	Number of litters in which > 0% of the sample successfully completed the reflex	Number of litters in which > 50% of the sample failed to complete the reflex	Number of litters in which > 0% of the sample failed to complete the reflex
Undisturbed controls	6	0	6*
Saline-gelatine vehicle	6	0	1
Low dose ACTH	8	2	5
High dose ACTH	6	0	1

*Significant difference compared with saline-gelatine vehicle and high dose ACTH (P = 0.007 and P = 0.015 respectively, Fisher Exact Probability)

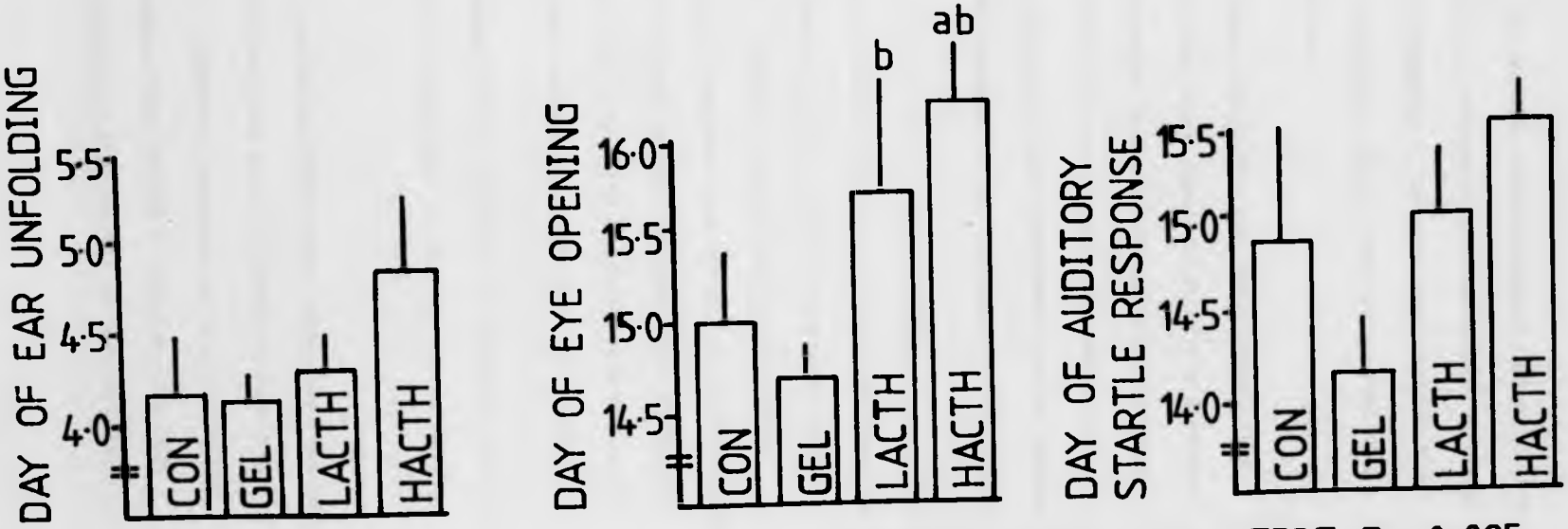
EXPERIMENT 6:2. The effects of ACTH administration during pregnancy upon late somatic-neurological development in juvenile offspring. Litters were derived from 6 undisturbed control mice, 7 saline-gelatine vehicle treated mice, 6 low dose ACTH treated mice and 6 high dose ACTH treated mice.

RESULTS. The effects of ACTH administered in two doses from days 12-17 inclusive of pregnancy, upon ear pinnae unfolding, eye opening and development of the auditory startle response are shown in Fig. 6.

EAR PINNAE UNFOLDING AND EYE OPENING. One way analysis of variance revealed that there were no significant differences between treatment groups in the mean day of unfolding of ear pinnae ($P = 0.28$). However, there were significant differences between treatment groups in the mean day of eye opening ($P = 0.029$). Further analysis of results revealed that offspring in litters from both low dose and high dose ACTH treated mice, showed delayed eye opening compared with offspring in litters from either control or saline-gelatine vehicle treated mice (statistical comparisons shown on Fig. 6).

AUDITORY STARTLE REFLEX. One way analysis of variance revealed that there were no significant differences between treatment groups in the mean day of appearance of the auditory startle response ($P = 0.19$). However, proportional analysis of results revealed that by postnatal day 15, 33% of control litters, 57% of vehicle treated litters, 33% of low dose ACTH treated litters and 0% of high dose ACTH treated litters, achieved a criterion of the whole litter sample displaying the auditory startle response. The difference in the proportion of litters from high dose ACTH treated mice displaying the auditory startle response was significant, compared with litters from vehicle treated mice, ($P = 0.049$ Fishers Exact Probability).

FIG. 6 ACUTE ACTH ADMINISTRATION DURING PREGNANCY AND INDICES OF LATE SOMATIC-NEUROLOGICAL DEVELOPMENT (DAYS OF EAR UNFOLDING, EYE OPENING AND AUDITORY STARTLE RESPONSE) IN JUVENILE OFFSPRING. MEANS ± S.E.M. ARE PRESENTED.



a DIFFERS FROM CON TTEST $P<0{\cdot}025$

b DIFFERS FROM GEL TTEST $P<0{\cdot}05$-$0{\cdot}001$

EXPERIMENT 6:3. The effects of chronic corticosterone administration during pregnancy upon neurological, neuromuscular and somatic development of neonatal and juvenile offspring. Litters were derived from 5 undisturbed control mice, 7 propylene glycol vehicle treated mice and 7 corticosterone treated mice.

RESULTS. The effects of chronic corticosterone administered from day 12 of pregnancy to parturition, upon development of the forelimb grasp, hindlimb grasp, body righting and negative geotaxis reflexes in neonatal offspring, are shown in Figs. 7 and 8 and tables 6:3 and 6:4 respectively. The effects of this treatment upon ear pinnae unfolding, eye opening and development of the auditory startle reflex are shown in Fig. 9.

FORELIMB GRASP REFLEX. Kruskal-Wallis analysis of variance revealed significant differences in flexion scores between treatment groups on postnatal day 1 (Fig. 7). Further analysis of results using Mann-Whitney U test showed that offspring from litters of corticosterone-treated mice displayed lower flexion scores, compared with offspring from litters of control mice ($P = 0.015$) and compared with offspring from litters of propylene glycol vehicle-treated mice ($P = 0.019$).

HINDLIMB GRASP REFLEX. Kruskal-Wallis analysis of variance revealed that there were no significant differences in flexion scores between treatment groups (Fig. 8).

BODY RIGHTING REFLEX. No significant differences were found between experimental groups in cumulative time taken to complete the body righting reflex (table 6:3). However, analysis of the proportion of pups within the litter sample that failed to complete the body righting reflex within the criterion time in 50% or more trials,

FIG.7 CHRONIC CORTICOSTERONE ADMINISTRATION DURING PREGNANCY AND DEVELOPMENT OF THE FORELIMB GRASP REFLEX IN NEONATAL OFFSPRING. MEANS ± S.E.M. ARE PRESENTED.

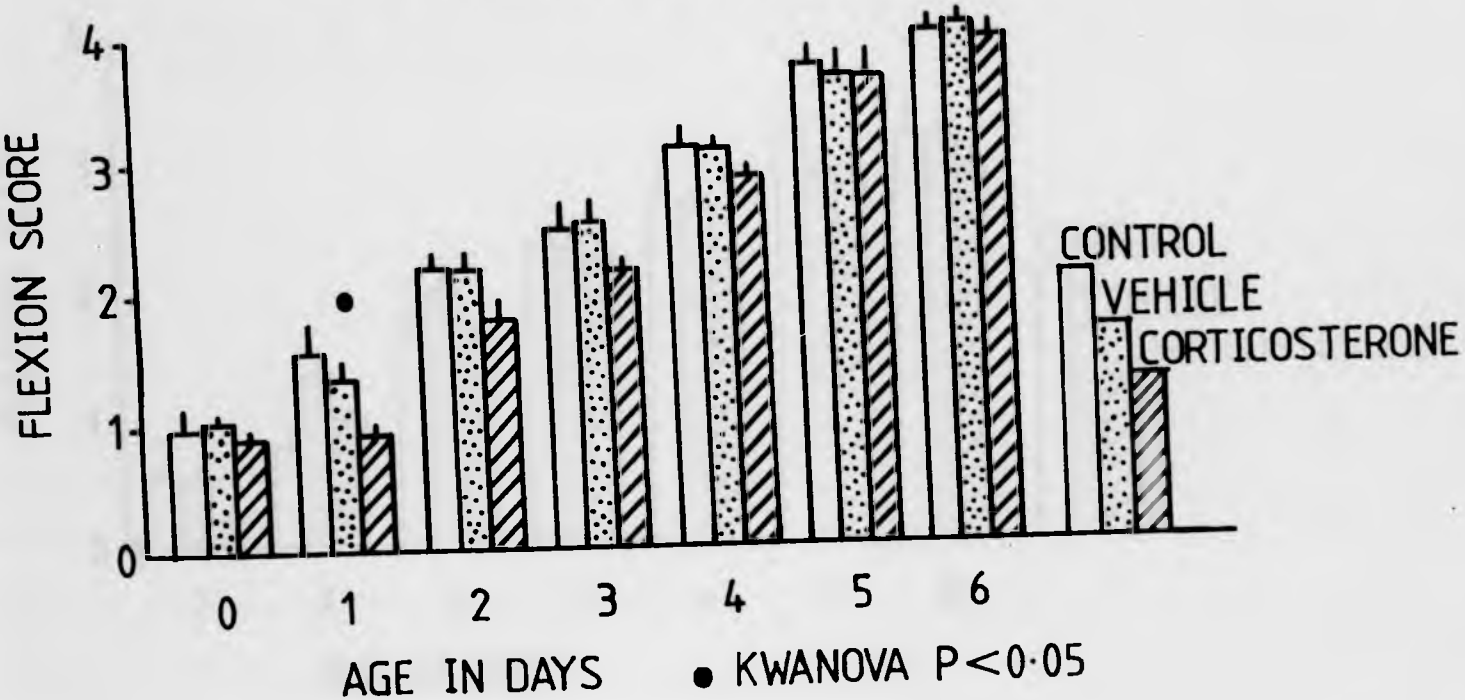


FIG. 8 CHRONIC CORTICOSTERONE ADMINISTRATION DURING PREGNANCY AND DEVELOPMENT OF THE HINDLIMB GRASP REFLEX IN NEONATAL OFFSPRING. MEANS ± S.E.M. ARE PRESENTED.

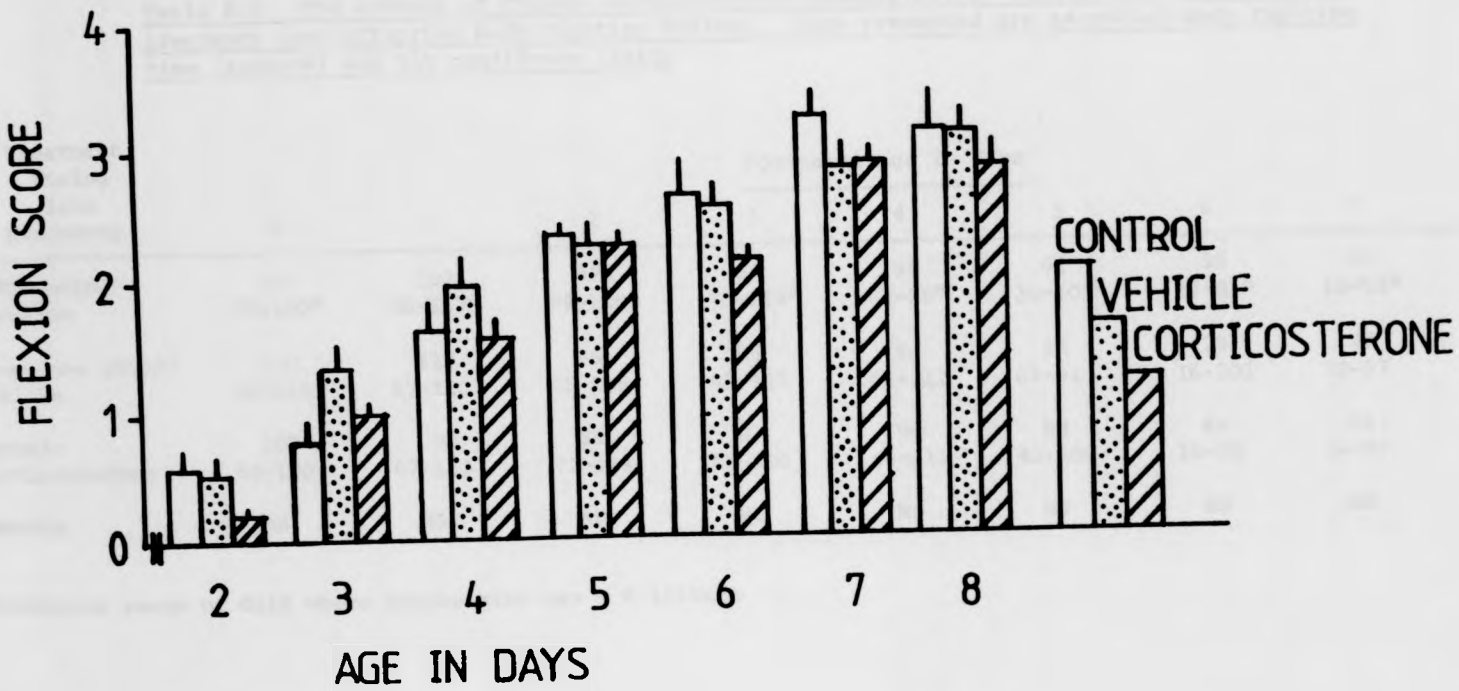


Table 6:3 The effects of chronic corticosterone administration during the final third of pregnancy upon offspring body righting reflex. Data presented are as median body righting time (seconds) and 95% confidence limits

Treatment during late pregnancy	Postnatal age in days								
	0	1	2	3	4	5	6	7	8
Undisturbed controls	102 75-109[a]	102 86-117[a]	97 59-109[a]	83 70-114[a]	98 66-99[a]	62 39-102[a]	59 22-81[a]	35 18-64[a]	16 13-35[a]
Propylene glycol vehicle	100 90-115	112 67-120	96 55-116	95 61-117	94 61-112	83 64-94	29 16-101	43 12-97	24 14-55
Chronic corticosterone	107 64-120	98 67-113	89 71-114	87 85-100	96 46-111	94 42-108	64 16-93	21 14-39	25 16-42
KWANOVA	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]indicates range of data where sample size was < 6 litters

revealed that offspring from litters of corticosterone-treated mice showed impairment of the body righting reflex. On postnatal day 0, 60% of control litters, 29% of vehicle-treated litters and 86% of corticosterone-treated litters, showed 50% or more failures in completing the righting reflex. The increased proportion of corticosterone litters failing to complete the reflex, was significant compared with vehicle-treated litters ($P = 0.05$, Fishers Exact Probability).

NEGATIVE GEOTAXIS REFLEX. There were no consistent effects of chronic corticosterone administration during pregnancy upon the time taken by pups in litters to complete the negative geotaxis reflex; results have not been presented. However, analysis of the proportion of pups within the litter sample that failed to complete this reflex within the criterion time, showed that more corticosterone-treated litters had at least 1 pup from the sample of 4 failing to complete the reflex on postnatal days 0-2 inclusive, compared with either control litters or vehicle-treated litters (table 6:4).

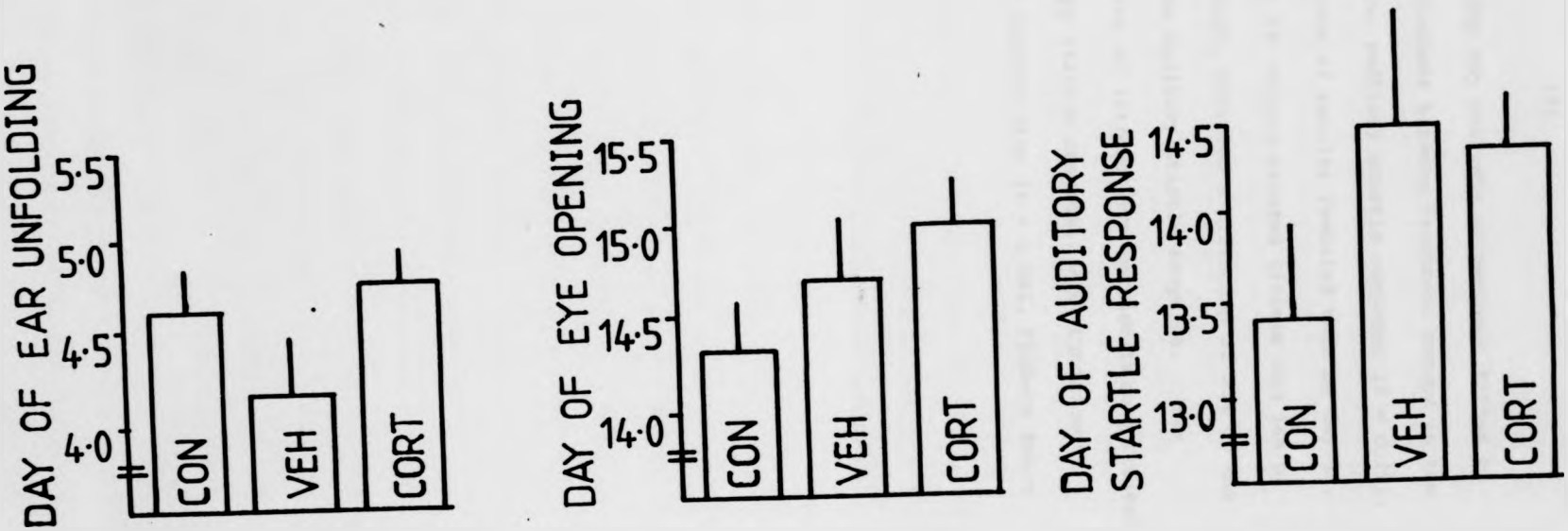
EAR PINNAE UNFOLDING AND EYE OPENING. One way analysis of variance failed to reveal any significant differences between treatment groups in the mean day of unfolding of ear pinnae ($P = 0.19$) or of eye opening ($P = 0.80$). Results are shown in Fig. 9. However, proportional analysis of results revealed that by postnatal day 13, 50% of control litters, 33% of propylene glycol vehicle-treated litters and 0% of corticosterone-treated litters had more than 25% of the pups showing evidence of eye opening. The difference in the proportion of litters from corticosterone-treated mice showing eye opening, compared with litters from control mice, was significant ($P = 0.045$, Fishers Exact Probability).

Table 6:4 The effects of chronic corticosterone administration during the final third of pregnancy upon development of the negative geotaxis reflex in neonatal offspring. Data presented are as numbers of litters

	Postnatal age in days		
	Day 0	Day 1	Day 2
Treatment during late pregnancy	Number of litters in which > 0% of the sample successfully completed the reflex	Number of litters in which > 50% of the sample failed to complete the reflex	Number of litters in which > 0% of the sample failed to complete the reflex
Undisturbed controls	4	0	2
Propylene glycol vehicle	3	3	4
Chronic corticosterone	0**	7***	7*

*significant difference compared with control P = 0.045 (Fisher Exact Probability)
**significant difference compared with propylene glycol vehicle P = 0.035 (Fisher Exact Probability)
***significant difference compared with control and propylene glycol vehicle P = 0.035 - P = 0.0013 (Fisher Exact Probability)

FIG. 9 CHRONIC CORTICOSTERONE ADMINISTRATION DURING PREGNANCY AND INDICES OF LATE SOMATIC-NEUROLOGICAL DEVELOPMENT (DAYS OF EAR UNFOLDING, EYE OPENING AND AUDITORY STARTLE RESPONSE) IN JUVENILE OFFSPRING. MEANS ± S.E.M ARE PRESENTED.



AUDITORY STARTLE REFLEX. One way analysis of variance failed to reveal any significant differences between treatment groups in the mean day of appearance of the auditory startle response ($P = 0.29$). However, proportional analysis of results revealed that by day 12, 80% of control litters, 66% of vehicle-treated litters and 14% of corticosterone-treated litters, achieved a criterion of 50% of the litter sample displaying the auditory startle response. The difference in the proportion of litters from corticosterone treated mice displaying the auditory startle response, was significant compared with litters from control mice ($P = 0.045$, Fishers Exact Probability).

DISCUSSION

Chevins (1981) reported that offspring in litters from mice chronically crowded during late pregnancy showed impaired development of reflexes. These animals were slower to complete the body righting reflex, showed reduced limb flexion and retarded development of the auditory startle response. Similar results have been reported in pups from rats restrained during pregnancy (Barlow, Knight and Sullivan, 1978). Taken together, these results suggest that stress during pregnancy deleteriously affects offspring neurological and neuromuscular development. One explanation of the results from these studies is that the described effects may be mediated by hyperactivity of the maternal pituitary-adrenocortical axis during pregnancy. In this study this hypothesis was tested and the consequences of ACTH or corticosterone administration during pregnancy, for offspring reflex ontogeny, were examined. It was found that ACTH effectively reproduced the effects of stress during pregnancy. ACTH treated litters showed reduced limb flexion, retarded development of the body righting and auditory startle reflexes and delayed eye opening. Whilst the high dose ACTH administration regime was found to be most effective in retarding aspects of neurological and neuromuscular development overall, reversals in effectiveness of ACTH between reflexes and occasions where ACTH treated litters showed apparently accelerated development of reflexes, were detected. These results can be considered as random effects, possibly resulting from the mode of testing, and do not detract from the majority of results.

There was difficulty associated with the experiments reported in this chapter concerning the collection of quantitative data and the statistical analysis of results: large variance within and between

litters was encountered, and this may account for the failure of some results to achieve significance. Employing the litter as the unit of variance was a necessary procedure which controlled within litter variance, and this technique of results analysis, largely ignored in the existing literature, is recognised as essential to any study of early development (Abbey and Howard, 1973). The subjective nature of some of the measurements was also a source of inaccuracy. For this reason all the experiments in this chapter were performed "blind" so that at no stage was it known as to which treatment group any litter belonged (D. Bosworth applied numerically coded labels to all cages).

Several lines of evidence suggest that the harmful effects of ACTH are not direct, but in turn mediated by adrenocortical products. ACTH does not cross the placenta (Chapter 2). The deleterious effects of ACTH administration during pregnancy upon somatic development can be prevented if the maternal adrenals are removed (e.g. Velardo, 1957; see discussion in Chapter 5). Additionally, administration of synthetic glucocorticoids (e.g. prednisolone) during pregnancy produced effects similar to those described here resulting from ACTH, impairing both somatic development and reflex ontogeny in neonatal offspring (Gandelman and Rosenthal, 1981; Gandelman and Guerriero, 1982). These reports supply evidence that foetal glucocorticoid exposure delays later neurological and neuromuscular development. The possibility that the similarity of the effects of stress and ACTH administration during pregnancy, are a result of foetal exposure to an endogenous glucocorticoid, was examined by assessing whether corticosterone administration during pregnancy could reproduce the described effects of stress and ACTH upon offspring reflex ontogeny. Corticosterone, being the dominant

glucocorticoid in mice, is the most obvious teratogenic compound, as neither androgen nor progestogen administration during pregnancy were found here to deleteriously influence general body development of offspring (Chapter 5).

Offspring in litters from mice treated chronically with corticosterone during pregnancy showed retarded reflex ontogeny. These animals showed reduced forelimb flexion, delayed development of the body righting, negative geotaxis and auditory startle reflexes and also showed later eye opening. Corticosterone therefore reproduces the effects of stress and ACTH administration during pregnancy, upon offspring neurological and neuromuscular development. This finding is consistent with the working hypothesis, that the effects of stress during pregnancy are mediated by activation of the maternal pituitary-adrenocortical axis, and exposure of the offspring *in utero* to products of this system.

It has been previously stated in Chapters 2 and 5 that corticosterone can cross the placenta in rodents (Xarrow, Philpott and Denenberg, 1970) but that a mechanism exists to prevent excessive exposure of the foetal rodent to corticosterone : the placenta converts this compound to the less potent 11-dehydrocorticosterone (Michaud and Burton, 1977). It is likely that in conditions of stress or after ACTH or corticosterone treatment, relatively large quantities of corticosterone enter foetal circulation. The reader is reminded that the effects of perinatal glucocorticoid exposure upon general body development, and the metabolic and endocrine effects of corticosterone exposure upon the developing animal have already been discussed in Chapter 5, but they relate strongly to these present results. Corticosterone exerts a catabolic effect on protein and is reported to decrease muscle mass (Seene and Viru, 1982). Corticosterone

suppresses the secretion of PRL, thyrotropin and insulin (e.g. Gala, Kothari and Haisenleder, 1981; Pamenter and Hedge, 1980; Billaudel and Sutter, 1982) which are all required for normal body development (e.g. Jost, 1979). Consequently, disruption of the metabolic mechanisms controlling body (and organ) growth, and catabolism of protein during a critical time of maximal growth and development of body, brain and other organ systems, will have general deleterious effects upon development.

As the neurological tests employed in this study require for their operation an undefinable degree of body development and strength, the effects of ACTH and corticosterone administration during pregnancy upon offspring reflex ontogeny, may be partially a result of their effects upon the body. For example, the body righting and negative geotaxis reflexes require both strength and neuromuscular co-ordination for their operation, as well as development of neural apparatus. The limb grasp reflexes require less strength but still demand a degree of neuromuscular development, whilst the auditory startle response requires sensory development and opening of the external auditory meatus (A.K. Palmer, Huntingdon Research Centre, personal communication) but also tests alertness. It is difficult to conclusively separate the effects of foetal corticosterone exposure upon somatic development, from those upon neurological development, but there is evidence that glucocorticoids specifically affect neural tissue in the developing rodent. Klepac (1982) reports altered nucleic acid ratios in rat brain following prenatal dexamethasone treatment. Howard (1965) and Takahashi, Goto, Sudo and Suzuki (1982) report altered nucleic acid ratios and decreased protein content of rodent brain following postnatal administration of corticosterone. Glucocorticoids inhibit brain growth (Devenport and Devenport, 1983a, b)

and retard biochemical maturation of the brain (Cotterell, Balasz and Johnson, 1972) but are also known to accelerate maturation of the retina (Beato and Doenecke, 1980). Foetal exposure to corticosterone at a time when brain growth and development is maximal, but incomplete in the mouse (Rodier, 1980) may well impair later functional development or desynchronise the development of different neural systems with other organs, resulting in the effects upon reflex ontogeny reported here.

Although there have been previous studies investigating the effects of glucocorticoid manipulation (stress or synthetic hormone treatment) during pregnancy upon offspring reflex ontogeny (Barlow, Knight and Sullivan, 1978; Gandelman and Rosenthal, 1981) such studies have not examined the possibility that results may be mediated by other factors. The results reported here seem not to be caused by undernutrition or premature birth. Neither are postnatal factors upon development instrumental in producing these results, since all litters were fostered to untreated mothers at birth, thereby controlling certain variables in maternal care and postnatal nutrition.

In conclusion, the deleterious effect of ACTH administration during pregnancy (Velardo, 1957; Yang, Yang and Lin, 1969; Robson and Sharaf, 1952) and perinatally (Monder, Yasukawa and Christian, 1981) upon offspring development have been previously reported, but this is the first study of the effects of ACTH administration during pregnancy upon offspring neurological development. The experiments in this study were designed to test the hypothesis that the retarded neurological development of offspring from rodents exposed to stressors during pregnancy, can be replicated by maternal pituitary-adrenocortical activation, and that ACTH or corticosterone

administered exclusively during pregnancy may also retard offspring neurological development. Further, whilst the potential neurological hazard of perinatal glucocorticoid exposure has been recognised (Weichsel, 1977) there is little knowledge of the possible consequences of glucocorticoid exposure exclusively during the prenatal period, other than from studies using synthetic compounds in high doses (Gandelman and Rosenthal, 1981; Gandelman and Guerriero, 1982). The results from this study strongly suggest that exposure of the foetus to elevated concentrations of naturally occurring glucocorticoids (corticosterone) presents a neurological hazard, and that compounds from the maternal pituitary-adrenal system may naturally regulate development of foetal body and brain.

CHAPTER 7

EFFECTS UPON THE ONSET OF PUBERTY AND THE ADULT OESTROUS CYCLE IN FEMALE OFFSPRING

INTRODUCTION

Most studies that have investigated the effects of stress during pregnancy upon reproductive function and correlates of sexual differentiation, have examined the male offspring and used the rat as the experimental animal (see Chapter 8). The results from such studies, reporting both behavioural and endocrine abnormalities, suggest that stress during pregnancy detrimentally affects the process of sexual differentiation of the brain in male offspring. There have been fewer studies of the effects of stress during pregnancy upon reproductive development and function in female offspring, and these will now be reviewed.

In the mouse, offspring from animals crowded during pregnancy show lower levels of sexual receptivity, compared with offspring from individually housed animals (Allen and Haggett, 1977). The more acute stress of restraint during pregnancy delays vaginal opening and lengthens the vaginal oestrous cycle, but increases behavioural receptivity of female offspring in mice (Politch and Herrenkohl, 1984a). However, in the rat restraint during pregnancy impairs behavioural receptivity (Dahlöf, Hard and Larsson, 1977) lengthens the vaginal oestrous cycle (Herrenkohl and Politch, 1978) increases spontaneous abortions and other reproductive deficiencies (Herrenkohl, 1979) and reduces post-partal prolactin secretion in adult female offspring (Herrenkohl and Gala, 1979). Other studies report that restraining rats during pregnancy does not influence the timing of vaginal opening (Barlow, Knight and Sullivan, 1978) or affect behavioural

receptivity (Beckhardt and Ward, 1983) of female offspring.

It is apparent from the above that there are contradictions in the literature concerning the effects of stress during pregnancy upon female offspring reproductive development. This can be explained by different studies employing different methods, species and strains of animal. The need for a more thorough and consistent study of the effects of stress during pregnancy upon female offspring, is further supported by the incomplete nature of some results. For example, Politch and Herrenkohl (1984a) have reported that offspring from restrained mice show delayed vaginal opening compared with offspring from non-stressed mice and whilst this result suggests that puberty is retarded in these animals, it is not known whether this is because of a delay in body weight gain, which is an important factor influencing sexual development (Meijs-Roeloffs and Moll, 1978) or due to endocrine abnormalities. Vaginal opening is only one criterion of puberty and more thorough studies include determination of first oestrus and body weights at these developmental events. Additionally, the validity of combining stages of the oestrous cycle, according to the methods of Herrenkohl and Politch (1978) who combined oestrus and metoestrus phases, is open to question.

That there is little empirical evidence that the pathology in offspring of stressed rodents is mediated by maternal pituitary-adrenocortical activity, has been previously outlined (Chapters 1 and 2). Manipulation of the maternal pituitary-adrenal system during pregnancy can affect development of the foetal adrenal (Jones, Lloyd and Wyatt, 1953; Milkovic, Milkovic and Paunovic, 1973; Milkovic, Milkovic, Sencar and Paunovic, 1970) but evidence suggesting that such treatment influences reproductive development of female offspring is limited. Monder, Yasukawa and Christian (1981) have reported

that perinatal ACTH treatment delays vaginal opening, but this study is also subject to the criticisms previously outlined.

This study is a more detailed investigation of the consequences of stress during pregnancy for sexual development of female offspring. The onset of puberty in experimental animals was examined by determining the day of vaginal opening and first oestrus and recording body weights at these development stages. The effects of crowding stress during pregnancy upon the oestrous cycle of adult offspring and the ability to mate and maintain pregnancy was also examined. The hypothesis that any deleterious effects of crowding during pregnancy upon reproductive development and function in female offspring, are mediated by maternal pituitary-adrenocortical activation, was empirically tested according to the rationale of the general working hypothesis (see Chapters 1 and 2). An attempt was made to reproduce the effects of crowding by administration of a series of hormones.

METHODS

Animal husbandry and treatments followed the procedure outlined in Chapter 3. At weaning (postnatal day 21) females used in experiments investigating the onset of puberty were housed in large cages, in groups of 3-6, according to treatment. Offspring from a single litter were usually group housed, although when necessary females from several litters of similar age and the same treatment, were used in order to standardise numbers. Care was taken to standardise housing density as this is a factor known to influence sexual maturation and the oestrous cycle in female rodents (Drickamer and McIntosh, 1980; Bronson and Chapman, 1968; McKinney, 1972; Nichols and Chevins, 1981b). Similarly, males are known to influence

the oestrous cycle (McKinney, 1972; Nichols and Chevins, 1981b) and therefore care was taken to avoid contamination of cages containing females by male bedding. Bedding was regularly changed (7-10 day intervals).

From postnatal day 25 females were observed daily between 1000-1200 hrs for vaginal opening. The second successive day of vaginal opening was set as a criterion for genuine and permanent vaginal patency. Female weight at vaginal opening (and first oestrus) was recorded. From the day of vaginal opening smears were taken daily by vaginal lavage, dried and stained with Giemsa (BDH, 1:20 in water for approximately 30 minutes). Vaginal smears were staged according to Bingel and Schwartz (1969). Following experiments to determine puberty, female mice were re-housed in groups of 8-10 by treatment, each cage containing a random sample of females from different litters.

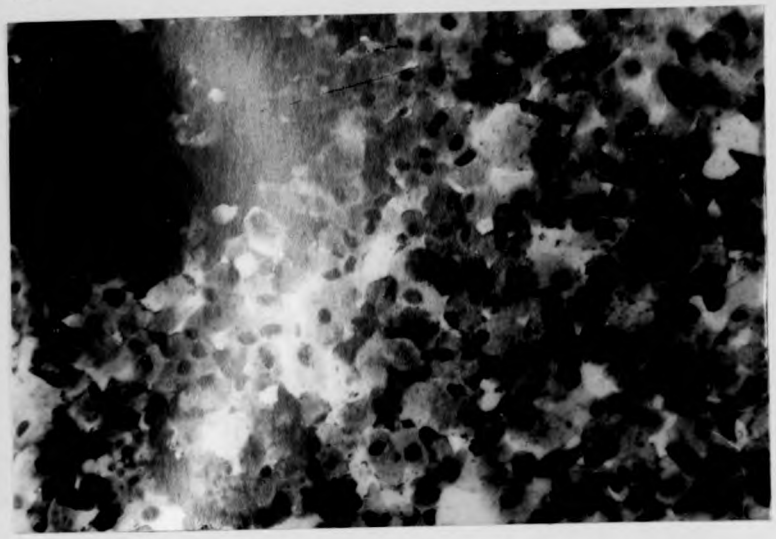
At 8-9 weeks of age, female offspring used in experiments investigating the adult oestrous cycle were housed individually in small cages. One experiment (experiment 7:5) also investigated the oestrous cycle of female offspring housed in groups of 5 in large cages. These females were left undisturbed for 7-10 days after which vaginal smears were taken daily between 1000-1200 hrs for 21 days. Plates II-V show the typical appearance of cycle stages observed in this study.

A study was made of the onset and maintenance of pregnancy in female offspring from crowded mice (experiment 7:3). At vaginal pro-oestrus, females were housed in small cages with sexually experienced males. For 20 minutes sexual behaviour was observed. The length of time to mating (appearance of vaginal plug) pregnancy length, litter size and litter weights were recorded.

PLATE II. Vaginal pro-oestrus. Presence of nucleated vaginal epithelial cells (x 312)

PLATE III. Vaginal oestrus. Cornification of vaginal epithelial cells (x 312)

II



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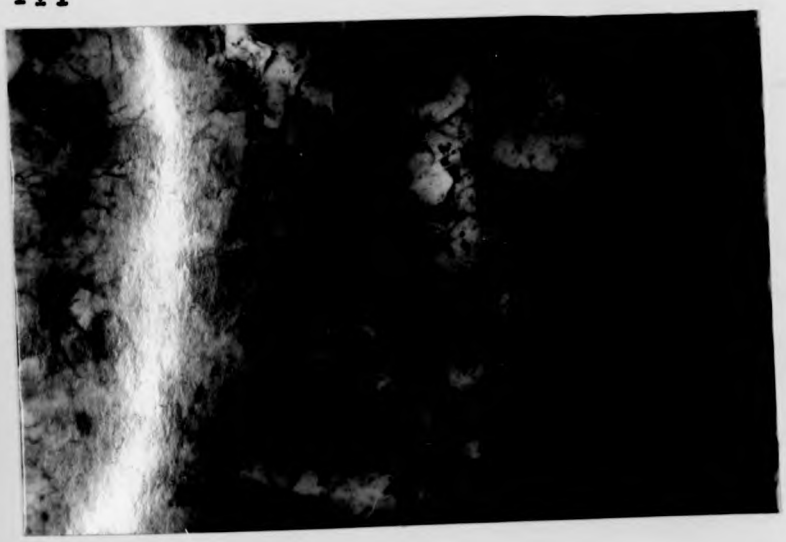

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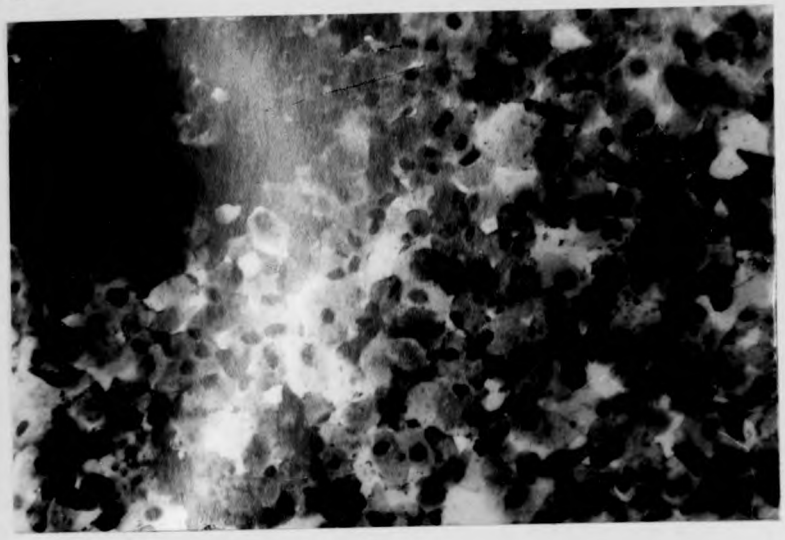

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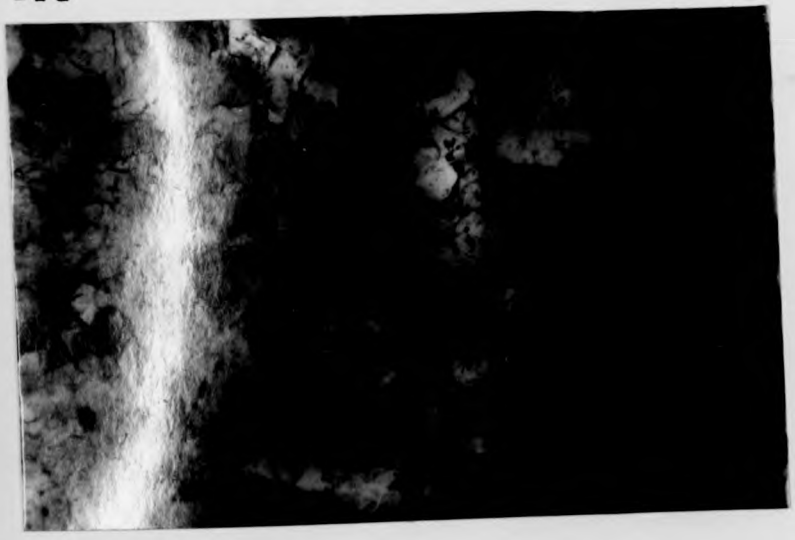

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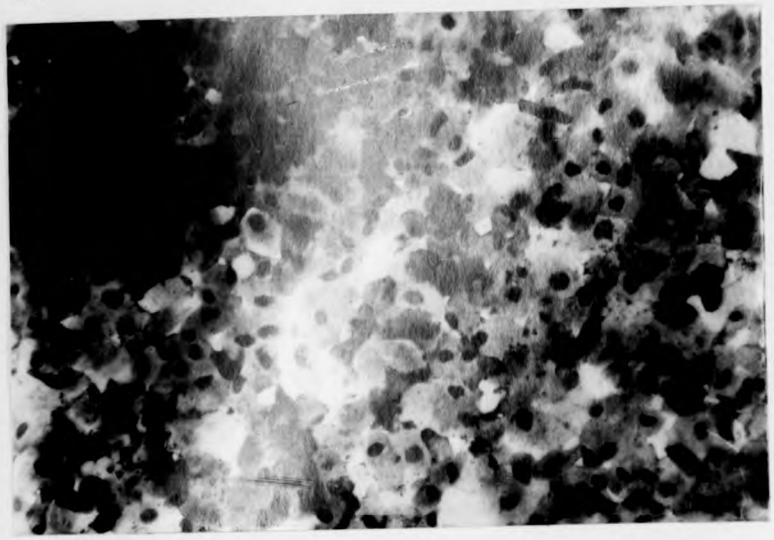

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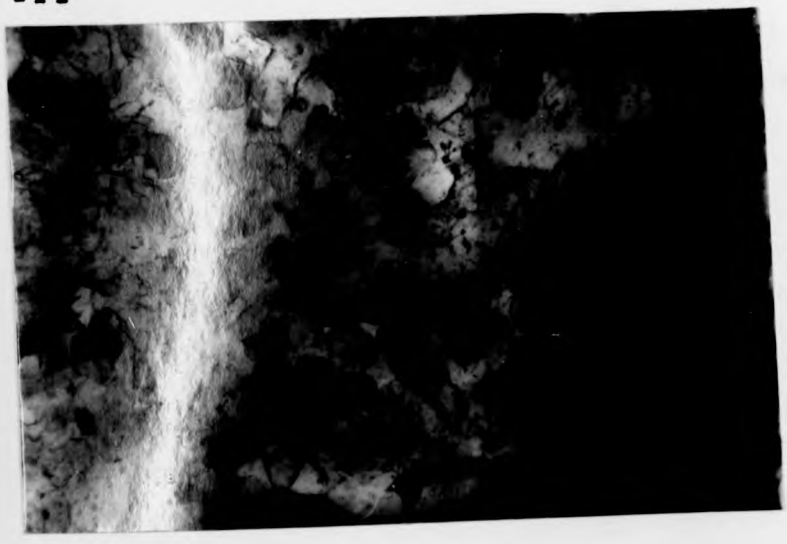
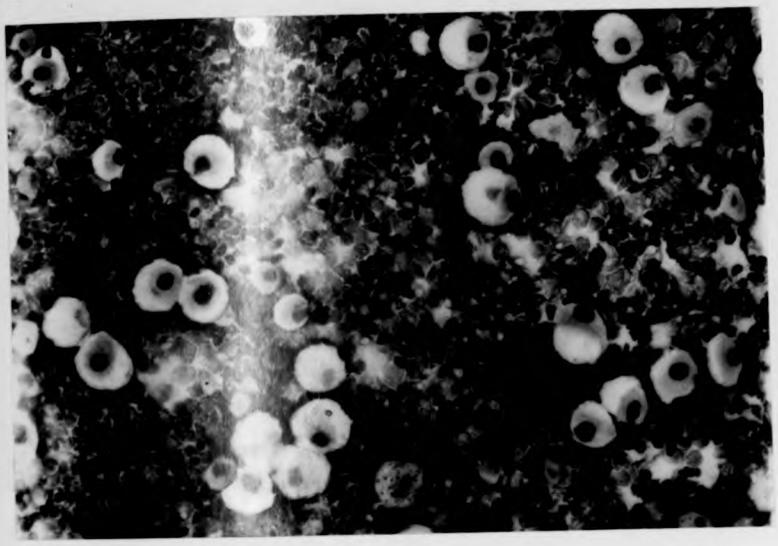


PLATE IV. Vaginal metoestrus. Large number of leucocytes and remnants of cornified cells (x 312).

PLATE V. Vaginal dioestrus. Presence of decreasing numbers of leucocytes and other cellular debris (x 312)

IV



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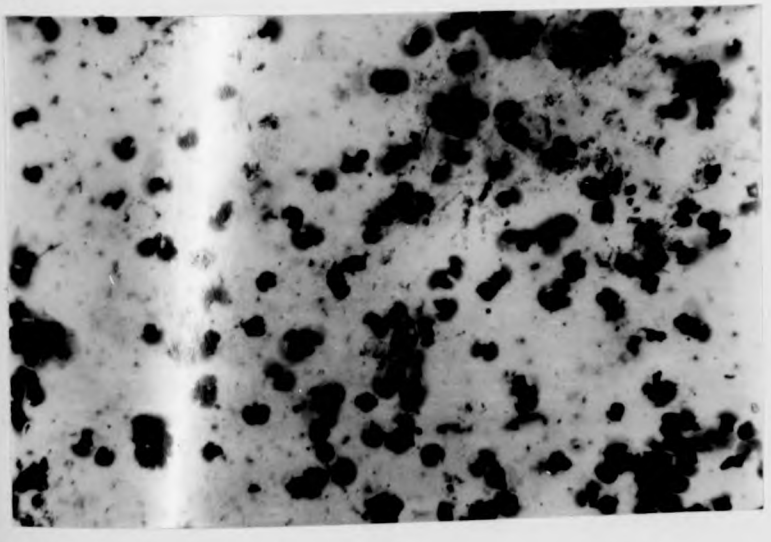

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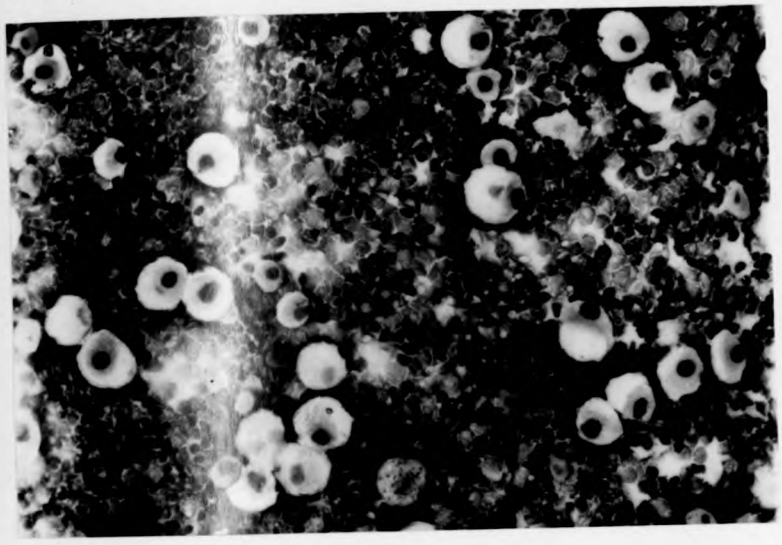

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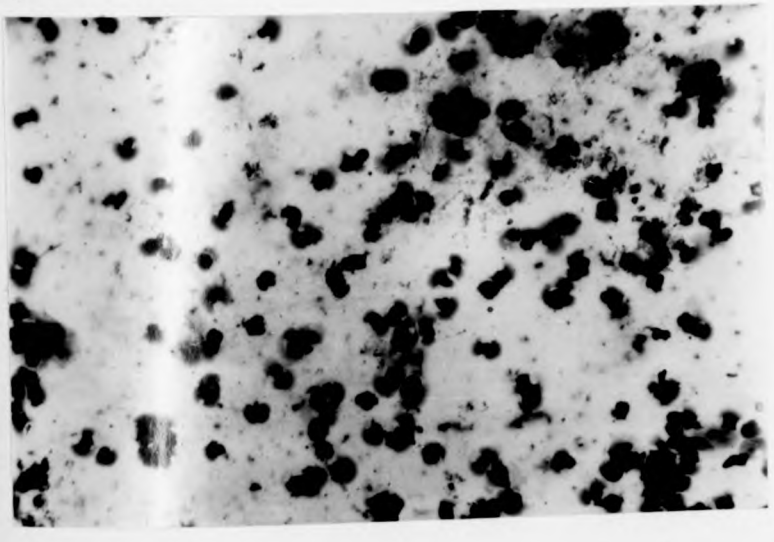


EXPERIMENT 7:1. The effects of chronic crowding during pregnancy upon the onset of puberty in female offspring. Offspring were derived from 9 control mice and 9 crowded mice.

RESULTS. The effects of chronic crowding during late pregnancy upon the onset of puberty in female offspring are shown in tables 7:1 and 7:2. Female offspring from crowded mice showed a significant delay of vaginal opening and first oestrus, and were heavier at these developmental stages, compared with offspring from control mice. Further analysis using proportional data revealed that significantly fewer female offspring from crowded mice showed vaginal opening or first oestrus on postnatal days 28 and 31 respectively, compared with offspring from control mice.

Table 7:1. The effects of chronic crowding during the final third of pregnancy upon the onset of puberty in female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Day of vaginal opening	Weight at vaginal opening	Day of first oestrus	Weight at first oestrus
Undisturbed controls n = 31	30.64 ±0.39	20.67 ±0.30	31.16 ±0.47	21.01 ±0.33
Chronically crowded n = 38	31.87* ±0.34	21.82** ±0.19	32.34* ±0.37	22.23** ±0.18

*significant difference compared with control $P < 0.025$ (t-test)
**significant difference compared with control $P < 0.001$ (t-test)

Table 7:2. The effects of chronic crowding during the final third of pregnancy upon the proportion of female offspring showing vaginal opening and first oestrus. Data given are as numbers of animals

Treatment during late pregnancy	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vaginal opening						
Undisturbed controls n = 31	7	11	14	21	24	27
Chronically crowded n = 38	0**	9	12	13**	24	29
First oestrus						
Undisturbed controls n = 31	5	9	13	18	22	26
Chronically crowded n = 38	0*	6	12	13*	20	25

*significant difference compared with control $P < 0.03$
**significant difference compared with control $P < 0.005$. Fisher Exact Probability tests.

EXPERIMENT 7:2. The effects of chronic crowding during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from 8 control mice and 8 crowded mice.

RESULTS. The effects of chronic crowding during late pregnancy, upon the oestrous cycle of adult female offspring are shown in table 7:3. Statistical differences were found between experimental and control groups in the mean number of days on which pro-oestrus stages were recorded. Females from mice crowded during pregnancy showed decreased incidence of pro-oestrus stages over the 21 day observation period, compared with offspring from control mice. There were no differences between treatment groups in the mean number of days of oestrus, metoestrus or dioestrus, or in the length of cycles. Proportional analysis revealed that 86% of offspring from crowded mice showed cycles without an apparent pro-oestrus stage, compared to 46% of offspring from control mice ($P = 0.025$ - Fishers Exact Probability).

Table 7:3. The effects of chronic crowding stress during the final third of pregnancy upon the oestrous cycle in adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				Cycle length (Days)
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	
Undisturbed controls n = 15	3.60 ±0.32	6.40 ±0.34	6.73 ±0.33	4.27 ±0.27	5.11 ±0.18
Chronically stressed n = 15	2.60* ±0.27	6.80 ±0.34	7.07 ±0.37	4.47 ±0.39	4.20 ±0.21

*significant difference with control P < 0.025 (t-test)

EXPERIMENT 7:3. The effects of chronic crowding during pregnancy upon attainment of pregnancy, length of pregnancy and size and weight of litters born to adult female offspring. Offspring were derived from 8 control mice and 8 crowded mice.

RESULTS. The effects of chronic crowding during late pregnancy upon fertility of adult female offspring are shown in table 7:4. Following housing with sexually experienced males, fewer crowded mice (54%) were mounted compared with controls in a 20 min test pairing (92% - P = 0.04, Fisher Exact Probability). There were no significant differences between crowded or control offspring in the mean time taken to mate, as monitored by appearance of vaginal plugs. There were no significant differences in the mean length of pregnancy between crowded or control offspring, but proportionally more offspring from crowded mice (80%) had pregnancies of less than 18 days compared with offspring from control mice (27% - P = 0.05, Fisher Exact Probability). There were no differences in the size or weight of litters born to offspring from crowded or control mice.

Table 7:4. The effects of chronic crowding stress during the final third of pregnancy upon parameters of reproduction of adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Appearance of vaginal plug (days)	Length of pregnancy (days)	Litter size (no)	Day 0 body weight (g)
Undisturbed controls n = 12	1.08 ±0.34	18.55 ±0.13	12.00 ±0.48	1.64 ±0.03
Chronically stressed n = 9	1.61 ±0.48	17.81 ±0.42	11.22 ±0.49	1.59 ±0.03

EXPERIMENT 7:4. The effects of ACTH administration during pregnancy upon the onset of puberty in female offspring. Offspring were derived from 7 control mice, 6 saline-gelatine vehicle-treated mice, 5 low dose ACTH-treated mice and 7 high dose ACTH-treated mice.

RESULTS. The effects of acute ACTH administration during late pregnancy upon the onset of puberty in female offspring are shown in tables 7:5 and 7:6. Female offspring from mice treated with either dose of ACTH during pregnancy, showed significantly delayed mean day of vaginal opening compared with offspring from control and vehicle-injected mice. There were no significant differences between experimental groups in the mean day of first oestrus or in mean body weights at vaginal opening or first oestrus. Proportional analysis of data revealed that significantly fewer female offspring from ACTH-treated mice showed vaginal opening on postnatal days 31-35 inclusive, or first oestrus on postnatal days 31-34 inclusive compared with offspring from control and vehicle-injected mice. However, fewer offspring from control mice displayed vaginal opening and first oestrus on postnatal days 31 and 32 compared with offspring from vehicle-injected mice.

Table 7:5. The effects of acute ACTH administration during the final third of pregnancy upon the onset of puberty in female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Day of vaginal opening	Weight at vaginal opening	Day of first oestrus	Weight at first oestrus
Undisturbed controls n = 24	36.08 ±0.87	20.70 ±0.38	38.29 ±1.00	21.78 ±0.28
Saline-gel vehicle n = 22	35.00 ±1.04	21.25 ±0.34	36.59 ±1.14	21.81 ±0.33
Low dose ACTH n = 19	38.37[ab] ±0.85	20.03 ±0.31	39.74 ±0.85	20.78 ±0.22
High dose ACTH n = 21	38.10[b] ±1.02	21.13 ±0.39	38.81 ±0.95	21.49 ±0.39
1WANOVA	P = 0.045	N.S.	N.S.	N.S.

a significant difference compared with control $P < 0.05$ (t-test)
b significant difference compared with vehicle $P < 0.01$ (t-test)

Table 7:6. The effects of acute ACTH administration during the final third of pregnancy upon the proportion of female offspring showing vaginal opening and first oestrus. Data given are as numbers of animals

Treatment during late pregnancy	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 39
Vaginal opening							
Undisturbed controls n = 24	2	3	7	11	14	15	19
Saline-gel vehicle n = 22	8[a]	8[a]	10	12	14	14	17
Low dose ACTH n = 19	0[b]	0[b]	2[b]	2[ab]	4[ab]	7	12
High dose ACTH n = 21	2[b]	2[b]	3[b]	4[ab]	7[b]	10	13
First oestrus							
Undisturbed controls n = 24	2	2	5	6	9	10	11
Saline-gel vehicle n = 22	7[a]	7[a]	8	9	9	10	16[a]
Low dose ACTH n = 19	0[b]	0[b]	2[b]	2[b]	3	4	7[b]
High dose ACTH n = 21	2[b]	2[b]	2[b]	2[b]	4	8	12

a significant difference compared with control (P = 0.04-P = 0.003)
b significant difference compared with vehicle (P = 0.04-P = 0.01)
Fisher exact probability tests.

EXPERIMENT 7:5. The effects of ACTH administration during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from 12 control mice, 6 saline-gelatine vehicle-treated mice, 9 low dose ACTH-treated mice and 8 high dose ACTH treated mice.

RESULTS. The effects of acute ACTH administration during late pregnancy upon the oestrous cycle of individually housed and group housed female offspring, are shown in tables 7:7a and 7:7b respectively. Statistical differences were found between experimental groups when offspring were housed individually in the mean number of days of pro-oestrus stages. Females from low dose ACTH-treated mice showed decreased incidence of pro-oestrus stages, over the 21 day observation period compared with offspring from both control and vehicle-injected mice. There were no differences between treatment groups in the mean number of days of oestrus, metoestrus or dioestrus or in the mean length of cycles. Proportional analysis revealed that 12% of control offspring, 25% of offspring from saline-gel vehicle injected mice, 87% of offspring from low dose ACTH-treated mice and 37% of offspring from high dose ACTH-treated mice, showed cycles without an apparent pro-oestrus stage. Significant differences were found between control and low dose ACTH groups ($P = 0.004$) and vehicle and low dose ACTH groups ($P = 0.019$) in the proportion of females showing cycles without a pro-oestrus stage.

Statistical differences were found between experimental groups in the mean number of days of oestrus, when offspring were housed in groups of 5. Females from vehicle-injected mice, low dose ACTH treated mice and high dose ACTH-treated mice, all showed increased incidence of oestrus compared with offspring from control mice. There were no significant differences between the gel vehicle group

and either dose of ACTH in the mean number of days of oestrus. There were no significant differences between experimental groups in the mean number of days of pro-oestrus, metoestrus or dioestrus or in cycle lengths. Proportional analysis of data revealed that 30% of control offspring, 70% of offspring from saline-gel vehicle-injected mice, 90% of offspring from low dose ACTH-treated mice and 60% of offspring from high dose ACTH mice, showed cycles without a pro-oestrus stage. The difference between the control group and low dose ACTH group was significant ($P = 0.009$).

Table 7:7a. The effects of ACTH administration during the final third of pregnancy upon the oestrous cycle of adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days in 21 day test period in each cycle stage				
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	Cycle length (Days)
Undisturbed controls n = 8	3.63 ±0.32	6.63 ±0.73	5.13 ±0.35	5.63 ±0.42	5.60 ±0.38
Saline-gel vehicle n = 8	3.50 ±0.46	6.13 0.40	6.00 ±0.57	5.38 ±0.68	5.04 ±0.25
Low dose ACTH n = 8	2.00* ±0.33	6.38 ±1.12	4.38 ±0.68	8.25 ±1.45	6.38 ±0.86
High dose ACTH n = 8	3.38 ±0.32	6.75 ±0.52	5.38 ±0.32	6.38 ±0.26	5.33 ±0.26
lWANOVA	P = 0.01	N.S.	N.S.	P = 0.08	N.S.

*significant difference compared with control $P < 0.005$ and vehicle $P < 0.01$ (t-tests)

Table 7:7b. The effects of ACTH administration during the final third of pregnancy upon the oestrous cycle of adult female offspring housed in groups. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	Cycle length (Days)
Undisturbed controls n = 10	2.80 ±0.39	2.80 ±0.36	3.80 ±0.26	11.60 ±0.70	5.58[a] ±0.54
Saline-gel vehicle n = 10	1.90 ±0.31	3.90* ±0.35	3.30 ±0.52	11.90 ±0.83	6.98 ±0.97
Low dose ACTH n = 10	1.80 ±0.42	4.60*** ±0.31	4.30 ±0.50	10.20 ±0.93	6.68 ±0.61
High dose ACTH n = 10	2.30 ±0.37	3.70** ±0.34	4.20 ±0.33	10.70 ±0.63	6.73 ±0.66
lWANOVA	N.S.	P = 0.006	N.S.	N.S.	N.S.

a 1 female failed to show a distinct cycle
* significant difference with control $P < 0.05$ (t-test, 2 tailed)
** significant difference with control $P < 0.05$ (t-test)
*** significant difference with control $P < 0.001$ (t-test)

EXPERIMENT 7:6. The effects of chronic corticosterone administration during pregnancy upon the onset of puberty in female offspring. Offspring were derived from 9 control mice, 7 propylene glycol vehicle treated mice and 10 corticosterone-treated mice.

RESULTS. The effects of chronic corticosterone administration during late pregnancy upon the onset of puberty in female offspring are shown in tables 7:8 and 7:9. Female offspring from mice treated with corticosterone during pregnancy showed significantly delayed vaginal opening compared with offspring from control and vehicle-treated mice. Significant differences were found between treatment groups in mean body weight at vaginal opening and mean day of first oestrus. Proportional analysis of data revealed that significantly fewer female offspring from corticosterone-treated mice showed vaginal opening on postnatal days 31-34 inclusive compared with offspring from control or vehicle-treated mice. Although significantly fewer female offspring from corticosterone-treated mice showed first oestrus on postnatal days 31, 33 and 34 compared with offspring from control mice, significantly fewer female offspring from vehicle-treated mice showed first oestrus compared with offspring from control mice. Both chronic corticosterone and propylene glycol vehicle administration during pregnancy retarded first oestrus.

Table 7:8. The effects of chronic corticosterone administration during the final third of pregnancy upon the onset of puberty in female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Day of vaginal opening	Weight at vaginal opening	Day of first oestrus	Weight at first oestrus
Undisturbed controls n = 37	31.13 ±0.35	21.25 ±0.23	31.70 ±0.41	21.48 ±0.22
Propylene glycol vehicle n = 31	32.00 ±0.52	20.35[c] ±0.30	34.16[a] ±0.64	21.13 ±0.31
Chronic corticosterone n = 34	33.35[ab] ±0.55	21.35[b] ±0.25	34.38[a] ±0.56	21.64 ±0.25
lWANOVA	P = 0.004	P = 0.018	P = 0.001	N.S.

a significant difference compared with control P < 0.001 (t-test)
b significant difference compared with vehicle P < 0.05 (t-test)
c significant difference compared with control P < 0.025 (t-test)

Table 7:9. The effects of chronic corticosterone administration during the final third of pregnancy upon the proportion of female offspring showing vaginal opening and first oestrus. Data given are as numbers of animals

Treatment during late pregnancy	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 39
Vaginal opening							
Undisturbed controls n = 37	9	13	23	30	32	33	37
Propylene glycol vehicle n = 31	4	10	19	21	24	26	30
Chronic corticosterone n = 34	4	8	9[a]	17[a]	19[ab]	22[ab]	32
First oestrus							
Undisturbed controls n = 37	8	12	20	25	30	31	37
Propylene glycol vehicle n = 31	3	5	6[a]	13[a]	14[a]	16[a]	28
Chronic corticosterone n = 34	4	8	9[a]	17	19[a]	22[a]	33

a significant difference compared with control (P = 0.04-P = 0.003)
b significant difference compared with vehicle (P = 0.04-P = 0.002)
Fisher Exact Probability tests.

EXPERIMENT 7:7. The effects of chronic corticosterone administration during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from the same pregnant mice used in experiment 7:6.

RESULTS. The effects of chronic corticosterone administration during late pregnancy upon the oestrous cycle of adult female offspring are shown in table 7:10. Statistical differences were found between experimental groups in the mean number of days of oestrus stages. Females from corticosterone-treated mice showed increased incidence of oestrus stages, over the 21 day observation period, compared with offspring from control mice. There were no differences between treatment groups in the mean number of days of pro-oestrus, metoestrus or dioestrus, in the mean length of cycles, or in the proportion of females showing cycles without a proestrus stage.

Table 7:10. The effects of chronic corticosterone administration during the final third of pregnancy upon the oestrous cycle of adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	Cycle length (Days)
Undisturbed controls n = 10	3.70 ±0.30	5.50 ±0.34	6.10 ±0.43	5.70 ±0.58	4.76 ±0.16
Propylene glycol vehicle n = 10	3.50 ±0.22	6.30 ±0.50	5.60 ±0.48	5.50 ±0.62	5.21 ±0.13
Chronic corticosterone n = 10	3.30 ±0.52	7.60* ±0.87	4.70 ±0.40	5.40 ±0.43	5.03 ±0.26
lWANOVA	N.S.	P = 0.06	N.S.	N.S.	N.S.

* significant difference compared with control P < 0.025 (t-test)

EXPERIMENT 7:8. The effects of progesterone administration during pregnancy upon the onset of puberty in female offspring. Offspring were derived from 6 control mice, 6 olive oil vehicle-treated mice, 5 low dose progesterone-treated mice and 6 high dose progesterone-treated mice.

RESULTS. The effects of acute progesterone administration during late pregnancy, upon the onset of puberty in female offspring are shown in tables 7:11 and 7:12. Female offspring from mice treated with either dose of progesterone during pregnancy showed significantly delayed mean day of vaginal opening. There were no significant differences between experimental groups in the mean day of first oestrus or in mean body weights at vaginal opening or first oestrus. Proportional analysis of data revealed that significantly fewer female offspring from progesterone-treated mice showed vaginal opening on postnatal days 31-34 inclusive or first oestrus on postnatal days 32-34 inclusive, compared with offspring from control and vehicle-injected mice. The higher dose of progesterone administered during pregnancy was most effective in retarding puberty.

Table 7:11. The effects of acute progesterone administration during the final third of pregnancy upon the onset of puberty in female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Day of vaginal opening	Weight at vaginal opening	Day of first oestrus	Weight at first oestrus
Undisturbed controls n = 16	33.37 ±0.76	21.50 ±0.47	34.31 ±1.03	21.73 ±0.48
Olive oil vehicle n = 23	33.69 ±0.69	21.71 ±0.46	34.78 ±0.87	22.36 ±0.32
Low dose progesterone n = 16	35.69* ±0.75	21.94 ±0.49	36.75 ±1.12	22.28 ±0.51
High dose progesterone n = 21	35.76* ±0.64	21.55 ±0.36	36.19 ±0.75	21.63 ±0.38
1WANOVA	P = 0.05	N.S.	N.S.	N.S.

* significant difference compared with control P < 0.025 (t-test)

Table 7:12. The effects of acute progesterone administration during the final third of pregnancy upon the proportion of female offspring showing vaginal opening and first oestrous. Data given are as numbers of animals

Treatment during late pregnancy	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 39
Vaginal opening							
Undisturbed controls n = 16	0	4	6	8	9	9	16
Olive oil vehicle n = 23	4	6	8	8	10	12	21
Low dose progesterone n = 16	0	1	1[ab]	4	4[a]	4	14
High dose progesterone n = 21	1	2	2[ab]	2[ab]	3[ab]	4[ab]	20
First oestrus							
Undisturbed controls n = 16	0	4	5	6	7	9	14
Olive oil vehicle n = 23	3	4	4	6	8	11	20
Low dose progesterone n = 16	0	1	1	4	4	4	13
High dose progesterone n = 21	1	2	2	2[a]	3[a]	4[ab]	18

a significant difference compared with control (P = 0.04-P = 0.007)
b significant difference compared with vehicle (P = 0.04-P = 0.029)
Fisher Exact Probability tests.

EXPERIMENT 7:9. The effects of progesterone administration during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from the same pregnant mice used in experiment 7:8.

RESULTS. The effects of acute progesterone administration during late pregnancy, upon the oestrous cycle of adult female offspring, are shown in table 7:13. Statistical differences were found between experimental groups in the mean number of days of oestrus stages. Females from high dose progesterone-treated mice showed increased incidence of oestrous stages, over the 21 day smearing period, compared with offspring from olive oil vehicle treated mice. There were no differences between treatment groups in the mean number of days of pro-oestrus, metoestrus or dioestrus, in the mean length of cycles, or in the proportion of females showing cycles without a pro-oestrus stage.

Table 7:13. The effects of acute progesterone administration during the final third of pregnancy upon the oestrous cycle of adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	Cycle length (Days)
Undisturbed controls n = 12	3.08 ±0.26	6.00 ±0.49	5.42 ±0.58	6.50 ±0.92	6.72 ±0.57
Olive oil vehicle n = 12	3.50 ±0.40	5.00 ±0.33	6.25 ±0.64	6.25 ±0.65	6.13 ±0.44
Low dose progesterone n = 12	3.25 ±0.35	5.66 ±0.31	5.50 ±0.29	6.58 ±0.34	5.44 ±0.10
High dose progesterone n = 12	2.50 ±0.36	7.00* ±0.41	5.50 ±0.34	6.00 ±0.41	5.71 ±0.28
1WANOVA	N.S.	P = 0.007	N.S.	N.S.	N.S.

* significant difference compared with vehicle P < 0.0005 (t-test)

EXPERIMENT 7:10. The effects of androstenedione or acute corticosterone administration during pregnancy upon the onset of puberty in female offspring. Offspring were derived from 8 control mice, 7 peanut oil vehicle-treated mice, 6 androstenedione-treated mice and 8 corticosterone-treated mice.

RESULTS. The effects of acute androstenedione and acute corticosterone administration during late pregnancy, upon the onset of puberty in female offspring are shown in tables 7:14 and 7:15. Female offspring from androstenedione-treated mice showed significantly accelerated vaginal opening and increased body weight at vaginal opening, compared with offspring from peanut oil vehicle-injected mice. Female offspring from androstenedione-treated mice also showed accelerated first oestrus compared with offspring from control and vehicle-injected mice, but there were no differences between experimental groups in body weights at first oestrus. Female offspring from androstenedione-treated mice showed evidence of vaginal abnormalities; during the peripubertal period these animals characteristically showed vaginal hyperaemia (see plates VI-IX). Proportionately more females from androstenedione-treated mice showed this abnormality compared with offspring from control mice ($P = 0.00008$), peanut oil vehicle ($P = 0.0029$) or corticosterone ($P = 0.0007$) treated mice.

Female offspring from corticosterone-treated mice showed delayed vaginal opening and first oestrus and were heavier at vaginal opening compared with offspring from control mice. Proportional analysis of data revealed that significantly fewer female offspring from corticosterone-treated mice showed vaginal opening on postnatal days 32-34 inclusive and day 39, or first oestrus on postnatal days 33-34 inclusive and day 39 compared with offspring from control mice.

Table 7:14. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon the onset of puberty in female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Day of vaginal opening	Weight at vaginal opening	Day of first oestrus	Weight at first oestrus
Undisturbed controls n = 36	33.53 ±0.51	20.66 ±0.41	36.03 ±0.80	21.94 ±0.35
Peanut oil vehicle n = 18	36.33* ±0.66	21.49 ±0.62	38.27 ±1.11	22.19 ±0.45
Acute androstenedione n = 24	33.54** ±0.54	22.10* ±0.47	34.42** ±0.84	22.36 ±0.54
Acute corticosterone n = 24	38.00*[a] ±1.19	22.23* ±0.33	39.66*[a] ±1.21	22.83 ±0.36
1WANOVA	P = 0.0001	P = 0.035	P = 0.002	N.S.

* significant difference compared with control $P < 0.025$ (t-test)
** significant difference compared with vehicle $P < 0.005$ (t-test)
a significant difference compared with androstenedione $P < 0.001$ (t-test)

Table 7:15. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon the proportion of female offspring showing vaginal opening and first oestrus. Data given are as numbers of animals

Treatment during late pregnancy	Day 30	Day 31	Day 32	Day 33	Day 34	Day 39
Vaginal opening						
Undisturbed controls n = 36	4	10	17	21	22	35
Peanut oil vehicle n = 18	0	2	2[a]	2[a]	3[a]	16
Acute androstenedione n = 24	3	6	7	13	17[b]	23
Acute corticosterone n = 24	4	4	4[a]	6[a]	8[a]	15[a]
First oestrus						
Undisturbed controls n = 36	2	5	11	15	16	29
Peanut oil vehicle n = 18	0	2	2	2[a]	3[a]	12
Acute androstenedione n = 24	3	6	7	9	12	21
Acute corticosterone n = 24	1	3	3	5[a]	5[a]	14[a]

a significant difference compared with control (P = 0.05-P = 0.00002)
b significant difference compared with corticosterone (P = 0.0003)
Fisher Exact Probability test.

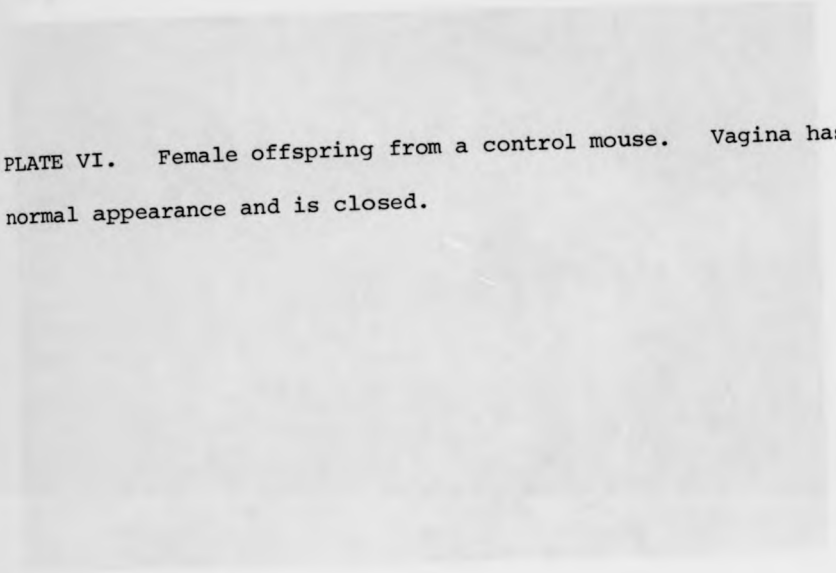


PLATE VI. Female offspring from a control mouse. Vagina has normal appearance and is closed.

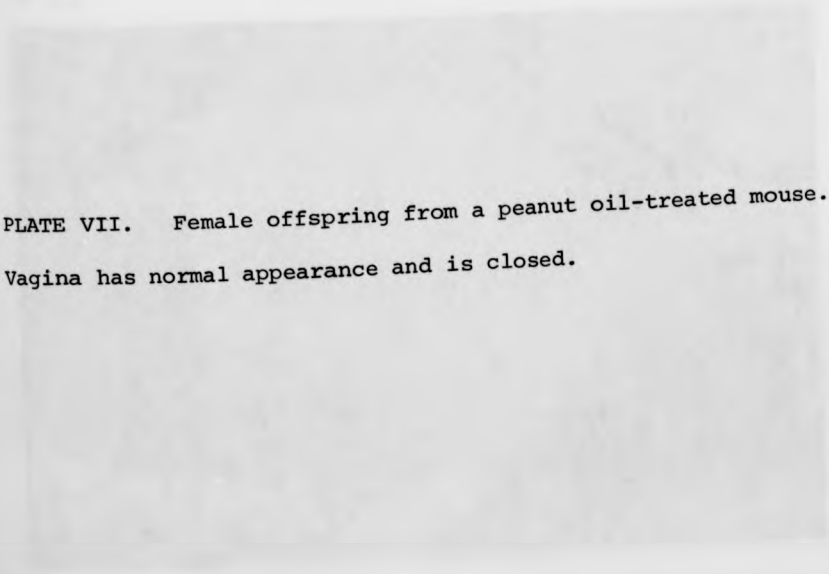
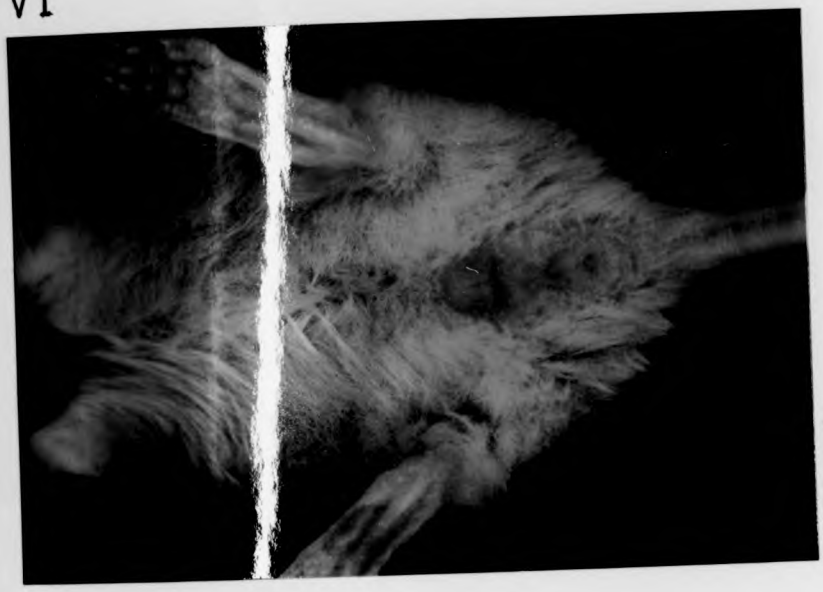


PLATE VII. Female offspring from a peanut oil-treated mouse. Vagina has normal appearance and is closed.

VI

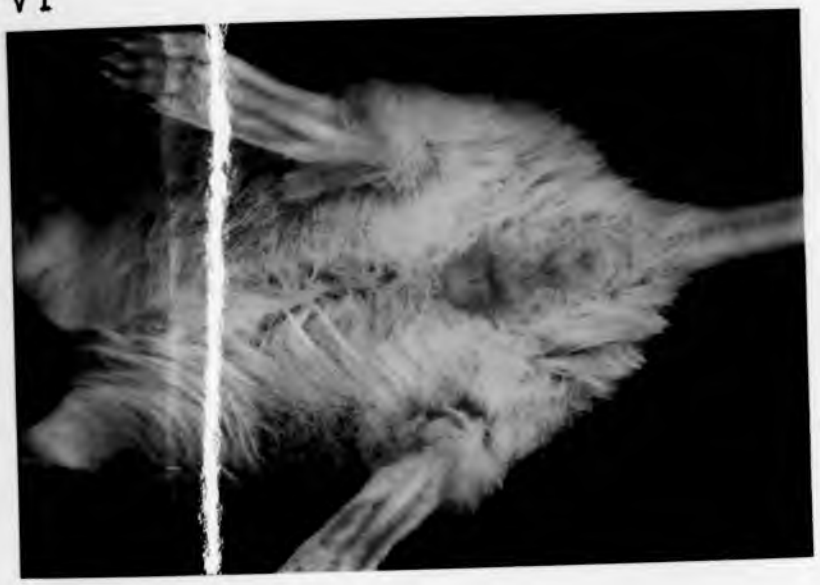


VII

VI

VII

VI

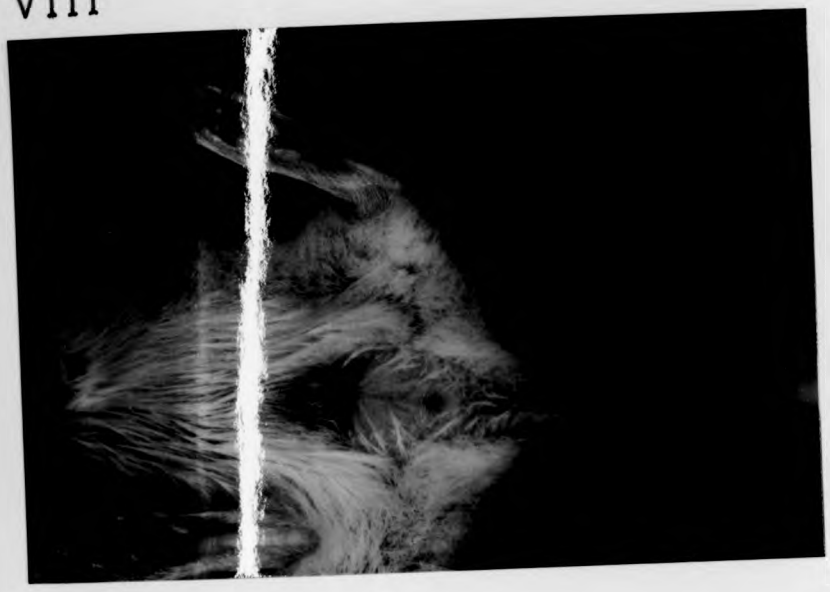


VII

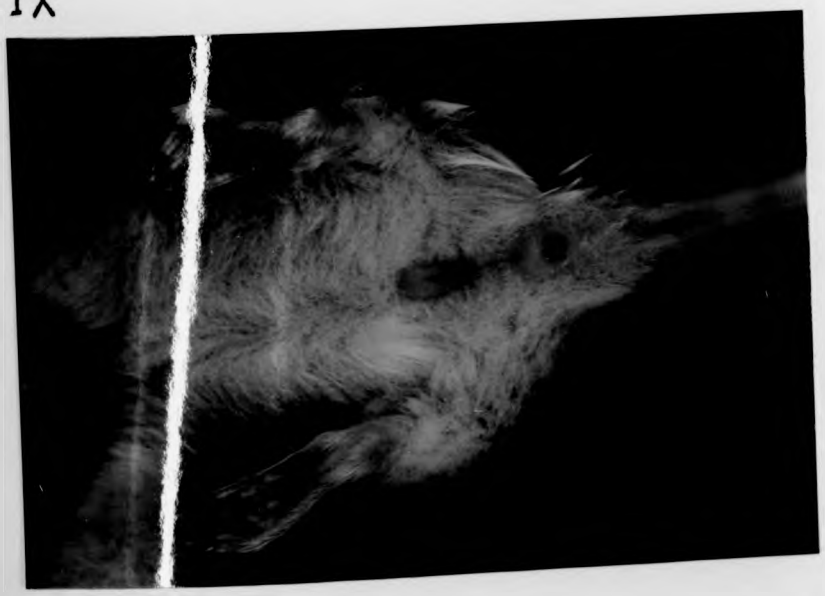
PLATE VIII. Female offspring from an androstenedione-treated mouse. There is distinct reddening of vaginal membranes (hyperaemia) as patency is approached.

PLATE IX. Female offspring from an androstenedione-treated mouse. Vaginal patency has been achieved and there is no evidence of hyperaemia.

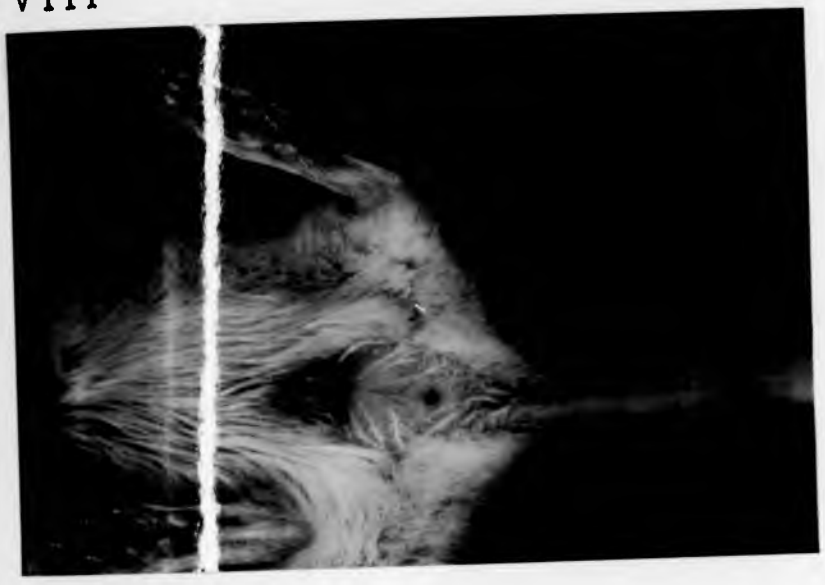
VIII



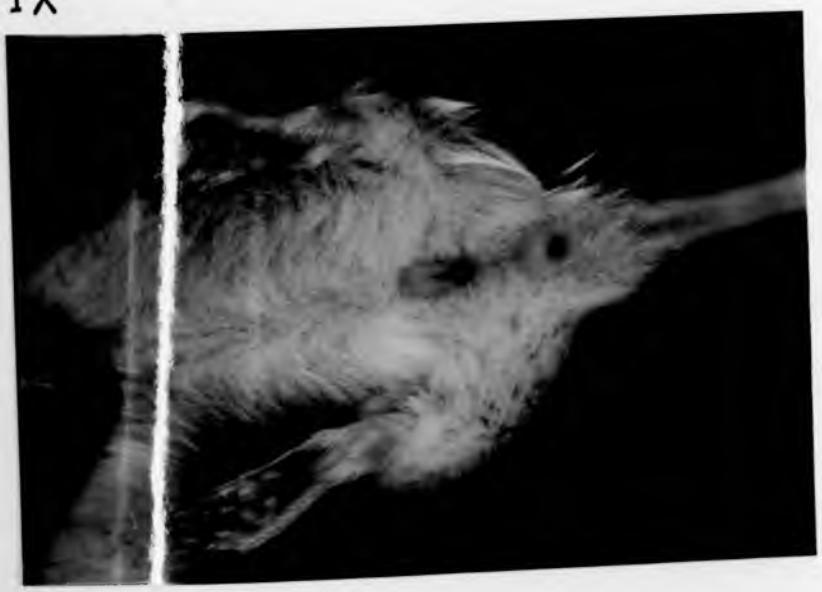
IX



VIII



IX



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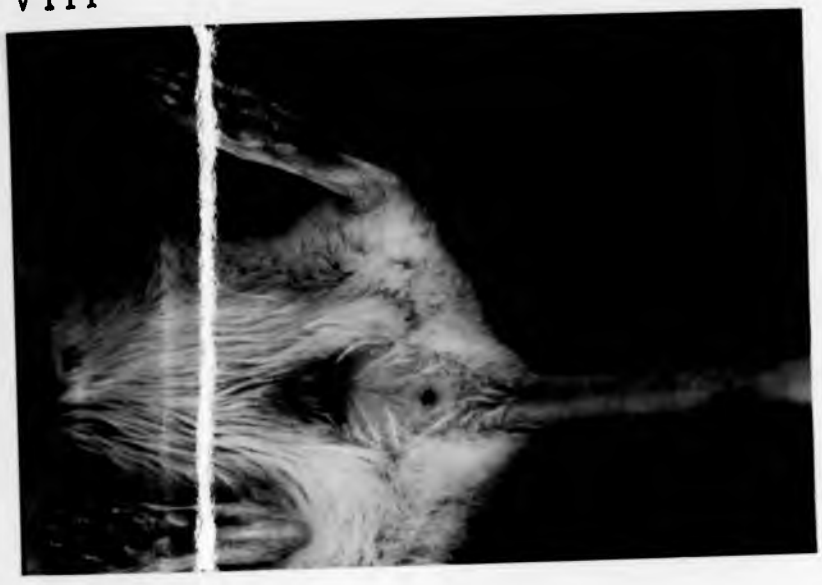

IX

EXPERIMENT 7:11. The effects of androstenedione or acute corticosterone administration during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from the same pregnant mice used in experiment 7:10.

RESULTS. The effects of acute androstenedione and acute corticosterone administration during late pregnancy, upon adult oestrus cycle in female offspring are shown in table 7:11. Statistical differences were found between experimental groups in the mean number of days of pro-oestrus, oestrus and dioestrus stages and in the length of cycles. Female offspring from androstenedione-treated mice showed decreased incidence of pro-oestrus and dioestrus and increased incidence of oestrus, over the 21 day smearing period, compared with offspring from control mice. Proportional analysis revealed that 43% of offspring androstenedione-treated mice compared with 0% of offspring from control, peanut oil vehicle-injected or corticosterone-treated mice, showed persistent oestrus lasting for 6 or more days ($P = 0.001$). Additionally, 36% of females from androstenedione-treated mice failed to show a distinct cycle over the 21 day smearing period compared with 0% of offspring from control, peanut oil injected or corticosterone treated mice ($P = 0.02$). Similarly, proportionately more offspring from mice treated with androstenedione during pregnancy showed cycles without a pro-oestrus stage: 21% of control offspring, 14% of offspring from peanut oil vehicle-injected mice, 93% of offspring from androstenedione-treated mice and 36% of offspring from corticosterone treated mice showed cycles without a pro-oestrus stage. The difference between offspring from androstenedione-treated mice and control offspring was significant ($P = 0.0001$). Offspring from corticosterone-treated mice showed increased incidence of dioestrus stages, over the 21 day smearing period compared with offspring from

control mice. There were no differences between control, peanut oil vehicle or corticosterone treatment groups in the mean number of days of pro-oestrus, oestrus or metoestrus or in the length of cycles.

Table 7:16. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon the oestrous cycle of adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	Cycle length (Days)
Undisturbed controls n = 14	3.93 ±0.29	5.57 ±0.36	6.43 ±0.27	5.07 ±0.29	5.15 ±0.17
Peanut oil vehicle n = 14	3.64 ±0.25	5.36 ±0.36	6.07 ±0.49	5.85 ±0.42	6.14 ±0.85
Acute androstenedione n = 14	1.78** ±0.50	11.57*** ±1.06	5.14 ±0.58	2.50*** ±0.53	6.46[a] ±0.63
Acute corticosterone n = 14	3.71 ±0.19	5.07 ±0.35	5.71 ±0.29	6.42* ±0.44	4.95 ±0.10
lWANOVA	P < 0.0001	P < 0.0001	N.S.	P < 0.0001	N.S.

a 5 females failed to show a distinct cycle (excluded from cycle length analyses)
* significant difference compared with control P < 0.01 (t-test)
** significant difference compared with control P < 0.001 (t-test)
*** significant difference compared with control P < 0.0005 (t-test)

EXPERIMENT 7:12. The effects of adrenalectomy coupled with chronic crowding during pregnancy upon the oestrous cycle of adult female offspring. Offspring were derived from 3 adrenalectomised crowded mice, 5 adrenalectomised control mice, 5 Sham-operated crowded mice and 4 Sham-operated control mice.

RESULTS. The effects of bilateral adrenalectomy on day 9 of pregnancy and chronic crowding during late pregnancy, upon the oestrous cycle of adult female offspring are shown in table 7:17. Statistical differences were found between experimental groups in the mean length of cycles. Offspring from Sham-operated crowded mice and offspring from adrenalectomised crowded mice showed shortened cycle lengths, compared with offspring from Sham-operated control mice and adrenalectomised control mice. There were no significant differences between experimental groups in the mean number of days of pro-oestrus, oestrus, metoestrus or dioestrus stages, but offspring from Sham-surgery crowded mice showed decreased incidence of pro-oestrus stages.

Table 7:17. The effects of chronic crowding stress and adrenalectomy during pregnancy upon the oestrous cycle in adult female offspring. Data given are as means ± S.E.M.

Treatment during late pregnancy	Number of days of 21 day test period in each cycle stage				Cycle length (Days)
	Days of pro-oestrus	Days of oestrus	Days of metoestrus	Days of dioestrus	
Sham-surgery controls n = 10	3.50 ±0.17	4.80 ±0.39	5.30 ±0.54	7.30 ±0.62	5.75 ±0.41
Sham-surgery stressed n = 11	2.54 ±0.53	5.27 ±0.74	5.18 ±0.64	7.91 ±0.91	4.85[a] ±0.06
Adrenalectomy controls n = 11	3.45 ±0.34	5.45 ±0.45	5.27 ±0.38	6.64 ±0.53	5.32 ±0.30
Adrenalectomy stressed n = 9	3.66 ±0.37	5.33 ±0.55	4.78 ±0.49	7.22 ±1.27	4.46* ±0.14
lWANOVA	N.S.	N.S.	N.S.	N.S.	P = 0.016

a 2 females failed to show a distinct cycle
* significant difference compared with Sham-surgery controls $P < 0.01$ and adrenalectomy controls $P < 0.025$ (t-test)

DISCUSSION

The results of experiment 7:1 show that the stress of chronic crowding during the final third of pregnancy delays the sexual development (as assessed by monitoring vaginal opening and first oestrus) of the female offspring. This was not due to delayed body weight gain, as offspring from crowded mice were heavier at puberty compared with offspring from control mice. The finding that crowding during pregnancy retards the onset of puberty in female offspring, compares well with a recent study reporting delayed vaginal opening in female offspring born to mice that were restrained during pregnancy (Politch and Herrenkohl, 1984a) and with other developmental consequences known to result from stress during pregnancy (see Chapter 5).

The permanency of the effects of crowding during pregnancy upon female offspring reproductive development and function, is demonstrated by the detection of abnormal oestrous cycles in adulthood (experiment 7:2). Specifically, offspring from stressed mice show fewer occasions of pro-oestrus and more cycles without a detectable pro-oestrus stage. It is most likely that this result reflects a shortening, rather than absence, of the pro-oestrus stage such that the 24-hourly testing regime failed to detect this phase of the cycle. Even within the control group, some females show cycles apparently lacking in this short stage, which in rats normally lasts 18 hours (Feder, 1981). The mouse oestrous cycle is notably erratic (Bingel and Schwartz, 1969) but can be compared with that of the rat. Although this study found no statistically significant differences in the frequency of other cycle stages or in cycle lengths, between offspring from crowded or control mice, the results are broadly in line with those of Herrenkohl and Politch (1978) in their study on rats. Additionally, although female offspring from crowded mice

were not significantly slower to mate (as monitored by the appearance of vaginal plugs) and did not show abnormal pregnancies, compared with offspring from control mice (experiment 7:3) they were mounted less in a 20 minute test period. This result suggests that these females are less attractive, or less behaviourally receptive, and compares well with previous studies of the behavioural receptivity of female offspring from crowded mice (Allen and Haggett, 1977).

The working hypothesis of this thesis has been that products from the maternal pituitary-adrenocortical system, released in conditions of stress, produce the effects of stress during pregnancy upon parameters of offspring development. Hormones known to be secreted by this system were administered to pregnant mice to attempt to reproduce the effects of stress. Similarity of the effects of a hormone treatment with those resulting from crowding, is taken as evidence to support the working hypothesis and to identify possible teratogenic agents.

ACTH administration during pregnancy significantly delayed one parameter of puberty, vaginal opening, but did not significantly delay first oestrus in female offspring (experiment 7:4). There were no differences in body weights at either of these times between offspring from ACTH-treated or control mice, and the possibility that the delay in sexual maturation observed in offspring from ACTH-treated mice, is due to delayed body weight gain cannot be excluded. Offspring from ACTH-treated mice show severely reduced body weights at weaning compared with controls (Chapter 5) and this is likely to persist peripubertally. Despite the inability to exclude general maturational factors as causing the retardation of sexual development evident in female offspring from ACTH-treated mice, it is clear that this hormone has a general deleterious effect upon offspring development.

Monder, Yasukawa and Christian (1981) have shown that perinatal administration of ACTH retards vaginal opening and other developmental milestones, but their study failed to examine other correlates of puberty.

The results from experiment 7:5 show that individually housed female offspring from mice treated with ACTH during pregnancy, show fewer occasions of pro-oestrous stages in the adult cycle. This result indicates that the effects of ACTH administration during pregnancy upon offspring sexual development persist into adulthood, is very similar to the effect produced by crowding and indicates an effect specifically on reproductive hormones. There were no detectable differences in the oestrous cycles of offspring from ACTH-treated or control mice when housed in groups. In this housing condition, all cycles were lengthened regardless of treatment group.

It is not surprising that the results from the experiments of the effects of ACTH administration during pregnancy upon offspring reproductive development and function, do not reproduce those from the study of the consequences of crowding with complete fidelity, since the hormone dosage and administration regime can only crudely imitate the endocrine response of a stressed animal. Despite this, the similarity of the consequences for reproductive development of ACTH administration during pregnancy, with those of crowding, generally support the working hypothesis. Both crowding and ACTH administration during pregnancy delays vaginal opening and disrupts the adult oestrous cycle. The likely endocrine causes of these phenomena and the control of puberty and reproductive cycles, are reviewed in detail later. As the intact ACTH molecule does not normally cross the placenta (Chapter 2) the effects of crowding and ACTH administration during pregnancy are probably due to foetal exposure to an ACTH

driven steroid and this possibility was examined.

Female offspring from mice treated chronically with corticosterone during late pregnancy (experiment 7:6) also showed significantly delayed vaginal opening, compared with offspring from control or vehicle treated mice. The results were confused by the finding that the control and vehicle groups showed differences in the timing of first oestrus. Additionally, the possibility that the retardation of vaginal opening is due to delayed weight gain once more cannot be excluded, as there were no differences between groups in body weights at this development stage. However, adult offspring from mice chronically treated with corticosterone during pregnancy did show significantly increased incidence of oestrus stages in their cycles (experiment 7:7). The increased incidence of oestrus was not an effect observed in the cycles of offspring from either crowded or ACTH treated mice, but was reported by Herrenkohl and Politch (1978) in the oestrous cycles of offspring from rats restrained during pregnancy.

Additional study was made of sexual development and function in female offspring from corticosterone treated mice: in these experiments the effects of acute corticosterone administration was studied. Acute corticosterone administration during pregnancy also delayed parameters of puberty. Offspring from mice treated in this way showed delayed vaginal opening and first oestrus, and were heavier at vaginal opening compared with offspring from control mice (experiment 7:10). Although these results are in general more convincing of an effect of corticosterone treatment during pregnancy upon female offspring reproductive development, than those yielded from the experiment of the effects of chronic corticosterone treatment, they are unfortunately complicated by the finding that the vehicle group differed from controls

with respect to vaginal opening. Finding completely inert vehicles in which to deliver hormones was a problem. Corticosterone is difficult to dissolve, and whilst this compound readily dissolves in propylene glycol (a solvent compatible with minipumps) and forms a stable suspension in peanut oil, both of these compounds seem to have specific effects upon the onset of puberty in females. Consequently, that corticosterone is the agent responsible for the effects of crowding or ACTH administration during pregnancy upon sexual development of the female offspring is still open to debate.

The effects of acute corticosterone administration during pregnancy upon the oestrous cycle of female offspring (experiment 7:11) were found to be different from the effects of chronic corticosterone administration. Rather than showing increased incidence of oestrus stages, female offspring from mice acutely treated with corticosterone during pregnancy, showed increased incidence of dioestrus stages, compared with offspring from control mice. If this effect is genuine, then the teratogenic effect of corticosterone upon the oestrous cycle appears to be variable with dosage and chronicity of the administration regime. Acute corticosterone administration during pregnancy did not affect the oestrous cycles of female offspring in a similar manner to either crowding or ACTH.

Both progesterone and androstenedione were also tested as steroids responsible for producing the effects of crowding during pregnancy upon the onset of puberty and the oestrous cycle. Like corticosterone, these compounds are secreted from the adrenal cortex under the influence of ACTH (Chapter 2).

Female offspring from mice treated with progesterone (either dose) showed significantly delayed vaginal opening compared with control mice (experiment 7:8). Although these animals also showed delayed

first oestrus, the results failed to achieve statistical significance and there were no significant differences between treatment groups in body weight at either vaginal opening or first oestrus. Experiment 7:9 investigated the oestrous cycles of these animals. Female offspring from mice treated with the higher dose of progesterone during pregnancy showed significantly increased incidence of oestrus compared with offspring from control mice, and this result was most similar to the effects of chronic corticosterone, rather than to crowding or ACTH administration during pregnancy.

The final steroid administered during pregnancy to reproduce the effects of crowding upon female offspring sexual development and function was androstenedione. The onset of puberty in female offspring from androstenedione treated mice was accelerated when compared with offspring from peanut oil-treated mice or unchanged when compared with control offspring (experiment 7:10). Whilst this result seems to exclude this compound as an agent responsible for producing the effects of crowding during pregnancy upon female offspring sexual maturation, caution must be exercised in interpreting results as differences were found between control and peanut oil groups. Although the onset of puberty was not delayed in female offspring from androstenedione-treated mice, other reproductive abnormalities were detected. A large proportion of these animals showed hyperaemia of the vaginal membranes throughout the peripubertal period (see plates VI-IX). This condition was not observed in any other treatment group. Additionally, offspring from mice treated with androstenedione during pregnancy showed severe abnormalities of the oestrous cycle: these animals showed decreased incidence of pro-oestrus and dioestrus stages, increased incidence of oestrus stages and lengthened cycles (experiment 7:11). These results extend from the effects of stress during pregnancy as found in experiment

7:2, but when compared with the results of Herrenkohl and Politch (1978) in addition to those observed in this study, they are similar to the general consequences of stress during pregnancy. Although no previous studies have examined reproductive development and function in female offspring from rodents treated during late pregnancy with androstenedione (or corticosterone or progesterone) comparisons can be drawn with other studies reporting the consequences of prenatal androgenisation of females. Female mouse foetuses exposed to testosterone from adjacent males (Vom Saal and Bronson, 1980) and female offspring offspring from hamsters treated with testosterone during pregnancy (Landauer, Attas and Liu, 1981) show lengthened oestrous cycles in adulthood and increased ano-genital distances (see also Chapter 2).

As a further examination of the hypothesis that maternal adrenal products are responsible for producing the effects of stress during pregnancy upon female offspring reproductive development and function, an experiment (experiment 7:12) was conducted to study whether maternal adrenalectomy prevents the observed effects of crowding. Whilst the offspring from adrenalectomised and stressed mice showed no abnormalities of the oestrous cycle (and in fact showed significantly shortened cycle lengths) neither did offspring from Sham-surgery and stressed mice. That offspring from adrenalectomised and stressed mice showed no abnormalities of the oestrous cycle supports the central hypothesis but the results from this experiment should be regarded with caution because of the failure to detect any effects of stress alone. A high mortality rate was experienced in the adrenalectomised group, which reduced the number of litters to be used for experimental purposes. Additionally, it is possible that fragments of adrenal regenerated over the 10 day period to birth of

litters. Together these factors make results suspect, and further work is necessary to establish whether adrenalectomy during pregnancy has any effect on reproduction in female offspring, or whether the maternal adrenal gland is required for the effects of crowding stress upon female offspring sexual development and function. Overall, the results from the experiments reported in this chapter give no indisputable evidence in support of, or against the working hypothesis. However, it is still necessary to examine the mechanisms controlling puberty and the oestrous cycle, and how the various treatments during pregnancy may cause disruption of these processes.

There are 3 known major influences upon the onset of puberty in female rodents: body weight during the peripubertal period, hypothalamo-pituitary-ovarian function and hypothalamo-pituitary-adrenal function. Correlational studies reveal that a critical body weight needs to be attained prior to appearance of the secondary sexual correlates of puberty (vaginal opening and first oestrus) in rats (Meijs-Roelofs and Moll, 1978). This seems also to be the case for humans (Johnson and Everitt, 1980). In order to exclude somatic factors from causing the retardation of puberty, increased body weight at puberty should be shown. Female offspring from crowded mice show increased body weight at puberty, as well as delayed puberty, and slower body weight gain can therefore be excluded as causing the observed retardation of sexual development. However, this was not the case in experiments in which hormones were administered and ACTH, corticosterone and progesterone treatment during pregnancy may delay sexual development by delaying body weight gain instead of, or in addition to more specific mechanisms. As previously mentioned, administration of hormones individually can only crudely imitate the endocrine response of a stressed animal, and the compounds used may

well exert general effects on body development (Chapter 5). However, an endocrine cause seems to be indicated in producing retarded sexual maturation in female offspring born to crowded mice.

The secondary sexual correlates of puberty, vaginal epithelial proliferation, and cell cornification are dependent upon the action of oestrogens (Emmens, 1962; Ramaley, 1979; Feder, 1981). A delay in the onset of puberty ultimately suggests disruption of oestrogen secretion, either in terms of timing or quantity of hormone secretion. The secretion of oestrogens from maturing ovarian follicles is under the influence of Follicle Stimulating Hormone (FSH). FSH concentrations rise in pituitary and plasma of rats approaching puberty under the influence of Gonadotrophin releasing hormone (GnRH or LHRH; Chappel, Ulloa-Aguirre and Ramaley, 1983; Hompes, Vermes, Tilders and Schoemaker, 1982). FSH stimulates ovarian development in peripubertal rodents (Meijs-Roelofs, Osman and Kramer, 1982; Uilenbroek, Arendsen de Wolff-Exalto and Welschen, 1976) and is essential for the normal onset of puberty in rodents (Ramaley, 1979, 1982). It is therefore a possibility that female offspring from mice crowded during pregnancy show retarded sexual development because of some alteration to the normal pattern of FSH secretion.

FSH secretion is itself promoted by prolactin (PRL; Voogt, Clemens and Meites, 1969) and PRL is thus also involved in the control of the onset of puberty. Inhibitory dopaminergic systems control the secretion of PRL: dopamine and PRL concentrations vary inversely at the pro-oestrus and oestrus stages of the cycle (Advis, Simkins, Chen and Meites, 1978). In the rat, PRL activates the peripubertal ovary (Andrews and Ojeda, 1981) and influences follicular activity and development (Uilenbroek, van der Schoot, Besten and Lankhorst, 1982). PRL levels in the plasma of female rats rise steadily until

puberty (Becu and Libertun, 1982). Direct confirmation of the role of PRL in controlling puberty has come from studies investigating the consequences for sexual development of manipulating this hormone. Administration of PRL to immature female rats accelerates puberty (Lung and Docke, 1981; Wuttke and Gelato, 1976) whilst suppression of PRL (but not of FSH or LH) by administration of bromoergocriptine to immature rats, delays puberty (Advis, Smith-White and OJeda, 1981). PRL probably exerts its effects on puberty by influencing FSH and consequently oestrogen secretion, and these compounds in turn regulate the secretion of PRL through a negative feedback loop (D'agata, Aliffi, Maugeri, Mongioi, Vicari, Gulizia and Polosa, 1982). Although in rats PRL accelerates puberty, and PRL removal retards puberty, there is the suggestion of a reverse effect in the mouse: male pheromones accelerate puberty in females possibly by suppressing PRL secretion (Keverne and De la Riva, 1982). Nevertheless, alteration of normal patterns of PRL secretion may account for the delays of sexual maturation evident in female offspring from mice stressed during pregnancy. The possible causes of the delay of sexual maturation and disruption of the oestrous cycle is discussed later.

The third major factor controlling puberty is the adrenal gland. The adrenal is an important "timer" initiating the onset of puberty (Gorski and Lawton, 1973; Macfarland and Mann, 1977; Meijs-Roelofs and Moll, 1978; Ramaley, 1979, 1982) and corticosterone is particularly important for this process (Meijs-Roelofs and Moll, 1978). Adrenal steroids are known to influence gonadotrophin secretion (e.g. Mann, Jackson and Blank, 1982; Mann, Korowitz and Barraclough, 1975; Mann and Barraclough, 1973; Weber, Ooms and Vreeburg, 1982). More specifically, corticosterone and related compounds can suppress PRL secretion (Gala, Kothari and Haisenleder, 1982; Bratusch-Marrain,

Vierhapper, Waldhausl and Nowotny, 1982). Thus adrenal influences upon reproduction are likely to be mediated via gonadotrophins.

The endocrine control of the oestrous cycle is complex and has previously been introduced in discussing the mechanism controlling puberty. Feder (1981) reviews the literature regarding oestrous cycles in rodents and mammals. However, it is necessary to point out that in addition to gonadotrophins and sex steroids, the adrenal may also be involved in controlling the oestrous cycle, since fluctuations in corticosterone concentrations in plasma have been detected throughout the oestrous cycles of mice (Nichols and Chevins, 1981a) and other rodents.

Having discussed the factors controlling puberty and the oestrous cycle, it is now necessary to examine how the various treatments mediate their effects. The model proposed in this study is that the effects of maternal stress are mediated through ACTH and adrenal steroids and there is some support for this hypothesis. The questions remaining to be answered are what are the ultimate endocrine causes of the described effects upon reproductive development and function, in offspring from crowded mice.

The delay in the onset of puberty, and the shortening of the pro-oestrus stage of the oestrous cycle observed in offspring from crowded mice suggests that these animals have some disruption of oestrogen secretion and consequently gonadotrophins. Neuroendocrine development is initiated during intra-uterine life. In both rats and mice gonadotrophin regulating structures develop and are functional during prenatal life and the foetus can secrete FSH, LH and PRL (Daikoku, Adachi, Kowano and Wakabayashi, 1981; Jenkin, McMillen and Thorburn, 1979; Pointis and Mahoudeau, 1976) there is thus the potential for disruption of the development of such structures

by factors exerting an influence during critical periods. In fact, Bhanot and Wilkinson (1982) have shown that prenatal haloperidol exposure retards the onset of puberty in female rats by increasing dopaminergic inhibition of PRL secretion during postnatal life. There are several lines of evidence to suggest that offspring from stressed rodents are PRL-deficient. Firstly, female offspring from rats restrained during pregnancy have increased dopamine concentrations in the arcuate nucleus (Moyer, Herrenkohl and Jacobwitz, 1978). This structure is concerned with the control of gonadotrophins including PRL (Arimura and Findlay, 1974). Secondly, such females in adulthood exhibit reduced post-partal circulatory PRL concentrations (Herrenkohl and Gala, 1979) whilst male offspring from restrained rats show decreased secretion of both PRL and corticosterone in response to ether administration (Politch and Herrenkohl, 1978). The hypothesis that hypoprolactinaemia may be a cause of the delay in puberty and shortening of the pro-oestrus stage is attractive because as well as explaining the observed results through a reduction of oestrogens (via FSH) it also fits the general working hypothesis (that adrenocortical products from the mother mediate the effects of crowding during pregnancy upon the offspring) in that PRL secretion is inhibited by corticosterone (Gala, Kothari and Haisenleder, 1982). Exposure to increased concentrations of corticosterone during prenatal life (following crowding, or ACTH or corticosterone treatment during pregnancy) may specifically alter PRL secretion later in life.

A second possible explanation of the cause of the retardation of puberty and disruption of the oestrous cycle in offspring from crowded mice concerns adrenal function in these animals. Offspring from restrained rats show adrenal atrophy (Dahlöf, Hard and Larsson, 1978) and decreased stress secretions of corticosterone (Politch, Herrenkohl

and Gala, 1978). As the adrenal is important in reproductive development and function, altered adrenal output can be expected to disrupt these processes. Offspring from rodents treated with ACTH (e.g. Milkovic, Milkovic, Sencar and Paunovic, 1970) or corticosterone and related glucocorticoids (e.g. Edward-Davis and Plotz, 1954; Skebelskaya, 1968; Klepac, 1982) also show adrenal atrophy, and therefore the adrenal underdevelopment shown in offspring from stressed rodents is probably due to maternal pituitary-adrenocortical activity, and foetal exposure to corticosterone. The extent to which the effects of crowding during pregnancy (and also ACTH or corticosterone treatment) upon female offspring sexual maturation and function are due to alterations in PRL secretion or adrenal output is unknown.

Another possible endocrine cause of the effects upon puberty and the oestrous cycle concerns ovarian development. Prenatal glucocorticoid (dexamethasone) exposure has been shown to alter nucleic acid ratios in ovary (Klepac, 1982) and this may influence the endocrine capability of this gland. Alteration of the ovary's sex steroid output may therefore also be implicated in producing the retardation of puberty and disruption of the oestrous cycle in offspring from mice crowded or treated with ACTH or corticosterone during pregnancy. Little study has been made of the endocrine status of offspring from stressed rodents. A worthwhile area for future study would be to examine gonadotrophin, sex steroids and pituitary-adrenal hormone concentrations in these animals at all stages of development and reproductive condition.

A more general hypothesis has been suggested by Ward and Weisz (1980) to account for the alterations in sexual differentiation and reproductive behaviour in male offspring from rats restrained during pregnancy, but which can be applied to the results of this

study, concerns the desynchronisation of brain development from endocrine development. Ward and Weisz (1980) showed an acceleration of the foetal testosterone surge in offspring from stressed rats. If a general consequence of prenatal stress is to desynchronise neuroendocrine development (particularly of systems controlling sexual differentiation and reproduction) then this phenomenon may be a cause of the abnormalities detected in sexual maturation and function in female offspring from crowded mice. At present it is not known what factors desynchronise brain development from the development of other organ systems, but it is known that crowding (Chevins, unpublished) ACTH or corticosterone administration during pregnancy (Chapter 6) retards development of the central nervous system and the development of reflexes in offspring.

Even though no single hormone administration regime completely matched the effects of crowding during pregnancy upon the onset of puberty or the oestrous cycle of offspring, and therefore cannot be considered as sole maternal agent mediating the effects of stress, some treatments did have effects upon the offspring which have yet to be discussed. Both progesterone and androstenedione treatment during pregnancy had an effect either on puberty or the oestrous cycle of the offspring, and as in the case of stress there are several mechanisms by which these effects may have been caused.

Progesterone is known to inhibit FSH and LH secretion in rats (Weber, Ooms and Vreeburg, 1982). Wuttke and Gelato (1976) report that progesterone treatment can retard puberty (vaginal opening) by suppressing PRL secretion. Progesterone treatment prenatally as a result of administration during pregnancy in this study has also been shown to retard vaginal opening, and this may be due to an effect on PRL secretion or more generally on other neuroendocrine regulating

systems which have previously been stated to be vulnerable to alteration during foetal development. Progesterone administration during pregnancy is also known to decrease brain nucleic acid concentrations and brain growth (Coyle, Anker and Cragg, 1976; Snyder, Hull and Roth, 1979) and this action may cause a desynchronisation of brain from endocrine development or damage to developing neuroendocrine structures, the possible effects of which have previously been discussed. Additionally, there is evidence that offspring from rats treated with progesterone during pregnancy show decreased plasma corticosterone concentrations in early life (Roudier, Portha and Picon, 1982) and this phenomenon may also be implicated in causing the delay in puberty and disruption of the oestrous cycle, evident in offspring from mice treated with progesterone during pregnancy as reported here.

The final steroid administered during pregnancy to attempt to reproduce the effects of crowding during pregnancy, upon the onset of puberty and the oestrous cycle of female offspring, was androstenedione. In contrast to the effects of all other treatments, offspring from androstenedione-treated mice showed at least a normal timing for the onset of puberty. It can be deduced that androstenedione is not the maternal agent mediating the effects of stress during pregnancy upon the onset of puberty in female offspring. Additionally, these animals showed severe abnormalities of the oestrous cycle, and most striking was the predominance of oestrus stages in the cycle. These results suggest that offspring from androstenedione-treated mice hypersecrete oestrogens, which is further supported by the finding that these animals showed hyperaemia of vaginal membranes. So how may androstenedione treatment during pregnancy influence the oestrous cycle of female offspring? Androstenedione administration during pregnancy is known to masculinise the sexual responses of male

offspring (Gilroy and Ward, 1978) and prenatal androgen exposure masculinises female rodents and lengthens their oestrous cycles (e.g. Landauer, Attas and Liu, 1981; Vom Saal and Bronson, 1980). The process of sexual differentiation is well understood and has been reviewed in Chapter 2. Essentially, androgens are aromatised to oestrogens in neural tissue and it is these latter compounds that masculinise the brain: testosterone being converted to oestradiol and androstenedione being converted to oestrone (Naftolin and Ryan, 1975). The effects of androstenedione administration during pregnancy upon the oestrous cycles of female offspring may be caused by exposure of the foetal brain to oestrone, and interaction of this compound with developing oestrogenic receptors which are present in the foetal rodent hypothalamus (Maclusky, Lieberburg and McEwen, 1979). Masculinisation of this structure could well result in altered hypothalamo-pituitary-ovarian output, which would be detected in oestrous cycles. A more peripheral effect of androgens is that they interfere with ovarian oestradiol secretion (Bagnell, Mills, Costoff and Mahesh, 1982) and therefore the abnormal oestrous cycles of female offspring from androstenedione treated mice may be caused by altered ovarian oestrogen biosynthesis and secretion. The severity of the effects of androstenedione treatment during pregnancy upon the oestrous cycle of the female offspring must also exclude this compound as the mediating agent producing the effects of crowding. However it still remains a possibility that a more natural dose may have yielded more meaningful results.

It is apparent from the results of this study that the evidence in support of the hypothesis that the effects of stress during pregnancy are mediated by maternal pituitary-adrenal products, is inconclusive. Although all the hormone treatments during pregnancy produced elements

of the syndrome described in female offspring from crowded mice, no one treatment completely reproduced the effects of crowding. This may be a reflection of the inadequacy of the hormone treatment regimes which neither match closely the endocrine response to crowding, nor severely interfere with reproductive development of female offspring. Certainly, a single teratogenic steroid has not been identified. In fact, it is probable that female offspring from crowded mice are exposed to abnormal concentrations of many different steroids *in utero* and together these steroids alter reproductive development. If this is so then it will be very difficult to artificially reproduce the hormonal factors which cause the prenatal stress syndrome. Alternatively, oestrogens secreted from the maternal adrenal in conditions of stress may mediate the effects of crowding. The consequences of oestrogen treatment during pregnancy for female offspring sexual development was not studied here, but oestrogens must be considered prime candidates for future work because of their importance in brain catecholamine metabolism (Breur, Schneider, Wandscheer and Ladosky, 1978) sexual differentiation, and their influence in control of gonadotrophin secretion. Further, oestradiol administration during pregnancy in rats is known to cause hypo-prolactinaemia in the offspring (Kuhn and Bollen, 1981; Kuhn, Bollen and Darras, 1982) and it is likely that sexual development may also be influenced. Finally, although this study was unable to supply firm evidence as to whether pituitary-adrenal products mediate the effects of crowding during pregnancy upon parameters of reproductive development in female offspring, it has shown that females are particularly susceptible to prenatal hormonal factors altering sexual development and function, and this contrasts with the robustness of males examined in the following chapter.

CHAPTER 8

EFFECTS UPON SEXUALLY DIFFERENTIATED BEHAVIOUR IN MALE OFFSPRING

INTRODUCTION

Whilst the effects of stress during pregnancy upon male offspring sexual behaviour are well known in the rat (Dahlöf, Hard and Larsson, 1977; Dunlap, Zadina and Gougis, 1978; Götz and Dörner, 1980; Masterpasqua, Chapman and Lore, 1976; Meisel, Dohanich and Ward, 1979; Ward, 1972) the involvement of the maternal and foetal pituitary-adrenal axes remains uncertain (Chapman and Stern, 1978). Male offspring from rats crowded (Dahlöf, Hard and Larsson, 1977) or restrained (Chapman and Stern, 1978; Dahlöf, Hard and Larsson, 1978; Herrenkohl and Whitney, 1976; Meisel, Dohanich and Ward, 1979; Ward, 1972) during the final third of pregnancy show augmented feminine sexual responses. Male offspring from rats restrained during pregnancy (Dunlap, Zadina and Gougis, 1978; Götz and Dörner, 1980; Ward, 1972) or avoidance conditioned before mating (Masterpasqua, Chapman and Lore, 1976) show impaired masculine copulatory responses.

Similarly, Chapman and Stern (1978) and Dahlöf, Hard and Larsson (1978) report that male offspring from rats restrained or crowded during pregnancy show morphological abnormalities of the genitalia: these animals show decreased ano-genital distances and this is attributed to interrupted androgenisation of the genital system. In fact, restraint during pregnancy is known to accelerate the foetal male's testosterone surge (Ward and Weisz, 1980) and reduce plasma testosterone concentrations in the neonatal male offspring in rats (Dörner, 1980). Additionally, restraining rats during pregnancy has been shown to reduce stress-induced secretion of prolactin and corticosterone (Politch, Herrenkohl and Gala, 1978)

and alter catecholamine neurotransmitter concentrations in discrete brain regions (Moyer, Herrenkohl and Jacobwitz, 1978). Together, these results show that stress during pregnancy alters endocrine function, and particularly the control of the hypothalamo-pituitary-gonadal system during the perinatal period, which is a critical period for sexual differentiation of the brain (see Chapter 2).

It has been suggested that this behavioural "demasculinisation" and "feminisation" of male offspring from rats stressed during pregnancy, is mediated by stress-induced activation of the maternal pituitary adrenocortical axis (Ward, 1972; Dahlöf, Hard and Larsson, 1977). Manipulation of the maternal pituitary-adrenal system during pregnancy can affect development of the foetal adrenal (Jones, Lloyd and Wyatt, 1953; Milkovic, Milkovic and Paunovic, 1973; Milkovic, Milkovic, Sencar and Paunovic, 1970) but until recently, there has been little evidence that maternal pituitary-adrenal manipulation during pregnancy can influence masculine sexual behaviour of the adult male offspring. Chapman and Stern (1978) originally examined this possibility by injecting pregnant rats daily with one of two doses of ACTH, and observing the sexual responses of the adult male offspring. They reported that although the male offspring showed augmented feminine sexual responses, such subjects did not display severe decrements to masculine sexual responses, except for increased post-ejaculatory intervals. However, more recently Stylianopoulou (1983) using similar doses of ACTH to Chapman and Stern, and Rhees and Fleming (1981) using much higher doses, have shown more severe decrements in the masculine sexual responses of adult male offspring in rats.

The studies mentioned so far have all used the rat as the experimental animal. In the mouse, there is no clear evidence that stress during

pregnancy impairs the sexual responses of male offspring. Male offspring from mice crowded during pregnancy show lengthened ejaculatory latencies (Allen and Haggett, 1977) but Politch and Herrenkohl (1984a) report that male offspring from mice restrained during pregnancy show no impairment of masculine copulatory responses, even though these animals were behaviourally feminised. Some study has been made of whether manipulation of the maternal pituitary-adrenal system, by administration of ACTH or corticosterone, can reproduce the effects of stress during pregnancy, upon male offspring behaviour patterns. Politch and Herrenkohl (1984b) have shown that ACTH or corticosterone acetate administration during pregnancy decreases copulatory activity of male offspring in mice, and this is similar to the effects of stress. Similarly, Simon and Gandelman (1977) report that male offspring from ACTH-treated mice are less likely to display aggression, and this result represents the disruption of a second testosterone-dependent sexually dimorphic pattern of behaviour. There has been no study of the effects of stress during pregnancy upon male offspring aggression in any species.

This study is a more detailed investigation of the consequences of stress during pregnancy for the expression of testosterone-dependent behavioural responses of male offspring. Masculine sexual behaviour in experimental animals, elicited by a sexually experienced female primed with oestradiol and progesterone, was examined. Aggressive behaviour (both attack and threat responses) of experimental animals, directed to anosmic standard opponent males, was also examined. The hypothesis that any deleterious effects of crowding during pregnancy, upon masculine sexual and aggressive responses are mediated by maternal pituitary-adrenocortical activation, was tested according to the rationale of the general working hypothesis

(see Chapters 1 and 2). Similarity of the effects of hormone treatment during pregnancy upon male offspring behaviour, with those resulting from crowding, is evidence to support the working hypothesis.

METHODS

Animal husbandry and treatments followed the procedure outlined in Chapter 3. At weaning (postnatal day 21) males used in behavioural experiments were re-housed, 8-10 animals per large cage according to treatment, in such a way that at least one representative from each litter was contained in each group. At 10-12 weeks of age, males from one such group cage were rehoused individually in small cages, for use in behavioural experiments. Subjects were thus random representatives of each individual litter.

Tests of male sexual behaviour were commenced 24 hours after individual housing. An experienced female was placed into a large neutral cage containing clean sawdust and covered with clear perspex, and after 10 minutes habituation a test male was introduced. All females used were 18-24 weeks of age, had produced one litter, and had been made sexually receptive with injections of 35 µg β-Oestradiol -3-benzoate (Sigma) 48 hours before use, and 100 µg progesterone (Sigma 6-8 hours before use). The steroid vehicle was olive oil (Sigma). The injection quantity was 0.1 ml, and injections were administered intramuscularly. This method of hormonally priming females was based on Mosig and Dewsbury (1976) and is designed to ensure standardised receptivity of females.

The resulting interaction between the experimental male and the receptive female was originally observed for a period of 80 minutes but in later experiments this period was extended to 100 minutes. The following measures were recorded: mount latency, number of mounts,

intromission latency, number of intromissions and number of animals ejaculating. After 60 minutes duration of behavioural interaction only latencies and number of animals ejaculating were recorded. It was possible to observe 4 interacting pairs at once, in which case care was taken to study experimental males from the different treatment groups. All behavioural assessment took place in red light between 1500 and 2100 hours. After testing, males were returned to individual housing. If any treatment was found to impair copulation, males were tested again (9-13 days later) following a series of at least 5 daily subcutaneous injections of testosterone propionate. The results section gives details of the duration of such treatment, the dose was 500 µg testosterone propionate (Sigma) in 0.1 ml olive oil (Sigma). The same procedures of behavioural assessment were employed (op. cit.). The rationale of this procedure was to examine whether deficits in masculine copulatory responses were caused by lowered levels of testosterone and whether testosterone therapy could restore sexual behaviour.

Tests of male aggressive behaviour were commenced after a period of isolation of the experimental males in small cages. (The results section gives details of the duration of isolation.) Male mouse aggression increases after only 24 hours of isolation (O'Donnell, Blanchard and Blanchard, 1981). A standard opponent was placed into a large neutral cage containing clean sawdust and covered in clear perspex, and after 5 minutes habituation, a test male was introduced. Standard opponents were group housed males aged approximately 11 weeks and rendered anosmic by nasal perfusion with 4% zinc sulphate solution. Standard opponents were treated twice with this solution in the 7 days prior to their use. Anosmic males rarely attack other mice (Brain, Benton, Childs and Parmigiani,

1981). The standard opponents had approximately 50 µl of male mouse urine applied to rump and base of tail (Hamilton microlitre syringe and brush were used). This urine was pooled from approximately 5 isolated and sexually experienced mice, and collected during the 24 hour period prior to use. Urine was stored in 5 ml, air tight specimen tubes and refrigerated at 4°C until use. Application of urine was developed in this study because of the variability of aggression between experiments and to increase the proportion of males that fight. Application of urine immediately prior to aggression tests dramatically increases the probability and intensity of aggression directed to these animals - probably due to the presence of an aggression-facilitating pheromone in the urine of male mice (Ingersoll, Bobotas, Ching-Tse and Lukton, 1982). An alternative procedure employed in later experiments to facilitate aggression was to isolate experimental animals earlier in life, which decreases the probability of prior experience of aggression during group housing and may be a more effective isolation procedure (Dr. P.F. Brain, Dept. Zoology, University of Swansea, personal communication).

Standard opponents were used once or twice only, and where they were used twice these were not in consecutive tests, nor in presentations to test males of the same experimental treatment. Care was also taken to avoid bias through order effects in second use of standard opponents.

The resulting interaction between the experimental male and the standard opponent was observed for 5 minutes via remote closed circuit T.V. monitor, and video taped for later detailed analysis. Behavioural tests were conducted under red light between 1500 and 2100 hours. The following measures were recorded: latency to

attack, number of discrete biting attacks, number of bites and cumulative attack time. A composite aggression score (+1 point for each sniff, bite and tail rattle) was also calculated. Sniffs were scored when targeted to the genital area of the standard opponent and tail rattles recorded as each distinct bout of tail rattling. These measures of aggression are based on those employed by Brain and Poole (1974), Brain, Nowell and Wouters (1971), Brain (1972), Brain and Nowell (1970b). Also recorded were rough grooms and upright threats as defined by Simon and Gandelman (1981).

If any treatment was found to impair aggression a second experiment was conducted on identically treated animals that had not been previously used in tests of aggression. During isolation males received daily injections of testosterone propionate (500 µg in 0.1 ml olive oil, subcutaneously). The rationale of this procedure was to examine whether deficits in aggression were caused by lowered levels of testosterone and whether testosterone therapy could restore this behaviour.

EXPERIMENT 8:1. The effects of chronic crowding during pregnancy upon copulatory behaviour of adult male offspring and the influence of testosterone therapy in adulthood. Offspring were derived from 8 control mice and 9 crowded mice. After an initial behavioural test males remained in individual housing for a further 9 days, during the last 5 days of which each male received a daily injection of testosterone propionate.

RESULTS. The effects of chronic crowding from days 12-17 of pregnancy upon the masculine sexual responses of male offspring are shown in table 8:1. Male offspring from crowded mice showed lengthened mount and intromission latencies, and lower numbers of intromissions compared with male offspring from control mice. Additionally, fewer male offspring from crowded mice achieved ejaculation in the test period (Fisher exact probability, $P = 0.04$). Testosterone propionate treatment prior to a repeat test abolished all significant differences in sexual behaviour between males from crowded and control mice, and significantly reduced mount latency of males from crowded mice compared with their initial levels.

Table 8:1. The effects of chronic crowding stress during the final third of pregnancy upon male offspring copulatory vigour and the influence of testosterone propionate (TP) treatment prior to behavioural assessment. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)		Number of mounts		Intromission latency(s)		Number of intromissions		Number of animals ejaculating	
	Untreated	After TP	Untreated	After TP	Untreated	After TP	Untreated	After TP	Untreated	After TP
Undisturbed controls n = 10	649 438-977	447 188-697	31 22-47	39 20-61	1305 477-2772	1142 488-2748	21 1-31	14 1-50	4	6
Chronically stressed n = 10	1051* 535-3121	552** 245-976	17 1-34	26 1-51	2951* 1028-4800	1237 460-4800	4* 0-15	13 0-46	0*	3

* significant difference compared with control untreated $P < 0.05$-$P < 0.025$ (MWU)
** significant difference compared with stressed untreated $P < 0.025$ (MWU)

EXPERIMENT 8:2. The effects of chronic crowding during pregnancy upon aggressive behaviour of adult male offspring. Offspring were derived from 8 control mice and 8 crowded mice, and at approximately 10 weeks of age were individually housed, remaining in isolation for 16 days prior to behavioural testing.

EXPERIMENT 8:3. The effects of chronic crowding during pregnancy upon aggressive behaviour of adult male offspring and the influence of testosterone therapy in adulthood. Offspring were derived from 9 control mice and 9 crowded mice, and at approximately 10 weeks of age were individually housed, remaining in isolation for 21 days, during the last 5 days of which, each male received a daily injection of testosterone propionate.

RESULTS. The effects of chronic crowding from days 12-17 of pregnancy upon the aggressive responses of male offspring and the proportion of animals that display aggression (experiment 8:2) are shown in tables 8:2 and 8:3 respectively. Male offspring from crowded mice showed lengthened attack latencies and lower numbers of attacks, bites, sniffs, tail rattles, rough grooms and upright threats compared with offspring from control mice (table 8:2). They also spent less time attacking and achieved lower composite aggression scores. Additionally, fewer male offspring from crowded mice displayed attacks, rough grooms or upright threats (table 8:3). In experiment 8:3 there were no significant differences in the number of male offspring from crowded or control mice displaying aggression (table 8:3) or the aggressive responses of these animals (table 8:4) following testosterone propionate treatment prior to testing. However, male offspring from crowded mice did display general though statistically non-significant reductions in the

frequency of aggressive responses. Further analysis of these results, based only on animals that displayed aggression, revealed deficits in aggression remaining even after testosterone propionate treatment (table 8:5). Fighting male offspring from crowded mice showed lengthened attack latencies and lower numbers of attacks, bites, tail rattles and rough grooms compared with offspring from control mice. They also spent less time attacking and achieved lower composite aggression scores.

Table 8:2. The effects of chronic crowding stress during the final third of pregnancy upon male offspring attack and threat behaviour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression score
Undisturbed controls n = 15	14 5-39	7 1-21	26 3-46	35 3-52	16 14-24	13 8-29	3 1-6	4 1-13	52 43-92
Chronically stressed n = 15	300* 14-300	0* 0-7	0** 0-13	0** 0-18	17 13-28	5** 0-12	0* 0-3	1* 0-4	28** 24-44

* significant difference with control $P < 0.025$ (MWU)
** significant difference with control $P < 0.01$ (MWU)

Table 8:3. The effects of chronic crowding stress during the final third of pregnancy upon the proportion of male offspring displaying attack and threat behaviour and the influence of testosterone propionate (TP) treatment prior to behavioural assessment. Data given are as numbers of animals

Treatment during late pregnancy	Number of males displaying attacks	Number of males displaying tail rattles	Number of males displaying rough grooms	Number of males displaying upright threats
EXPERIMENT 2				
Undisturbed controls n = 15	12	13	12	13
Chronically stressed n = 15	6**	9	6**	8*
EXPERIMENT 3				
Undisturbed control + TP n = 10	6	6	3	7
Chronically stressed + TP n = 10	6	6	6	5

* significant difference with control P = 0.047
** significant difference with control P = 0.026 (Fisher Exact Probability test)

Table 8:4. The effects of chronic crowding stress during the final third of pregnancy upon male offspring attack and threat behaviour and the influence of testosterone propionate treatment prior to behavioural assessment. Data given are as medians and 95% confidence limits. Analysis based on all animals

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression score
Undisturbed controls n = 10	63.5 21-300	9.5 0-25	11.5 0-46	21.0 0-72	15.0 4-18	8.5 0-17	0.0 0-4	2.5 0-10	39.0 14-66
Chronically stressed n = 10	222.0 36-300	1.5 0-12	1.5 0-22	2.5 0-30	13.5 8-15	2.0 0-10	1.0 0-1	0.5 0-7	16.0 13-36

Table 8:5. The effects of chronic crowding stress during the final third of pregnancy upon male offspring attack and threat behaviour and the influence of testosterone propionate treatment prior to behavioural assessment. Data given are as medians and 95% confidence limits. Analysis based on animals displaying aggression

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression scores
Undisturbed controls	23.5 15-80	19.0 2-26	31.0 2-47	50.0 5-77	15.0 4-18	15.5 3-22	4.0 4-5[a]	9.0 2-13	59.0 23-73
Chronically stressed	73.5** 36-298	4.0** 1-17	7.0* 1-28	13.0* 2-46	13.5 8-15	6.0** 2-10	1.0** 1-4	7.0 1-9[a]	37.5** 13-49

a indicates range of data where N = < 6 (see table 8:3)
* significant difference with control P < 0.05 (MWU)
** significant difference with control P < 0.025 (MWU)

EXPERIMENT 8:4. The effects of ACTH administration during pregnancy upon copulatory behaviour of adult male offspring and the influence of testosterone therapy in adulthood. Offspring were derived from 5 control mice, 4 saline-gelatine vehicle treated mice, 5 low dose-ACTH-treated mice and 5 high dose ACTH-treated mice. After an initial behavioural test males remained in individual housing for a further 13 days, during the last 6 days of which each male received a daily injection of testosterone propionate.

RESULTS. The effects of ACTH administration from days 12-17 of pregnancy upon the masculine sexual responses of male offspring are shown in table 8:6. Male offspring from high dose ACTH-treated mice showed lengthened intromission latency and lower numbers of mounts and intromissions compared with male offspring from control mice. The lower dose of ACTH had no detectable behavioural influence except a marginally significant reduction on the number of animals ejaculating compared with untreated controls (Fisher exact probability P = 0.06). Male offspring from saline-gelatine vehicle injected mice did not show significantly different behavioural responses compared with male offspring from undisturbed control mice. Testosterone propionate treatment prior to a repeat test did not improve the sexual responses of male offspring from high dose ACTH-treated mice; the number of mounts and intromissions remained below control levels.

Table 8:6. The effects of ACTH administration during the final third of pregnancy upon male offspring copulatory vigour and the influence of testosterone propionate (TP) treatment prior to behavioural assessment. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)		Number of mounts		Intromission latency(s)		Number of intromissions		Number of animals ejaculating	
	Untreated	After TP	Untreated	After TP	Untreated	After TP	Untreated	After TP	Untreated	After TP
Undisturbed controls n = 9	776 372-1188	529 288-1012	44 26-74	40 31-59	1046 521-1247	569 446-1427	26 13-29	24 8-37	5	6
Saline-gel vehicle n = 9	678 433-978	547 336-778	24 18-42	51 28-70	1417 667-2403	838 448-1932	12 0-28	18 5-27	3	3
Low dose ACTH n = 9	562 432-844	472 344-1146	37 30-63	33 18-64	1012 650-1602	1146 452-4800	20 7-43	12 0-32	1	2
High dose ACTH n = 9	1039 440-4800	642 306-4800	25[a] 0-29	17[ab] 0-38	2194[a] 1274-4800	1418 306-4800	4[a] 0-14	3[a] 0-26	3	3
KWANOVA	N.S.	N.S.	P = 0.029	P = 0.039	P = 0.019	N.S.	P = 0.017	N.S.		

a significant difference compared with control untreated $P < 0.05$-$P < 0.001$ (MWU)
b significant difference compared with control after TP $P < 0.01$ (MWU)

EXPERIMENT 8:5. The effects of ACTH administration during pregnancy upon aggressive behaviour of adult male offspring. Offspring were derived from 5 control mice, 4 saline-gelatine vehicle treated mice, 5 low dose ACTH-treated mice and 5 high dose ACTH-treated mice. At approximately 7 weeks of age males were individually housed and remained in isolation for 28 days prior to behavioural testing.

RESULTS. The effects of ACTH administration from days 12-17 of pregnancy, upon the aggressive responses of male offspring and the proportion of animals that display aggression are shown in tables 8:7 and 8:8 respectively. Male offspring from ACTH-treated mice showed no significant decrements in aggressive behaviour, compared with male offspring from control or vehicle-injected mice. There were no differences in the proportion of males from ACTH-treated mice showing aggression, compared with males from control or vehicle-injected mice.

Table 8:7. The effects of acute ACTH administration during the final third of pregnancy upon male offspring attack and threat behaviour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression scores
Undisturbed controls n = 9	185 58-300	6 0-28	5 0-31	5 0-35	17 13-24	0 0-28	0 0-2	3 0-10	29 17-71
Saline-gel vehicle n = 9	114 34-300	11 0-23	18 0-27	27 0-38	10 2-19	19 0-31	2 0-9	4 0-14	48 11-67
Low dose ACTH n = 9	300 75-300	0 0-15	0 0-23	0 0-28	13 9-17	0 0-18	0 0-6	0 0-7	18 13-57
High dose ACTH n = 9	184 129-300	3 0-12	7 0-20	14 0-19	9 4-18	7 0-20	1 0-1	2 0-8	30 9-50
KWANOVA	N.S.	N.S.	N.S.	N.S.	P = 0.09	N.S.	N.S.	N.S.	N.S.

Table 8:8. The effects of acute ACTH administration during the final third of pregnancy upon the proportion of male offspring displaying attack and threat behaviour. Data given are as numbers of animals

Treatment during late pregnancy	Number of males displaying attacks	Number of males displaying tail rattles	Number of males displaying rough grooms	Number of males displaying upright threats
Undisturbed controls n = 9	5	4	4	6
Saline-gel vehicle n = 9	6	7	5	7
Low dose ACTH n = 9	3	4	3	4
High dose ACTH n = 9	6	7	5	6

EXPERIMENT 8:6. The effects of chronic corticosterone administration during pregnancy upon copulatory behaviour of adult male offspring. Offspring were derived from 9 control mice, 7 propylene glycol vehicle-treated mice and 10 corticosterone-treated mice.

RESULTS. The effects of chronic corticosterone administration from day 12 of pregnancy to parturition, upon the masculine sexual responses of male offspring are shown in table 8:9. Male offspring from mice treated chronically with corticosterone showed no significant decrements in sexual behaviour, compared with male offspring from control or propylene glycol-treated mice.

Table 8:9. The effects of chronic corticosterone administration during the final third of pregnancy upon male offspring copulatory vigour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)	Number of mounts	Intromission latency(s)	Number of intromissions	Number of animals ejaculating
Undisturbed controls n = 11	454 232-1632	39 19-80	1196 316-4800	15 0-56	4
Propylene glycol vehicle n = 11	583 272-1042	49 26-85	949 397-1531	30 6-51	2
Chronic corticosterone n = 11	729 268-1162	36 11-54	1254 717-4800	20 0-34	5
KWANOVA	N.S.	N.S.	N.S.	N.S.	

EXPERIMENT 8:7. The effects of chronic corticosterone administration during pregnancy upon aggressive behaviour of adult male offspring. Offspring were derived from the same pregnant mice used in experiment 6, but were not used in tests of copulatory behaviour. At approximately 9 weeks of age males were individually housed and remained in isolation for 17 days prior to behavioural testing.

RESULTS. The effects of chronic corticosterone administration from day 12 of pregnancy to parturition, upon the aggressive responses of male offspring and the proportion of animals that display aggression, are shown in tables 8:10 and 8:11 respectively. Male offspring from mice treated chronically with corticosterone showed no significant decrements in aggressive behaviour, compared with male offspring from control or propylene glycol-treated mice. There were no differences in the proportion of males from corticosterone-treated mice showing aggression compared with males from control or propylene glycol vehicle-treated mice.

Table 8:10. The effects of chronic corticosterone administration during the final third of pregnancy upon male offspring attack and threat behaviour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression score
Undisturbed controls n = 10	74 25-300	6 0-12	13.5 0-41	14.5 0-27	16.5 11-26	5.5 0-20	2 0-5	1.5 0-5	39.5 20-53
Propylene glycol vehicle n = 11	32 10-300	8 0-22	16 0-28	26 0-38	13 8-16	6 0-22	1 0-6	5 0-16	40 16-69
Chronic corticosterone n = 11	44 9-300	9 0-22	15 0-31	12 0-42	12 8-16	10 0-17	0 0-2	4 0-7	35 15-71
KWANOVA	N.S.	N.S.	N.S.	N.S.	P = 0.06	N.S.	N.S.	N.S.	N.S.

Table 8:11. The effects of chronic corticosterone administration during the final third of pregnancy upon the proportion of male offspring displaying attack and threat behaviour. Data given are as numbers of animals

Treatment during late pregnancy	Number of males displaying attacks	Number of males displaying tail rattles	Number of males displaying rough grooms	Number of males displaying upright threats
Undisturbed controls n = 10	7	8	7	8
Propylene glycol vehicle n = 11	8	8	8	8
Chronic corticosterone n = 11	8	9	5	9

EXPERIMENT 8:8. The effects of progesterone administration during pregnancy upon copulatory behaviour of adult male offspring. Offspring were derived from 6 control mice, 6 olive oil vehicle-treated mice, 5 low dose progesterone-treated mice and 6 high dose progesterone-treated mice. The test period was 100 minutes.

RESULTS. The effects of acute progesterone administration from days 12-17 of pregnancy, upon the masculine sexual responses of male offspring are shown in table 8:12. Male offspring from mice treated with progesterone showed no significant decrements in sexual behaviour, compared with male offspring from control or olive oil-treated mice.

Table 8:12. The effects of acute progesterone administration during the final third of pregnancy upon male offspring copulatory vigour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)	Number of mounts	Intromission latency(s)	Number of intromissions	Number of animals ejaculating
Undisturbed controls n = 10	746 441-1692	45 33-63	1032.5 601-1936	28.5 7-44	6
Olive oil vehicle n = 10	741 379-1364	33 15-88	1627 641-6000	6.5 0-29	3
Low dose progesterone n = 10	822 518-1593	40.5 17-84	1775 909-2069	11 7-31	3
High dose progesterone n = 10	751 294-1430	43.5 12-76	1003.5 294-6000	17 0-43	3
KWANOVA	N.S.	N.S.	N.S.	N.S.	

EXPERIMENT 8:9. The effects of progesterone administration during pregnancy upon aggressive behaviour of adult male offspring. Offspring were derived from the same pregnant mice used in experiment 6 and had been used in tests of copulatory behaviour. At approximately 10 weeks of age males were individually housed and remained in isolation for 21 days prior to behavioural testing.

RESULTS. The effects of acute progesterone administration from days 12-17 of pregnancy, upon the aggressive responses of male offspring and the proportion of animals that display aggression, are shown in tables 8:13 and 8:14 respectively. There were significant differences between experimental groups in measures of aggression. Male offspring from olive oil-treated mice showed lengthened attack latencies, reduced numbers of attacks, bites and tail rattles and spent less time attacking compared with male offspring from control mice. Male offspring from progesterone-treated mice show reduced number of sniffs of the opponent compared with offspring from olive oil-treated mice. Proportionately fewer male offspring from olive oil-treated mice displayed attacks, tail rattles or rough grooms compared with male offspring from control mice.

Table 8:13. The effects of acute progesterone administration during the final third of pregnancy upon male offspring attack and threat behaviour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression score
Undisturbed controls n = 10	36.5 16-300	8.5 0-13	16 0-30	19 0-37	15.5 10-18	8.5 0-15	1.5 0-6	5.5 0-12	46 16-66
Olive oil vehicle n = 10	300[a] 205-300	0[a] 0-4	0[a] 0-11	0[a] 0-14	20 12-25	0[a] 0-7	0 0-0	1 0-5	21.5 12-31
Low dose progesterone n = 10	62 6-300	8.5 0-21	16 0-46	15.5 0-47	11[b] 7-18	5 0-6	0 0-3	6.5 0-13	34 13-58
High dose progesterone n = 10	61 18-300	4.5 0-15	13 0-19	13 0-24	12[b] 1-17	6.5 0-20	0 0-2	4.5 0-11	28 17-43
KWANOVA	P = 0.037	P = 0.07	P = 0.051	P = 0.06	P = 0.043	P = 0.053	N.S.	N.S.	P = 0.086

a significant difference compared with controls ($P < 0.025$-$P < 0.01$)
b significant difference compared with vehicle ($P < 0.025$-$P < 0.01$)

Table 8:14. The effects of acute progesterone administration during the final third of pregnancy upon the proportion of male offspring displaying attack and threat behaviour. Data given are as numbers of animals

Treatment during late pregnancy	Number of males displaying attacks	Number of males displaying tail rattles	Number of males displaying rough grooms	Number of males displaying upright threats
Undisturbed controls n = 10	7	8	6	8
Olive oil vehicle n = 10	2*	3*	1*	6
Low dose progesterone n = 10	7	8	4	8
High dose progesterone n = 10	7	7	2	7

* significant difference compared with control P = 0.032-P = 0.027 Fisher Exact Probability test

EXPERIMENT 8:10. The effects of androstenedione or corticosterone administration during pregnancy upon copulatory behaviour of adult male offspring. Offspring were derived from 8 control mice, 7 peanut oil vehicle-treated mice, 6 androstenedione-treated mice and 8 corticsterone-treated mice. The test period was 100 minutes.

RESULTS. The effects of acute androstenedione and acute corticosterone administration from days 12-17 of pregnancy, upon the masculine sexual responses of male offspring are shown in table 8:15. Male offspring from mice treated with either androstenedione or corticosterone showed no significant decrements in sexual behaviour, compared with male offspring from control or olive oil-treated mice.

Table 8:15. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon male offspring copulatory vigour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)	Number of mounts	Intromission latency(s)	Number of intromissions	Number of animals ejaculating
Undisturbed controls n = 10	564.5 193-949	30 17-57	1174.5 559-6000	12.5 0-42	4
Peanut oil vehicle n = 10	779 504-1321	30 3-52	909 576-6000	15 0-38	5
Acute androstenedione n = 10	636 354-1244	31.5 22-48	1214.5 798-6000	14.5 0-23	5
Acute corticosterone n = 10	719.5 587-1881	25 10-60	1192 716-6000	18 0-43	3
KWANOVA	N.S.	N.S.	N.S.	N.S.	

EXPERIMENT 8:11. The effects of androstenedione or corticosterone administration during pregnancy upon aggressive behaviour of adult male offspring. Offspring were derived from the same pregnant mice used in experiment 10, and had been used in tests of copulatory behaviour. At approximately 8 weeks of age, males were individually housed and remained in isolation for 26 days prior to behavioural testing.

RESULTS. The effects of acute androstenedione and acute corticosterone administration from days 12-17 of pregnancy, upon the aggressive responses of male offspring and the proportion of animals that display aggression, are shown in tables 8:16 and 8:17 respectively. There were significant differences between measures of aggression between experimental groups. Male offspring from androstenedione-treated mice scored lower on the composite aggression score compared with male offspring from control, peanut oil and corticosterone-treated mice. Proportionately fewer male offspring from androstenedione-treated mice displayed attacks compared with male offspring from peanut oil-treated mice.

Table 8:16. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon male offspring attack and threat behaviour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Attack latency(s)	Number of attacks	Number of bites	Cumulative attack time(s)	Number of sniffs	Number of tail rattles	Number of rough grooms	Number of upright threats	Composite aggression score
Undisturbed controls n = 8	300 32-300	0 0-21	0 0-51	0 0-62	18.5 12-25	1.5 0-14	1 0-6	1 0-7	28.5 10-53
Peanut oil vehicle n = 9	252 207-300	2 0-5	7 0-16	10 0-21	17 11-31	3 0-15	1 0-2	4 0-7	31 15-56
Acute androstenedione n = 8	300 267-300	0 0-1	0 0-4	0 0-6	17 5-25	0 0-3	0 0-5	0.5 0-2	17.5[a] 6-25
Acute corticosterone n = 8	300 30-300	0 0-22	0 0-48	0 0-14	24 6-28	0.5 0-15	0 0-8	0 0-9	31.5 19-63
KWANOVA	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	P = 0.049

a significant difference compared with control, vehicle and corticosterone P = 0.032-P = 0.002 (MWU)

Table 8:17. The effects of acute androstenedione and acute corticosterone administration during the final third of pregnancy upon the proportion of male offspring displaying attack and threat behaviour. Data given are as numbers of animals

Treatment during late pregnancy	Number of males displaying attacks	Number of males displaying tail rattles	Number of males displaying rough grooms	Number of males displaying upright threats
Undisturbed controls n = 8	3	5	5	4
Peanut oil vehicle n = 9	6	6	6	6
Acute androstenedione n = 8	1*	3	3	3
Acute corticosterone n = 8	3	4	3	3

* significant difference compared with vehicle P = 0.034. Fisher Exact Probability test

EXPERIMENT 8:12. The effects of adrenalectomy coupled with chronic crowding during pregnancy upon copulatory behaviour of adult male offspring. Offspring were derived from 3 adrenalectomised crowded mice, 5 adrenalectomised control mice, 5 Sham-operated crowded mice and 4 Sham-operated control mice. The test period was 100 minutes.

RESULTS. The effects of bilateral adrenalectomy on day 9 of pregnancy and chronic crowding from days 12-17 of pregnancy, upon the masculine sexual responses of male offspring are shown in table 8:18. Male offspring from mice adrenalectomised or stressed during pregnancy showed no significant decrements in sexual behaviour, compared with male offspring from Sham-operated control or stressed mice.

Table 8:18. The effects of chronic crowding stress and adrenalectomy during pregnancy upon male offspring copulatory vigour. Data given are as medians and 95% confidence limits

Treatment during late pregnancy	Mount latency(s)	Number of mounts	Intromission latency(s)	Number of intromissions	Number of animals ejaculating
Sham-surgery controls n = 10	703 442-1123	36.5 22-59	928.5 588-6000	13 0-34	5
Sham-surgery stressed n = 10	685 541-1006	33.5 18-43	1243 700-6000	9.5 0-21	2
Adrenalectomy control n = 10	698 490-896	38.5 25-52	1220.5 746-3465	10 1-28	3
Adrenalectomy stressed n = 8	782 294-2792	27.5 1-95	1422.5 672-6000	14.5 0-28	4
KWANOVA	N.S.	N.S.	N.S.	N.S.	

DISCUSSION

The results of experiments 8:1 and 8:2 show that the stress of chronic crowding during the final third of pregnancy is detrimental to the differentiation of some sexually dimorphic patterns of behaviour in the male offspring. The finding that crowding during pregnancy impairs the copulatory responses of male offspring in mice, compares well with previous studies employing more artificial stressing procedures in the rat (Dahlöf, Hard and Larsson, 1977; Dunlap, Zadina and Gougis, 1978; Herrenkohl and Whitney, 1976; Masterpasqua, Chapman and Lore, 1976; Meisel, Dohanich and Ward, 1979; Ward, 1972). There are only two other reports of the effects of stress during pregnancy upon male offspring sexual behaviour in the mouse: Allen and Haggett (1977) report increased post-ejaculatory intervals in male offspring from crowded mice, and Politch and Herrenkohl (1984a) report no deficits in masculine sexual responses, but augmented feminine responses, in male offspring from restrained mice. In addition, crowding during pregnancy was also found to impair the expression of aggression in male offspring. This result extends the known effects of stress during pregnancy to another testosterone-dependent sexually dimorphic aspect of behaviour, which has not been previously studied. That both copulation and aggression are impaired in male offspring of mice crowded during pregnancy, suggests that the sexual differentiation of behaviour of the male rodent, is genuinely at risk if the pregnant female's environmental conditions are adverse or stressful, during the final stages of foetal development.

The described "demasculinisation" of behaviour (though lack of masculinisation would probably be a more accurate description, see Chapter 2) reflects either a failure of development of the brain

regions controlling the expression of these behavioural responses, or a failure of development of the pituitary-gonadal system. As the latter is ultimately under hypothalamic control, a developmental defect in the central nervous system is implicated in any case, and the question resolves itself to whether the lesion is located at a neurobehavioural site, a neuroendocrine site or both. An attempt was made to distinguish between these possibilities : testosterone propionate was administered to male offspring from both control and crowded mice prior to a repeat examination of copulatory behaviour (experiment 8:1). The rationale behind this technique was that if the damage was purely endocrine, testosterone replacement therapy should result in a restitution of normal patterns of masculine behaviour. Testosterone therapy abolished all significant differences in mating behaviour between male offspring from crowded and control animals, and although the single prior exposure to females in the first behavioural test may also have improved performance, this result strongly suggests that the lesion caused in male offspring from stressed mice is mainly endocrine. Stahl, Götz, Poppe, Amendt and Dörner (1978) and Dörner (1980) have, in fact, already demonstrated testosterone deficiency in male newborn rats whose mothers were restraint-stressed during pregnancy, and perinatal androgen therapy can restore masculine sexual responses in these animals (Dörner, Götz and Docke, 1983). Whether these deficits in circulatory testosterone persist into adulthood and occur in the male offspring from mice stressed during pregnancy, has yet to be determined.

In contrast, the effects of testosterone therapy upon the aggressive responses of male offspring from crowded mice (experiment 8:3) were less clear. First analysis of results indicated that

testosterone therapy could reinstate aggression, as no differences in the proportion of male offspring from crowded or control mice fighting, and no differences in the latencies of the number of component aggressive acts shown by these two groups, were apparent. However, when the analysis is confined only to those animals which display aggression, differences in the intensity of aggression between offspring from crowded and control mice are seen to remain after testosterone treatment. One interpretation of this result is that only a proportion of the male offspring from crowded mice show an androgen deficiency, and more permanent effects upon male offspring aggression may be due to damage to brain areas controlling this behaviour.

The effects of stress during pregnancy upon male offspring masculine behaviour, particularly sexual behaviour, has been suggested to be mediated by exposure of the offspring early in life to products from the maternal pituitary-adrenocortical axis (Ward, 1972; Dahlöf, Hard and Larsson, 1977). There has been little previous investigation of this hypothesis. Additionally, it was not clear in previous studies when in the perinatal period any factors influencing offspring behaviour exerted an influence; in this study fostering procedures were used to control certain post-natal influences. Chapman and Stern (1978) tested this hypothesis by administering ACTH to rats during pregnancy; examination of their male offspring revealed no impairment to masculine copulatory responses. In experiment 8:4, the effects of ACTH administration during pregnancy upon male offspring masculine copulatory responses was studied, and results suggest that contrary to the report by Chapman and Stern (1978) this treatment does impair male offspring copulation. Since this result was obtained, there have been

independent confirmations that male offspring from both rats (Rhees and Fleming, 1981; Stylianopoulou, 1983) and mice (Politch and Herrenkohl, 1984b) treated with ACTH during pregnancy, show deficiencies in masculine sexual responses. However, as with the case of stress, no study has investigated whether these behavioural deficiencies are a result of impaired testosterone secretion. In experiment 8:4, the effects of testosterone therapy was also studied, and results show that testosterone treatment did not completely restore sexual responses of offspring from ACTH-treated mice to control levels. It must be concluded that the ACTH administration regime produced effects that extend beyond those of crowding, and that male offspring from ACTH-treated mice have some permanent damage to brain areas influencing copulatory behaviour. In contrast to the decrements detected in copulation, aggression was not affected in male offspring from ACTH-treated mice (experiment 8:5). However, Simon and Gandelman (1977) reported that proportionately fewer male offspring from ACTH-treated mice attacked male opponents. Although doses of ACTH used in this study were identical to those of Simon and Gandelman (1977) other differences in methodology and strain of mouse could account for differences in results. Problems were encountered in this study in inducing mice to fight, and great variability in proportions of males fighting between experiments was encountered. Application of male mouse urine to standard opponents did increase the number and duration of attacks against these animals, but isolation earlier in life may also have improved aggression and decreased variability between experiments. Isolation earlier in life could not be used in this study because of housing and resource shortages.

There is overall strong evidence that ACTH administration

during pregnancy reproduces the effects of stress during pregnancy, upon male offspring copulation and aggression, and thus there is support for the working hypothesis that the maternal adrenal is required for the production of the described effects. As stated elsewhere, the intact ACTH molecule does not cross the placental barrier (Chapter 2) and as such the effects of crowding or ACTH administration during pregnancy upon male offspring masculine behaviour patterns are probably mediated by adrenal products. Unfortunately, in this study no steroid administration regime influenced any aspect of offspring behaviour. Corticosterone was administered to pregnant mice in both chronic and acute regimes and neither influenced male offspring copulation (experiments 8:6 and 8:10) or aggression (experiments 8:7 and 8:11). That corticosterone treatment during pregnancy does not affect male offspring copulation, contrasts with the report of Herrenkohl and Politch (1984b) who showed that administration of corticosterone acetate to pregnant mice depresses masculine sexual responses of male offspring. Similarly, in this study, no deficits in copulation (experiment 8:8) or aggression (experiment 8:9) were detected in male offspring from progesterone-treated mice. This result contrasts with one previous report that male offspring from rats treated with progesterone during pregnancy, show deficits in sexual behaviour and aggression (Hull, Franz, Snyder and Nishita, 1980). The differences in the results between previous studies and those reported here may be explained by methodological and other differences (e.g. species or strain differences, hormone and dosage differences). However, agreement with previous reports on the effects of androstenedione administration during pregnancy was obtained in this study: such treatment did not impair male offspring copulation (experiment 8:10)

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or aggression (experiment 8:11) and the former result agrees with that of Gilroy and Ward (1978) using the rat. Collectively, there is strong evidence that androstenedione is not the maternal adrenal steroid responsible for producing the effects of stress or ACTH treatment during pregnancy, upon male offspring masculine behaviour. Further study is required prior to conclusions that corticosterone or progesterone mediate the effects of stress or ACTH treatment during pregnancy. On the basis of the results from this study, the hypothesis that the effects of crowding or ACTH treatment during pregnancy, upon male offspring behaviour, are ultimately mediated by corticosterone, progesterone or androstenedione must be rejected.

It is difficult to comment on the study of the effects of adrenalectomy coupled with crowding during pregnancy, upon male offspring copulation (experiment 8:12)-few pregnant mice were used. Although offspring from adrenalectomised stressed mice showed patterns of copulation which did not differ from those of either adrenalectomised or Sham-operated controls, which is consistent with the working hypothesis, offspring from Sham-operated stressed mice did not show deficits in copulation compared with controls, as predicted from the results of experiment 8:1. More effective experiments are required to ascertain whether adrenalectomy can abolish the harmful effects of crowding during pregnancy upon male offspring sexual behaviour, and indeed whether the maternal adrenal and its products are involved in producing the described effects of these treatments.

As has been pointed out in Chapter 7, administration of essentially arbitrary doses of single hormones can only crudely imitate the hormonal milieu of a stressed animal, and it is not surprising that the steroid administration regimes did not reproduce

the effects of crowding or ACTH treatment during pregnancy with complete fidelity. It is a strong possibility that several steroids are responsible for producing the effects of crowding during pregnancy upon male offspring behaviour. Alternatively, an unidentified adrenal product may mediate the effects of crowding: in this study the effects of oestrogen administration during pregnancy were not studied. Recent evidence suggests that oestrogen (oestradiol 17-β) acts as an anti-androgen, which with or without testosterone, influences the sexual differentiation of brain and behaviour (Vom Saal, Grant, McMullen and Laves, 1983). Oestrogens are secreted from the adrenal (see Chapter 2) and their involvement in mediating the effects of crowding upon male offspring behaviour, is a possibility that remains to be studied. It is interesting to note that oestradiol administration during pregnancy results in hypoprolactinaemia in the offspring (Kuhn, Bollen and Darras, 1982) and reduced prolactin secretion has been reported in offspring from rats restraint stressed during pregnancy (Politch, Herrenkohl and Gala, 1978). The endocrine abnormalities and possible causes of the behavioural deficits of offspring from stressed rodents will now be discussed.

The organising influence of androgens for the expression of masculine behaviour (e.g. Grady, Phoenix and Young, 1965) and sexual differentiation is well known (see Chapter 2). Testosterone is required during the perinatal period and in adulthood for the normal expression of masculine behaviour. There is evidence that offspring from stressed rats show abnormal pituitary-gonadal activity: Ward and Weisz (1980) report an acceleration of the normal testosterone surge in male foetuses of restrained rats. Additionally, neonatal offspring from rats restrained during pregnancy show reduced plasma testosterone concentrations compared with offspring from non-stressed

rats (Stahl, Götz, Poppe, Amendt and Dörner, 1978; Dörner, 1980). Thus the abnormal patterns of sexual behaviour in offspring from stressed rats may be caused by altered testosterone secretion early in life. In fact, Ward and Weisz (1980) postulate that stress during pregnancy mistimes the development of endocrine systems from the brain, and it is this phenomenon that results in the decrements of masculine sexual behaviour in male offspring, at least in rats. Whether this is the only cause of the deficits in masculine behaviour in offspring from rodents stressed during pregnancy is open to question: reduced testosterone secretion in adulthood may also be a cause, and this possibility was experimentally examined in this thesis. To date there is no direct evidence that male offspring from rodents stressed during pregnancy show reduced secretion of testosterone in adulthood. However, adult male offspring from restrained rats are reported to show reduced secretion of prolactin (PRL) and corticosterone in conditions of stress (Politch, Herrenkohl and Gala, 1978). PRL is a pituitary hormone that is suppressed by corticosterone and other glucocorticoids (e.g. Gala, Kothari and Haisenleder, 1981; see Chapter 7) and thus PRL deficiency in prenatally stressed male offspring may be due to *in utero* exposure to corticosterone from the maternal adrenal. Foetal exposure to naturally occurring glucocorticoids is also known to impair development of the foetal adrenal (e.g. Milkovic, Milkovic, Sencar and Paunovic, 1970). More importantly, if decreased stress-induced secretion of a hormone is indicative of a defect in the secretory mechanism of the hormone, then reduced PRL and corticosterone secretion may be involved in the cause of decreased masculine behaviour in the male offspring from rodents stressed during pregnancy. PRL is known to induce testicular testosterone biosynthesis in the rat

(Baranao, Legnani, Chiauzzi, Bertini, Suescun, Calvo, Charreau and Calandra, 1981; Waeber, Reymond, Reymond and Lemarchand-Beraud, 1983) and in humans (Slonim, Glick, Island and Kasselberg, 1982). Hypoprolactinaemia early in life or in adulthood could therefore result in some loss of testosterone biosynthesis and secretion, and consequently the normal expression of testosterone-dependent behaviour patterns would be at risk. It can be stated in the absence of other evidence, that hypoprolactinaemia may be the cause of the deficiencies in masculine behaviour reported here in male offspring from crowded mice. Hypoprolactinaemia is one hypothesis suggested to account for the results obtained in other chapters, but adrenal underdevelopment has also been suggested as a cause of observed results. Adrenal underdevelopment, known in offspring from crowded or restrained rats (Dahlöf, Hard and Larsson, 1978) may also cause the deficits in masculine sexual behaviour reported in these animals: corticosterone secretion is correlated with copulatory activity in male rats and this might suggest some causal relationship between this hormone and sexual responses (Bronson and Desjardins, 1982; Szechtman, Lambrou, Gaggiula and Redgate, 1974). Whether the deficits in testosterone dependent sexually-dimorphic aspects of behaviour reported here are due to deficits in PRL throughout life is open to debate. A worthwhile area for future study is to examine the endocrine status of prenatally stressed offspring, particularly in the mouse, both early in development and in adulthood. It has already been suggested that a deficit in behaviour may be due to abnormal development of the brain region concerned with controlling this behaviour and this may account for the ineffectiveness of testosterone therapy restoring aggression in male offspring from crowded mice. A permanent effect on the brain is also suggested by

the ineffectiveness of testosterone therapy in inducing a restitution of copulation in male offspring from ACTH treated mice. Whether this apparent effect on the brain is due to actual damage or a result of incomplete androgen masculinisation of neural and neuroendocrine structures (perhaps resulting in a permanent insensitivity to androgen or impaired control of the pituitary-gonadal system) is unknown, and this is worthy of future study. It is clear however, that crowding and other stressful procedures, if applied to the pregnant rodent, threaten to disrupt the sexual differentiation and development of male offspring. Finally, as with the results in Chapter 7, this study was unable to supply firm evidence as to whether adrenal products mediate the effects of crowding, and further study is necessary prior to excluding an extra-adrenal mechanism of mediation, producing the described effects of stress during pregnancy.

CHAPTER 9

GENERAL DISCUSSION

Living in high population densities is well known to influence the endocrine activity of rodents: crowding is reported to depress pituitary-gonadal activity in male mice (Bronson, 1973; Jean-Faucher, Berger, De Turckheim, Veyessiere and Jean, 1981) and to suppress reproductive cycles in female mice (McKinney, 1972; Nichols, 1980). Further, it has been suggested that the phenomena of regulation of reproductive development and efficiency, by environmental conditions and population density, is modulated by the hypothalamo-pituitary-adrenocortical axis (e.g. Christian, Lloyd and Davis, 1964; Christian, 1971) and this is an application of Selye's (1936, 1950) concept of stress. This thesis, and other work, suggests that environmental stress induced by crowding, affects the development and reproductive potential not only of the present, but also of future generations. Whilst it can be understood that adverse environmental conditions can influence the development, physiology and behaviour of mammals directly, a problem arises in explaining the changes in these same parameters, observed in the offspring of mammals exposed to environmental stress exclusively during pregnancy, in that the offspring are never in direct contact with the external environment, and therefore not stressed. In all organisms, the outcome of development is an interaction between genes and environment: in placental mammals, "the environment" includes the body chemistry of the mother while the developing foetal organism is supported *in utero*. Changes in the body chemistry of the maternal organism can be transmitted to the foetus. Further, certain compounds such as steroid hormones readily pass from maternal circulation to foetal circulation via the

placenta (see Chapter 2). This means that changes in the endocrine status of the mother will with all probability, produce changes of hormonal status in the unborn offspring.

A syndrome has been described in the offspring of mice subjected to the stress of chronic crowding during late pregnancy, which extends the previously known consequences of stress during pregnancy. This syndrome comprises increased perinatal mortality and low birth weight (Chapter 5) retarded onset of puberty and disrupted reproductive cycles in females (Chapter 7) and decrements in copulation and aggression in males (Chapter 8). The working hypothesis, that this syndrome is mediated by maternal pituitary-adrenocortical products was tested: ACTH, corticosterone, progesterone and androstenedione were administered singly to pregnant mice, to examine whether these hormones reproduced closely the effects of crowding. Evidence was obtained to suggest that the inhibition of somatic development in offspring from crowded mice was probably due to pituitary-adrenocortical activation during pregnancy, in that ACTH and corticosterone most effectively reproduced the effects of crowding upon this parameter of development (Chapter 5). In addition, both ACTH and corticosterone administration during pregnancy retarded neuromuscular development of offspring (Chapter 6) and this was a previously reported consequence of stress during pregnancy (Chevins, 1981). However, evidence that sexual differentiation of offspring from crowded mice was disrupted as a consequence of exposure to adrenocortical products was inconclusive: no steroid administration regime effectively reproduced the effects of crowding upon reproductive development and function in females (Chapter 7) or masculine behaviour in males (Chapter 8). In contrast, ACTH administration during pregnancy did produce similar effects to crowding upon the

reproductive development and function of females, and copulation in males. This finding, coupled with independent reports of the effects of ACTH administration during pregnancy (see Chapter 2 literature summary) supply evidence that the known consequences of stress during pregnancy (see Chapter 1 literature summary) are probably mediated by activation of the maternal pituitary-adrenal system. A schematic representation of the syndromes observed in this study in the offspring of mice crowded or treated with hormones during pregnancy, is given in Table 9:1.

In order to adequately construct and examine the working hypothesis, other factors which could possibly mediate the effects of crowding during pregnancy, upon offspring development were identified and investigated. In Chapter 4, the possibility that the crowding or hormone administration regimes used in this study, affected maternal food intake or altered the length of pregnancy, was investigated. No evidence was found that any treatment severely depressed food intake or (with perhaps the exception of chronic corticosterone) shortened pregnancy lengths. These factors can therefore be excluded from any hypothesis of mediation, although it is recognised that they may in combination aggravate the effects of the treatments employed in this study. Additional care was taken throughout this study to control, or at least minimise, postnatal influences upon offspring development. At birth, all litters were culled to a standard size and fostered to an untreated dam. This procedure partially controls for abnormal patterns of maternal behaviour and poor lactation which might result from the various treatments, and prevents continued exposure of offspring to hormones in the milk of natural mothers. It is important to realise that maternal behaviour is in part stimulated by the pup, and therefore

Table 9:1. Schematic representation of developmental syndromes evident in offspring from mice crowded, treated with ACTH, corticosterone, progesterone or androstenedione during late pregnancy.

	Treatment during pregnancy				
Alteration to offspring	CROWDING (STRESS)	ACTH	CORTICOSTERONE	PROGESTERONE	ANDROSTENEDIONE
Prenatal death	+*	0[2]	+	+	0
Postnatal death	0	+*	+	0	0
Somatic development	-*	-*	-	0	0
Neuromuscular development		-	-		
Onset of puberty (female)	-*	-	-	-	0
Oestrous cycle (female)	+*	+	+	+	+
Copulation (male)	-*	-*	0[2]	0[2]	0*
Aggression (male)	-	0	0	0	0

KEY: + = Effect present; enhanced or non-directional
- = Effect present; retarded or reduced
0 = No detectable effect
* = Agreement with other reports
2 = Disagreement with other reports

Table 9:1. Schematic representation of developmental syndromes evident in offspring from mice crowded, treated with ACTH, corticosterone, progesterone or androstenedione during late pregnancy.

	Treatment during pregnancy				
Alteration to offspring	CROWDING (STRESS)	ACTH	CORTICOSTERONE	PROGESTERONE	ANDROSTENEDIONE
Prenatal death	+*	O[2]	+	+	O
Postnatal death	O	+*	+	O	O
Somatic development	-*	-*	-	O	O
Neuromuscular development		-	-		
Onset of puberty (female)	-*	-	-	-	O
Oestrous cycle (female)	+*	+	+	+	+
Copulation (male)	-*	-*	O[2]	O[2]	O*
Aggression (male)	-	O	O	O	O

KEY: + = Effect present; enhanced or non-directional
- = Effect present; retarded or reduced
O = No detectable effect
* = Agreement with other reports
2 = Disagreement with other reports

it is still possible that the effects of crowding or hormone manipulation during pregnancy upon offspring development, may be influenced to some extent by altered maternal behaviour of the foster mother, due to some as yet unidentified property of the treated litter. However, it can be stated with confidence that any effects of crowding or hormone treatment during pregnancy upon offspring development, is effective mainly during intra-uterine life.

Assuming that the maternal adrenal cortex does play a major role in producing the syndrome evident in offspring from crowded mice (and offspring from ACTH-treated mice) what adrenal products are responsible? It has already been suggested in Chapters 7 and 8 that one possible reason for why no single steroid closely mimicked the teratogenic effects of stress, was that the dosage and administration regimes were crude and did not match the hormonal milieu of a stressed rodent with complete fidelity. It remains a possibility that more complex administration regimes may give firmer evidence to identify teratogenic hormones. There is no basis for the supposition that all the harmful effects of crowding during pregnancy are mediated by the same adrenocortical product or caused in the same way. The interaction of several adrenal steroids may be required, for example, to disrupt the process of sexual differentiation (see Chapters 7 and 8). Alternatively, a single and as yet unidentified adrenocortical product may be responsible for producing the entire syndrome evident in offspring from crowded mice. A strong candidate for such an agent is adrenal oestrogen, and a worthwhile area for future work is to examine the teratogenic activity of oestradiol and related compounds.

A possibility that cannot be excluded, is that the effects of crowding during pregnancy upon offspring development are mediated by

extra-adrenocortical hormones. Lieberman (1963) reported that offspring from rats crowded or treated with adrenaline during pregnancy showed increased activity levels, whilst offspring from rats treated with hydrocortisone or noradrenaline during pregnancy showed decreased activity levels. This result indicates that some components of the prenatal stress syndrome are reproduced, and therefore may be mediated by, adreno-medullary hormones. Alternatively, the prenatal stress syndrome may be mediated by extra-adrenal factors, for example by placental passage of ACTH fragments, or by enkephalins and opioid peptides. The neuroendocrinology of neuropeptide synthesis is now becoming understood to the extent that it is known that a single precursor peptide is common to the biosynthetic pathways of ACTH and the endorphins (e.g. Iversen, 1979). β-endorphin is known to be secreted along with ACTH during conditions of stress. Recently, Rhees, Badger and Fleming (1983) report that naloxone (an endorphin antagonist and facilitator of gonadotrophin release) fails to induce copulation in male offspring from rats stressed by restraint during pregnancy, but induces copulation in offspring born to non-stressed control rats. This result can be interpreted in two ways; the reduced copulation in male offspring from rats stressed during pregnancy is caused by an abnormality in gonadotrophin regulating structures, or by an abnormality of endorphin-naloxone receptor systems. The latter interpretation may intuitively suggest that endorphin may be implicated in mediating the effects of stress during pregnancy, upon this parameter of offspring development. However, Monder, Yasukawa and Christian (1980) reported that female offspring from ACTH treated mice show retarded vaginal opening and delays in other developmental milestones, and that this is caused by disturbances in naloxone-sensitive

receptors, since naloxone can prevent these teratogenic effects of ACTH. Hence ACTH administration during pregnancy is shown to be capable of affecting opioid receptor systems and this may influence male offspring sexual behaviour in Rhees, Badger and Flemings' (1983) study. A worthwhile area of future study is to examine these alternative, extra-pituitary-adrenocortical hypotheses of mediation of the teratogenic effects of stress, as well as more fully examining the "prenatal stress" syndrome and causes of each component effect.

In addition to more fully documenting a syndrome of abnormal development in offspring from rodents stressed during pregnancy, and testing the hypothesis that the maternal pituitary-adrenal system mediates the production of these developmental effects, this study has wider implications. This study has shown that naturally occurring hormones regulate aspects of foetal development, particularly body growth and endocrine development. In Chapters 6, 7 and 8 the effects of the various treatments upon parameters of development that are partially controlled by neuroendocrine or neural systems was reported. It seems probable that other more specific aspects of development controlled by brain and neuroendocrine systems are also affected, particularly those systems that are maturing during critical periods coinciding with the time of insult. In the mouse, many neural systems are not fully developed until after birth (Rodier, 1980) and there is the potential for damage to these structures. Additionally, the experiments reported in this thesis have investigated the teratogenic effects of stress and various hormones which have not been previously studied; Shepard (1976) catalogues known teratogenic agents and their effects, including hormones, but their known effects are largely upon morphology and there is little study of other aspects of development. For the

purposes of this study a "teratogenic effect" is not only the gross morphological malformation, but also the more subtle "pathology" of altered body, endocrine, brain or behavioural development resulting from insult during foetal life, which may only be detectable in postnatal life.

Hypoprolactinaemia has been suggested as a possible single common pathway underlying the cause of the syndrome detected in offspring from crowded mice, although other endocrine causes have been postulated. Reduced perinatal secretion of insulin and thyrotrophin and the metabolic action of corticosterone, may be a cause of the retarded development of body (Chapter 5) and brain (Chapter 6) and adrenal underdevelopment may be implicated in the cause of the consequences of stress during pregnancy upon offspring sexual development and behaviour (Chapters 7 and 8). An important general hypothesis of the causes of the syndrome, and one which is particularly relevant to sexual differentiation of behaviour (Chapter 8) is that stress during pregnancy may act to desynchronise brain from endocrine and other organ system development. This is an attractive hypothesis as it agrees well with one current theory of explanation of almost all teratogenic effects: it has been suggested that all morphological defects resulting from insult during embryonic-foetal life are a result of mistimed restorative growth of different cell populations (MRC News, 1982, No. 16 p. 7-8). Further, it would seem that the biochemical or toxic action of a teratogen is less important than previously thought: the major principles of teratogenesis are the period of development at which the insult is sustained; the nature and dose of the agent and the genetic constitution of the conceptus (e.g. Krauer, Krauer and Hytten, 1984). This would go some way in explaining why many substances have similar effects upon foetal

development (see Shepard, 1976) and why such alien substances as formaldehyde, when administered during pregnancy, can have effects upon the offspring strikingly similar to those produced by environmental stress (see Schnurer, 1963). In fact, it is worthy of note that because teratological studies often involve administration of alien substances, which may well be noxious or activate the stress response, the biochemical effect of the test substance may in some cases cause less damage than the inevitable exposure to stress hormones.

In Chapter 4, an attempt was made to study confounding factors (foetal undernutrition, prematurity at birth) that may influence the effects of stress, it remains a possibility that certain treatments applied early in life in particular undernutrition, influence development indirectly via stress. Specifically to undernutrition during early life, the possibility that abnormal development is mediated by stress, rather than nutrient loss alone, has already been recognised (Adlard and Smart, 1971). Of more direct relevance to this thesis, the complexity of interacting systems in mammalian development have not only made it difficult to design definitive experiments to elucidate mechanisms mediating the effects of crowding, but will also make difficult the identification of underlying causes of the developmental defects detected in the offspring.

The relevance of this study to the human condition must be discussed with reservation: the differences in gestation period, foetal development and general ontogeny, pharmacology and pharmokinetics, physiology, and behaviour even between different species of rodents are so great, as to make direct extrapolation from laboratory animal to humans difficult, if not impossible. Particularly in the field of teratology, results of experiments using laboratory animals are often not applicable to humans and

discussion of such problems can be found elsewhere (e.g. Lewis, 1983; Krauer, Krauer and Hytten, 1984). Nevertheless, it is useful to note that foetal exposure to hormones has similar consequences for humans as rodents (probably because of similarities in general endocrinology). In humans, ACTH administration during pregnancy results in foetal adrenal atrophy (Migeon, Prystowsky, Grumbach and Byron, 1956) and this is identical to the effect on rodents (e.g. Milkovic, Milkovic, Sencar and Paunovic, 1970). Similarly, stress during pregnancy has been claimed as a cause of homosexuality in human males (Dörner, Geier, Ahrens, Krell, Munx, Sieler, Kittner and Muller, 1980; Dörner, Schenk, Schmiedel and Ahrens, 1983). These latter studies were correlational (more homosexual men were born in the years spanning the second world war) rather than experimental, and so cannot provide "proof of cause", but they do agree quite well with the finding that male offspring from rats stressed by restraint during pregnancy, show augmentation of homosexual behaviour (Götz and Dörner, 1980). However, the vastly more powerful postnatal environmental and cultural influences on man must be seen as a more plausible cause of this surprising phenomenon. In humans, *in utero* exposure to sex steroids is reported to influence parameters of aggression in later life (Meyer-Bahlberg and Ehrhardt, 1982) and perinatal exposure to glucocorticoids is well recognised to present a neurological hazard (Weichsel, 1977; Sidhu, 1983) and these consequences of hormone exposure also occur in rodents. Thus, there is some basis for employing laboratory animals in these types of studies, such that conclusions for the human condition can be cautiously extrapolated from results.

To speculate further on the value of the animal model reported here, to medically related research, it has been demonstrated in

Chapter 5 that there is a high incidence of perinatal mortality in litters from mice crowded or treated with ACTH, corticosterone or progesterone during late pregnancy. An awareness of the role of the maternal pituitary-adrenal system in foetal development (which is generally lacking in the literature to date e.g. Van Assche and Robertson, 1981) may prove valuable for a further understanding of the causes of "small for dates" foetuses and sudden infant death syndrome (cot deaths) which are not well understood. The causes of sudden infant deaths remains an enigma to medical science. Current research is centred on respiratory patterns of infants and some success has been achieved in developing animal models reproducing airway sensory deprivation induced obstructive apnoea, which is a common post-mortem finding (although not a direct cause of death) of victims of sudden infant death syndrome (e.g. Abu-osba, Mathew and Thach, 1981). It is interesting to note that glucocorticoids accelerate lung maturation (Beato and Doenecke, 1981) and are used clinically, because of this property, to treat premature and "small for dates" babies (Sidhu, 1983). It has previously been noted that glucocorticoids also retard neurological development. It may prove valuable to change the framework of study, and attempt to identify factors which are manifest during prenatal life, rather than exclusively during postnatal life. It can be speculated that sudden infant death syndrome may be an analogous pathology in humans to the perinatal mortalities reported in litters from mice stressed, or treated with ACTH or corticosterone during pregnancy (Chapter 5) and may be caused by mistiming of brain from lung and other organ development, due to prenatal exposure to glucocorticoids or other hormones. This postulation would be worthy of investigation: initial studies could well involve attempts to correlate hormonal abnormalities during

pregnancy (or extremes in natural physiological variation) with the development and mortality rates of babies, thereby identifying additional factors in the epidemiology of this syndrome.

Finally, a more obvious conclusion from the results of this study is that stress and pituitary-adrenocortical activation during pregnancy, may be a mechanism adding to phenotypic variation within natural animal populations. It is already known that intrauterine position contributes to phenotypic variation of rodents without genetic variation (Vom Saal and Bronson, 1978, 1980). The stress induced in this study by crowding, can be related to natural circumstances (more so than that resulting from restraint) of rapidly growing populations, where resources shortages and intra-species conflicts may further add to the animals perception of, and reaction to, the adverse conditions. Therefore, rather than the syndrome described here in offspring from crowded mice being considered purely as "pathological", it may also be thought of as phenotypic variation, and even adaptation to the environment in which the parental generation exists.

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