Behavioural effects of oestradiol in the female rat

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BEHAVIOURAL EFFECTS OF OESTRADIOL IN THE FEMALE RAT

by

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A thesis submitted for the degree of Doctor of Philosophy
in the
University of Durham

Department of Psychology
Durham

January, 1979

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ACKNOWLEDGEMENTS

I am grateful to Professor F. V. Smith for the opportunity of working in the Department of Psychology, and for the excellent research facilities made available to me.

To Dr. Robert F. Drewett, who initially stimulated my interest in the hormonal bases of behaviour, for his guidance and critical appraisal of the work presented here, go my sincere thanks.

I am indebted to David Wastell, Dr. Arthur Still and Alan White, for much statistical advice, Cathy Thompson for computing assistance, and the Technical Staff for constructing all apparatus, especially Malcolm Rolling for graphics and photography.

To Marie Harper and Joan Emery for care and maintenance of the experimental animals, and much good humour during the course of this study; for technical assistance and their untiring help: Keith Kendrick, Angela Trew and David Harper. To all these people - a big "Thank you".

My thanks also to Professor Michael Morgan and Una Padel for helpful discussions; to Dr. Piet Wiepkema for communicating his recent findings to me, and to other members of the Behavioural Physiology Group, University of Groningen for their comments.

In spite of many delays and unfamiliar terminology, Judith Willis typed this thesis with much patience and care. Mrs. J. Poelstra-Hiddinga did some additional typing.

Finally, I would like to extend my deep gratitude to my parents for their constant support and encouragement throughout this work.

ABSTRACT

Spiteri, N. J. : "Behavioural Effects of Oestradiol in the Female Rat"

Using behavioural and physiological measures, this thesis investigates behavioural effects of oestradiol, and of two of its synthetic analogues, mestranol and ethynyl oestradiol, in the female rat. Two types of behaviour receive particular attention, feeding and sexual behaviour.

The first chapter describes some physiological and behavioural changes in the female rat over the oestrous cycle. Changes in meal size are seen during oestrus, or in ovariectomized rats treated with oestradiol. The effect of oestradiol on the gastric emptying of saline, glucose and fat loads was investigated (Chapter 2) to determine whether changes in stomach emptying were responsible for altered meal size. Only the emptying of fats from the stomachs of oestradiol treated rats was significantly reduced.

The role of oestradiol in motivational processes associated with sexual behaviour was also examined (Chapter 3). It was found that the performance of females, running to sexually active or castrate males in a straight runway, depended on their oestrous state, and the gonadal condition of the males. These were independent and additive effects.

The characteristics of the potent male that were rewarding to a female were then investigated by varying the odour and sexual activity of male rats (Chapter 4). The results suggest that both the odour and sexual activity of a potent male are rewarding to a female rat.

Ethynyl oestradiol and mestranol are synthetic oestrogens normally used in oral contraceptives. The experiments presented in Chapter 5 show that in female rats both of these steroids reduced food intake reliably, and more than oestradiol. Ethynyl oestradiol, but not mestranol, stimulated sexual behaviour. Furthermore, there was no interaction between oestrogen and progestogen components of oral contraceptives which influenced, in any way, the behaviours observed in this study. The final chapter reviews the effects of oral contraceptives on mental, somatic and behavioural changes in women.

CHAPTER 1

BEHAVIOURAL AND PHYSIOLOGICAL CHANGES DURING THE OESTROUS CYCLE

1.1 Introduction

This thesis investigates the effects of oestradiol on feeding and sexual behaviour in the female rat. In this chapter, a concise account of some behavioural and physiological changes, which take place during oestrus, is presented. Particular attention is given to physiological changes associated with, and to changes in, feeding and sexual behaviour.

1.2 Hormonal changes during the oestrous cycle

1.2.1 Oestradiol-17β and Progesterone

The oestradiol content of peripheral plasma has been measured by Brown-Grant, Exley and Naftolin (1970) using a competitive protein binding method, and Butcher, Collins and Fugo (1974) using Radio-immunoassay and competitive protein binding procedures. Brown-Grant et al. (1970) found that in rats with four day oestrous cycles (on a 14 hour light/10 hour dark schedule) plasma oestradiol levels were low (< 5 pg/ml) on the night of oestrus and throughout metoestrus. There was a gradual rise during dioestrus, until on the morning of prooestrus they reached a peak of about 28 pg/ml. The plasma oestradiol levels then dropped again until, by late prooestrus, they had nearly returned to the low levels eventually attained on the night of oestrus. Animals with five day cycles showed a broadly similar pattern. Butcher et al., however, demonstrated that the oestradiol peak could be as high as 90 pg/ml plasma at about noon of prooestrus.

On the morning of prooestrus (when oestradiol content of plasma is at its highest) progesterone secretion was very low (Hashimoto, Henricks, Anderson and Melampy, 1968: < 1 μg/hr/ovary; Butcher et al., 1974: < 4 ng/ml plasma); its highest peak (Hashimoto et al., 1968: 4 μg/hr/ovary; Butcher et al., 1974: 46 ng/ml plasma) occurred late on the same day.

Another slight rise in progesterone secretion was found on the day of metoestrus. Hashimoto et al. (1968) measured the secretion of pregn-4-ene-20-ol-3-one, which showed two equally high peaks, one on the day of prooestrus, coincident with the progesterone peak, and the other on the day of metoestrus.

Feder, Resko and Goy (1968) measured progesterone levels in systematic plasma, in rats with five-day oestrous cycles, using gas liquid chromatography. Progesterone content was significantly higher on the day of prooestrus than at any other stages during the cycle. A slight rise was also found during metoestrus and the first day of dioestrus.

1.2.2 Pituitary Hormones

Secretion of gonadotrophins and of Prolactin during the oestrus cycle has been well described (LH: Brown-Grant et al., 1970; Piacsek, Schneider and Gay, 1971; FSH and Prolactin: Gay, Midgley and Niswender, 1970; FSH, LH, and Prolactin: Butcher et al., 1974). Increased secretion of LH, FSH and Prolactin by the pituitary occurs during the prooestrus period. The increased secretion of gonadotrophins is coincidental with the progesterone surge which peaks 5 to 7 hours later. The pre-ovulatory elevation of LH occurred around 18.00 hrs on prooestrus (ca. 1700 ng/ml plasma), while the elevation of FSH was more gradual, rising to a peak (ca. 600 ng/ml plasma) at midnight and then declining slowly until noon of oestrus (Butcher et al., 1974). Ferin, Tempone, Zimmering and Van de Wiele (1969) demonstrated that in the adult rat the initiation of the LH release is under oestrogenic and not progestogenic control.

Prolactin showed a peak at about 15.00 hrs of prooestrus (257 ng/ml plasma) and a second, brief peak 24 hours later on the afternoon of oestrus (Butcher et al., 1974). These findings agree with the earlier report of Gay et al. (1970).

Corticosterone levels increase at prooestrus (Critchlow, Liebelt,

Bar-Sela, Mountcastle and Lipscomb, 1963), and there is some evidence that in its early stages the rise in progesterone in blood at the same time may be due to adrenal rather than ovarian secretion.

1.3 Sexual Behaviour

1.3.1 Changes during the oestrous cycle

The sexually receptive female rat will respond to mounting by a male by assuming a stationary, squat posture, while deeply arching her back in a concave manner, flexing her tail to one side. This exposes the female's genital regional to the thrusts of the male and also facilitates intromission. This posture is termed "lordosis", and its occurrence has been used to define sexual receptivity in the female rat. Other behaviour patterns also characteristic of the female rat at oestrus are ear-quivering, darting and hopping away from the male, and nipping and clawing at his face (Hemmingsen, 1933; Ball, 1937).

Changes in lordosis during the cycle have been described by Young, Boling and Blandau (1941); Blandau, Boling and Young (1941) and Kuehn and Beach (1963). The first two studies used a "manual test". They performed this test by clasping the animal with two fingers anterior to the iliac crests, while stimulating the vaginal area. It has been described by Ball (1937) as "useful in rough or non-quantitative work" and both Gerall and McCrady (1970) and Adler and Bell (1969) have shown that it gives false positive results - that is, females receptive by the manual test are not necessarily receptive when tested with a male. The data from these two studies are not therefore wholly satisfactory. Kuehn and Beach (1963) used a single group of female rats, which were tested with males at hourly intervals throughout the night of oestrus. It has been shown, however, that mating shortens the oestrous period by several hours (Blandau et al., 1941) and it may also enhance receptivity during the early stages of oestrus (Rodriguez-Sierra, Crowley and Komisaruk, 1975). A description

of the changes in sexual receptivity at oestrus free from both sources of possible error is given by Drewett (1973a). He showed that sexual receptivity was first apparent only after 14.00 hrs on the day of prooestrus, reached a peak at about midnight and then declined through the following day. The fully cornified smear marked the termination, rather than the height, of behavioural oestrus: and these observations replicate exactly the early finding of Young et al. (1941). According to Ter Haar (1972), the period of high sexual receptivity follows with a delay of about 12 hours peak plasma levels of oestradiol-17β.

Madlafousek and Hinak (1977) present an extensive inventory of sexual behaviour patterns in the female rat; but they only give a somewhat crude description of the changes in behaviour over the oestrus period, noting only the presence or absence of behaviours such as darting and hopping. They showed that the occurrence of the presenting posture and hopping were seen from about 23.00 hrs on the night of oestrus, whereas lordosis occurred much earlier (at about 18.00 hrs, late in prooestrus).

The fine details of the behavioural changes, other than lordosis, during prooestrus and oestrus have been examined by Wiepkema (unpublished observations, personal communication, 1978). He noted that there was an increase in darting, hopping and ear-quivering from late prooestrus, rising to a high plateau of several hours during the first half of the oestrous night. These behaviours then declined throughout the latter part of the night; so that only low levels of hopping, darting and ear-quivering were recorded on the morning of oestrus. Lordosis behaviour followed a similar trend.

1.3.2 Hormonal Control

The central role of oestrogens in the control of sexual behaviour is in no doubt. Although several oestrogens have been shown to be effective in stimulating sexual behaviour in the ovariectomized rat (oestradiol,

oestrone, oestriol and oestrone-3-sulphate, Beyer, Morali and Vargas, 1971) most workers have used oestradiol or its esters.

In an ovariectomized rat sexual receptivity at a level comparable with that found during a natural oestrus, can be reinstated with oestradiol alone. This does not occur if oestradiol is injected for short periods (24 or 48 hours) even in high doses (Boling and Blandaw, 1939; Beach, 1942) although some receptivity is restored by such treatment (Zemlan and Adler, 1977). If, however, doses as low as 1.5 μg (of oestradiol benzoate) are given daily, the Lordosis-to-Mount ratio reaches very high levels (80%) within 5 days (Davidson, Smith, Rodgers and Bloch, 1968a). This effect of oestradiol occurs in animals which are both ovariectomized and adrenalectomized; that is animals from whom all known sources of progesterone have been removed (Davidson, Rodgers, Smith and Bloch, 1968b).

Constant oestrous rats with blood levels of oestradiol much lower than those found during prooestrus (ca. 10 pg/ml, as against ca. 90 pg/ml) are also sexually receptive after they have been in the state for some time (Hardy, 1970), and Powers and Zucker (1969) have shown that in intact rats a single injection of 2 μg of oestradiol benzoate on the morning of (vaginal) oestrus reinstates sexual behaviour 24 hours later.

Powers (1970) investigated the role of progesterone in sexual receptivity by ovariectomizing female rats at various times before the onset of oestrus. He ovariectomized the rats at 9.00 a.m. on the morning of prooestrus, on the assumption that this treatment would eliminate the increase in progesterone after the oestrogen surge had already taken place, and tested them for receptivity at 9.00 p.m. This operative procedure led to considerable reduction in sexual receptivity, which was not due to postoperative stress as animals operated 6 hours before testing showed normal receptivity. But the critical question is whether such a procedure eliminated the progesterone surge <u>without</u> interfering with the oestrogen

surge in any important way, since if both were reduced the experiment would obviously not serve to demonstrate a critical role for progesterone. Some authors have found that large amounts of oestrogen are secreted until late on the day of prooestrus (see Yoshinaga et al., 1969; Butcher et al., 1974). Since hormone levels were not measured in the study by Powers, this question cannot be answered unequivocally.

In a recent study, Södersten and Hansen (1977) demonstrated that 4-day cyclic rats became sexually receptive 24 hrs after an injection of 1 or 2 μg of oestradiol benzoate on any day of the cycle, except on the second day after the display of spontaneous oestrus. When they ovariectomized the rats at the time of oestradiol treatment, receptivity was abolished, but it was restored again with injection of progesterone. Repeated treatment of sexually receptive intact rats with progesterone did not affect the duration of oestrus; and treatment with oestradiol benzoate on the day of oestrus prolonged the duration of receptivity. However, the dose of oestradiol used was higher than the dose needed for the induction of receptivity (100 μg vs 2 μg), in ovariectomized rats.

These results suggest that sexual receptivity in the intact rat cannot occur in the absence of oestradiol and progesterone, and that progesterone may not be associated with mechanisms terminating behavioural oestrus in the rat.

1.3.3 Satiation

Feedback from coitus, in addition to inducing hormonal changes (Taleisnik, Caligaris and Astrada, 1966) and, initially, facilitating sexual receptivity (Rodriguez-Sierra et al., 1975), is also responsible for the inhibition of sexual receptivity (Hardy and DeBold, 1972). The possibility that there might be a qualitative relationship between the amount of stimulation during mating and duration of heat in the female rat was suggested by Blandau et al. (1941). Hardy and DeBold (1972) tested

female rats at hourly intervals over a period of 10 hours, with a criterion of 10 mounts or attempted mounts per test. Rejection of males by females was higher, and "lordosis quotients" lower in females that received coital stimulation than in females wearing a vaginal mask. Further, they showed that unmasked, ovariectomized females that had been brought into heat with injections of oestradiol benzoate and progesterone, rejected the males as often as did unmasked intact females in natural heat. This suggests that rejection behaviour following intromission and ejaculation is independent of obvious hormonal change.

Marked shortening of behavioural oestrus following copulation has been reported in another species, the guinea-pig (Goldfoot and Goy, 1970). These authors attributed the termination of behavioural oestrus following copulation to an "afferent neural inhibitory process". They ruled out ovarian or hypophyseal participation in the coital inhibition of heat, because all their animals were both ovariectomized and hypophysectomized, and were brought into heat with oestradiol and progesterone.

A different measure of inhibition comes from a study measuring latencies with which female rats returned to the male for more copulation. Pierce and Nuttall (1961) showed that, in a situation in which the female could escape a male by jumping onto a platform and subsequently return, the female spent longer on the escape platform after an intromission than a mount, and after an ejaculation than an intromission.

Hardy and DeBold (1972) suggest that there may be two variables influencing rejection behaviour, a non-hormonal one (due to the irritative effect of vagino-cervical stimulation during coitus), and a hormonal one (related to the gradual decline of sexual receptivity - Hardy, 1970).

1.4 Food Intake

1.4.1 Changes during the oestrous cycle

During dioestrus, the female rat eats more and becomes less active.

Conversely during late prooestrus, the stage of the cycle during which the female is sexually receptive, and which follows peak plasma oestradiol-17β levels in circulation (Butcher, Collins and Fugo, 1974), food intake and body weight decrease while general activity increases (Brobeck, Wheatland and Strominger, 1947; Kennedy and Mitra, 1963; Rodier, 1971; Tarttelin and Gorski, 1971; Drewett, 1973b). The decrease in food intake is a result of a decrease in the size of the individual meals and a less than compensatory increase in the frequency of the meals (Kenney and Mook, 1974; Blaustein and Wade, 1976, 1977; Gale and Sclafani, 1978). Thus, rats in oestrus resume eating more quickly than rats in dioestrus.

Drewett (1973b) argues that the oestrous depression of feeding is a direct consequence of oestrogen production by the ovary, rather than an indirect effect of sexual receptivity. He shows that food intake may be depressed in ovariectomized rats by doses of oestradiol insufficient to produce either sexual receptivity or vaginal cornification. Further, he suggests that the "phasic" component during oestrus of the oestrogenic effect on feeding is effectively an artefact of the need for a long-term "tonic" component to allow the female to cope with the wide variations in energy demand produced by pregnancy and lactation.

The anorexic effect of the oestrogen peak during late prooestrus is restricted to a reduction in meal size (Drewett, 1974). This reduction may indicate increased sensitivity to the satiating signals normally responsible for meal termination.

Ovariectomy results in a transient increase in daily food intake which is characterised by an increase in the size of an individual meal, and a less than compensatory decrease in meal frequency (Kenny and Mook, 1974; Blaustein and Wade, 1976). When the daily food intake returns to pre-ovariectomy levels, the meal size remains elevated but the meal frequency decreases. Daily injections of oestradiol benzoate reverse the effects

observed after ovariectomy: there is a transient decrease in daily food intake which is accomplished by a decrease in meal size and a less than fully compensatory increase in meal frequency; meal size remains permanently suppressed as long as the daily injections are maintained. A further increase in meal frequency is responsible for the return to control levels of daily food intake. These effects are not observed when rats are injected with progesterone alone (Blaustein and Wade, 1977). These results suggest that oestradiol affects the mechanism(s) that terminate individual meals (Kenney and Mook, 1974; Blaustein and Wade, 1976). Thus the effect of ovarian oestrogens may be specific to short-term processes controlling meal size.

1.4.2 Ovarian control of gastrointestinal processes

Not much is known about the ovarian control of digestive processes. Wang (1923) suggested that oestradiol might have direct alimentary effects. For example, by slowing stomach emptying or increasing the rate of intestinal absorption. Wang stated that "changes in food intake ... may be due to the influence of ovarian hormone or hormones on the function of the stomach". That gastrointestinal mechanisms may be involved in the termination of short-term food intake receives support from the studies by Ehman, Albert and Jamieson (1971), Young, Gibbs, Antin, Holt and Smith (1974), and Snowdon (1975).

The basal secretion of gastric acid in the female rat is higher in dioestrus than in prooestrus or oestrus, and the response of the stomach to gastrin is also more sensitive. Ovariectomy eliminates the difference in secretory sensitivity between male and female rats (Omole, 1972), and injections of oestradiol-17β decrease the sensitivity to gastrin, histamine and carbachol.

Female rats absorb dextrose at a consistantly higher rate than males (Althausen, 1943), and castration of the males does not reduce this

difference. Ovariectomy of the female does not have an immediate effect: eighteen days after ovariectomy absorption is unaffected. Ovariectomizing females at three weeks of age and then testing them seven weeks later reduced the rate of absorption to the male level. Injection of oestrone (20 μg/day for ten weeks) into normal males increased their intestinal absorption of dextrose to the level of the females; but injection of 100 μg/day into adult males for fifteen days was ineffective (Althausen, 1943).

1.5 Water Intake

This follows the same general pattern as food intake, over the oestrous cycle, showing a depression during oestrus (Tarttelin and Gorski, 1971). It is likely that reduced water intake over oestrus is a consequence of the decrease in food intake. Another possibility is that several oestrogens, including oestradiol, are responsible for increased uterine water retention (Brotherton, 1976). Thus ovarian oestrogens may affect water consumption indirectly, by increasing water retention in the female rat, during oestrus.

CHAPTER 2

OESTRADIOL AND STOMACH EMPTYING IN THE FEMALE RAT

2.1 Introduction

Stomach emptying is a variable which often correlates with changes in meal patterns: vagotomy in rats maintained on a liquid diet (Snowdon and Epstein, 1970; Davis and Booth, 1974) and lesions to the lateral hypothalamus (Kissileff, 1970; De Castro and Balagura, 1974) give rise to a decrease in meal size and an increase in the rate of gastrointestinal transit (Snowdon, 1970; Ralph and Sawchenko, 1978). Conversely, ventromedial hypothalamic lesions result in an increase in meal size and a decrease in the rate of gastrointestinal transit (Balagura and Devenport, 1970; Becker and Kissileff, 1974; Ralph and Sawchenko, 1978). It has already been mentioned (in Chapter 1) that oestradiol injections in ovariectomized rats cause a decrease in meal size. It is possible, therefore, that oestradiol changes the rate of stomach emptying, leading to changes in the size of individual meals.

In a recent study, Blaustein and Wade (1977) intubated anaesthetised rats with a nutritionally complete liquid diet. They found that rats treated daily for two weeks with 2 μg oestradiol benzoate, and fasted for 24 to 28 hours, showed a 58% increase in intestinal distance travelled by the liquid diet, and a 32% decrease in gastric retention when compared with oil treated controls. In a second experiment, in which they used a slightly longer deprivation period (34 to 38 hours) and a single injection of oestradiol benzoate (5 μg per rat), they found no difference between treated and control groups. No information is given as to the amount of fluid secreted into the stomach. These experiments may be criticised on the fact that Blaustein and Wade used anaesthetised animals. It is known that changes in blood composition (nutrient and hormonal) as well as

intestinal activity occur in the anaesthetised state, which differs considerably from the normal condition, and this may have been a contributing factor to the equivocal nature of their results.

There is much evidence in the literature that the mechanisms involved in the regulation of stomach emptying of salts and sugars are different from those regulating the emptying of fats (e.g. see: Farrell and Ivy, 1926; Shay and Gershon-Cohen, 1934; Hunt and Knox, 1964; Knoebel, 1976). These mechanisms may be differentially affected by oestradiol. Therefore, in the following experiments the influence of oestradiol benzoate on the rate of stomach emptying was investigated using saline, fat and glucose loads in the ovariectomized female rat.

2.2 Experiment 1 : The effect of oestradiol benzoate on food intake after food deprivation in the female rat

Subcutaneous injections of oestradiol in the ovariectomized rat suppress food intake (Tarttelin and Gorski, 1971; Drewett, 1973a,b). The following experiment tested whether the anorexic effect of oestradiol benzoate manifested itself in ovariectomized rats which were food deprived for 24 hours, from the time of oestradiol benzoate administration to the replacement of food. A deprivation period is necessary in stomach loading experiments in order to ensure initial uniformity in the volume of stomach contents (effectively, the stomach would be empty), prior to loading. Thus, the information from this experiment was necessary in order to determine whether or not any changes in the mechanisms controlling meal size, caused by oestradiol, are independent of changes in deprivation.

2.2.1 Method

General procedures for ovariectomy and measuring food intake

1. Ovariectomy:

All the rats used in the following experiments were ovariectomized under ether anaesthesia. A small area of skin over the spine was shaved

and a short (1 to 2 cm) incision was made antero-posteriorly along the midline. Through this incision a second incision was made in the body wall on the right flank. The ovary was drawn out through this incision, and all the blood vessels leading to it together with the upper end of the uterine horn and some periovarian fat were tied off by means of a silk thread. The ovary, periovarian fat and part of the uterine horn were removed, the remainder of the horn and periovarian fat were replaced, and the incision was closed with a single silk suture. When both ovaries had been removed, the dorsal incision was closed with further silk sutures. The wound was cleaned with Hibitane antiseptic solution (I.C.I. - Gr.Br.). This technique has two main advantages: it is quicker than using two skin incision; and the dorsal skin wound is not over the flank incisions, and was therefore less prone to infection.

2. Food intake:

Rats were housed individually in plastic cages, measuring 42 x 30 x 17 cm. Coprophagia was prevented by a removable galvanised wire grid, 2 cm above the bottom of the cage. Dry, powdered food (Rat and mouse maintenance diet No. 1, BP Nutrition, Witham) was available in a metal food hopper (Figure 2.1), which was attached to the top of the cage, but easily removable. The bottom of the hopper was 1 cm above the metal grid. Rats differ in their feeding habits, some using their fore-paws to scoop up the food; but the particular design of the food hopper kept spillage down to a minimum. The rats could not carry away the powdered diet, and on average the spillage was about 0.2 grams daily. This spillage was collected on paper towelling placed beneath the grid under the hopper. The food hopper was routinely half-filled (a further precautionary measure to reduce spillage) with the diet. The food with any of the spillage was collected and weighed daily on a "Torbal" balance, to the nearest 0.1 g.

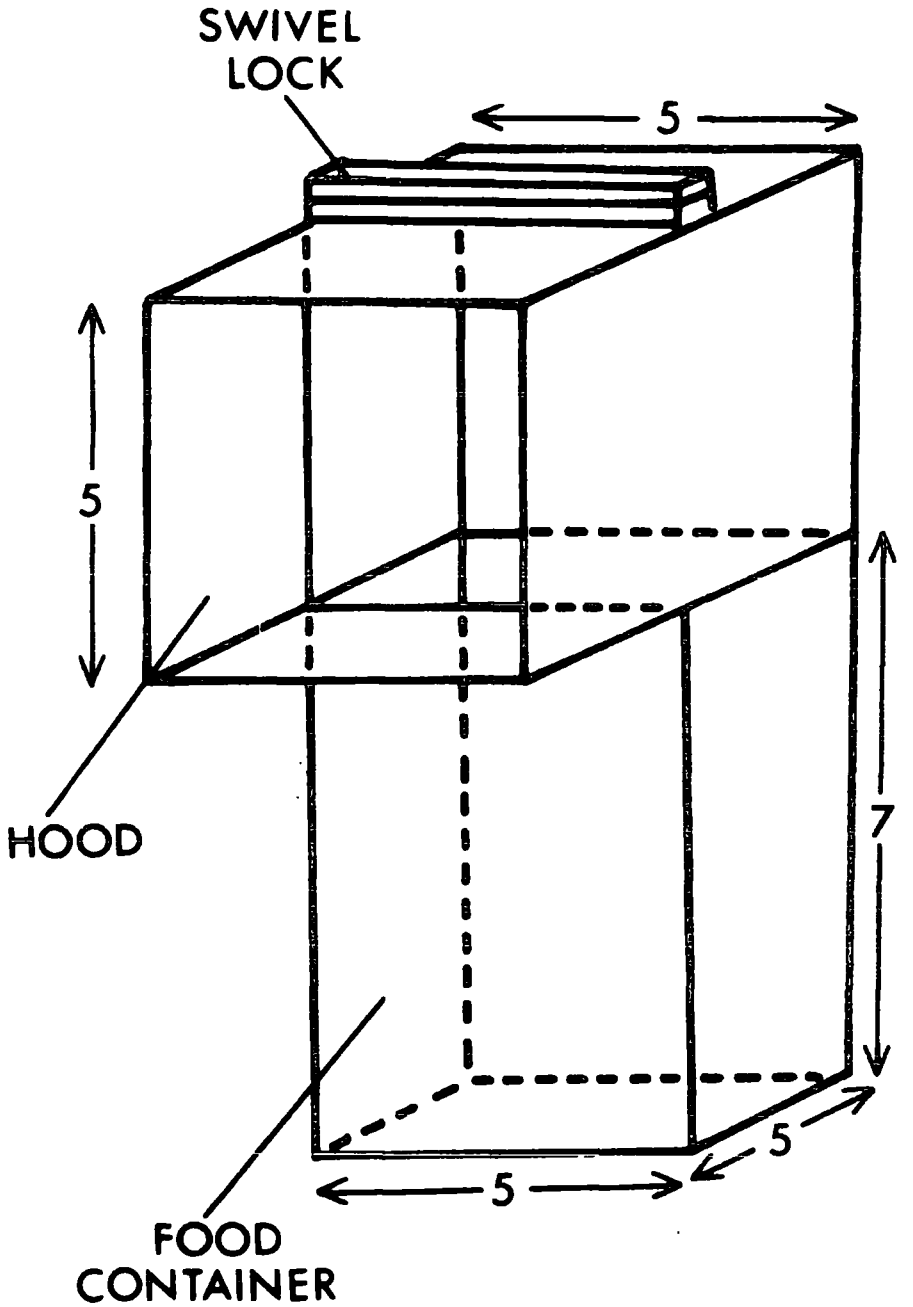


Figure 2.1

Experiment 1. The food hopper. Measurements are given in centimetres.

Experimental animals

The animals were 16 hooded female rats (Piebald VIROL-GLAXO strain), about 120 days of age, and weighing 253 $\pm$ 25 g. The rats were ovariectomized one month before the start of the experiment, in order to allow food intake and body weight to stabilise. Two weeks after ovariectomy the rats were transferred to individual plastic cages, and given powdered food and water ad lib. Previous to this, the animals lived in two group cages, eight animals in each cage, and were given maintenance diet and water ad lib. The animals were housed in a reversed light room having a 12 hr light-12 hr dark regime, the lights switching off at 12.00 hr GMT. The temperature was thermostatically controlled at +21^{o}C.

Experimental schedule

The experiment was run over a period of two weeks using a counterbalanced design. The rats were allocated to two groups using a tabled sequence of random numbers (Fisher and Yates, 1963), an experimental group treated with oestradiol benzoate using arachis oil as the vehicle for injection, and a control group which was treated with arachis oil only. Food intake was measured over one day preceding food deprivation. On the following day, all the rats were injected with either the hormone or the oil vector two hours after the onset of the dark cycle, at 14.00 hr, and the food removed immediately. Eight rats were injected with 5 µg oestradiol benzoate in 0.1 ml arachis oil, and the other eight rats with 0.1 ml arachis oil only. All the rats were injected again at 09.00 h on the following day with the same amount of hormone or oil vehicle. The injections were performed subcutaneously, in the nape of the neck, using a 25 ga disposable needle. The food was replaced five hours later at 14.00 h, 24 hours after the initial removal of the food hoppers, and food intake was measured over the subsequent 24 hour period. After this, seven treatment-free days allowed complete elimination of the hormone. During the second week, the treatment

for each group was reversed, so that the experimental animals in the first week now became the control animals and vice-versa. Each animal thus acted as its own control.

2.2.2 Results and Discussion

Figure 2.2 shows that female rats treated with oestradiol benzoate ate less than rats injected with the oil vehicle only. A matched-pairs t-test on the data showed that the difference in mean food intake between the hormone treated rats and the controls was significant at the 0.1% level ($t = 4.4332$; with $df. = 7$; $p < 0.001$).

The result of this experiment shows that the suppressive effect of oestradiol benzoate on food intake manifests itself even after twenty-four hours of food deprivation. Blaustein and Wade (unpublished observations, cited in Blaustein and Wade, 1977) reported that a single injection of 2 μg of oestradiol benzoate significantly altered meal size (reduced it) within 22 to 34 hours after administration. In this experiment, 5 μg of oestradiol benzoate was used, in order to ensure the effect of the hormone.

Oestradiol primarily affects the mechanism(s) that terminate short-term food intake (meal size); and the observed changes are not secondary to changes in total daily intake, nor do they seem to be secondary to changes in meal frequency (Blaustein and Wade, 1976, 1977). Since there is evidence for a gastrointestinal contribution to the termination of short-term food intake, the next experiments investigate the possibility that oestradiol benzoate treatment of ovariectomized rats causes a change in stomach emptying resulting in the alteration of meal size.

2.3 Experiment 2 : The effect of oestradiol benzoate on the stomach emptying of saline in the female rat

Shay and Gershon-Cohen (1934), in their radiographic studies on stomach emptying, noted that tap water left the stomach more slowly than isotonic saline. They concluded that in order to slow emptying (presumably

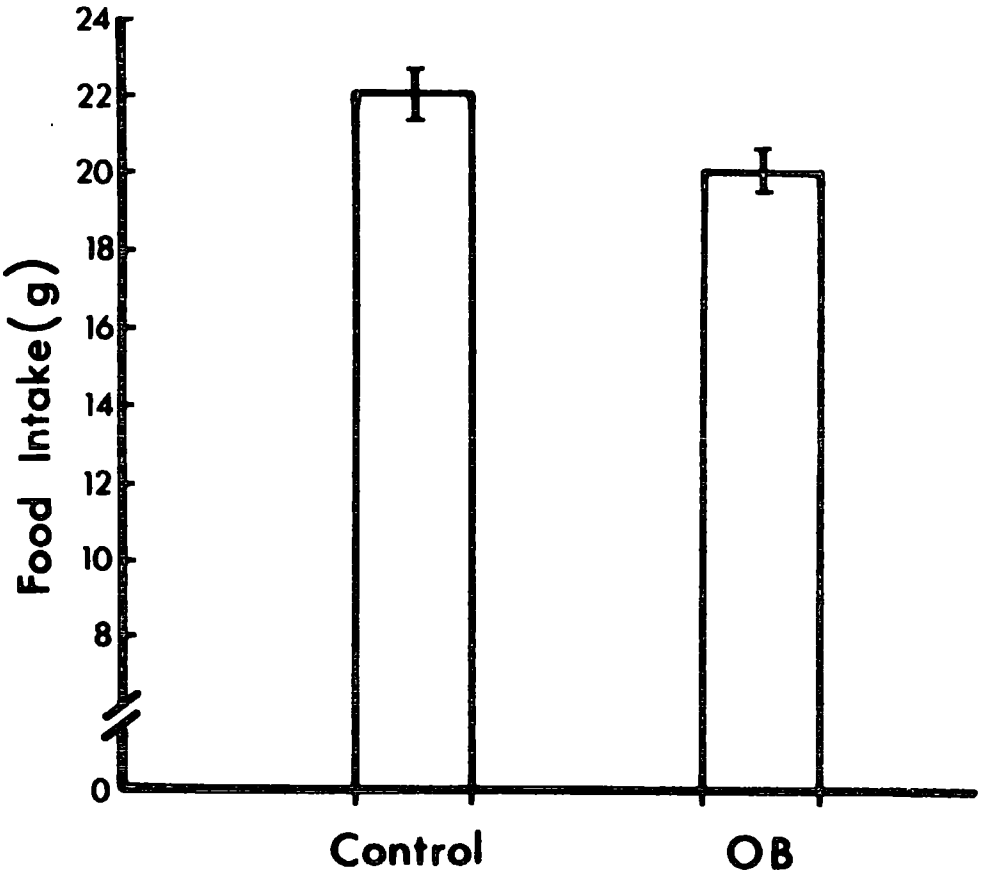


Figure 2.2

Experiment 1. Mean food intake (± standard error) of ovariectomized rats treated with oil only (control) or oestradiol benzoate (OB), after 24 h food deprivation.

relative to the emptying of tap water), hypertonic solutions of salts or glucose were necessary. Snowdon (1970) also showed that a 1.8% saline solution left the stomach more slowly than isotonic saline. This suggests that the rate of stomach emptying is influenced by the osmotic pressure of the ingested diet. The following experiment investigated the effect of oestradiol benzoate on the emptying of hypertonic saline from the stomach, in female rats.

2.3.1 Method

Experimental animals

The animals were 22 hooded female rats about 120 days of age. They were kept in a reversed light room and food and water were freely available. The rats were ovariectomized under ether anaesthesia, one month before the start of the experiment. The animals were handled often to reduce stress during stomach loading.

Experimental procedure

The rats were randomly allocated to two groups, and were food deprived and injected with 5 µg oestradiol benzoate in 0.1 ml arachis oil per rat or 0.1 ml oil/rat only, according to the procedure described in Experiment 1. The loading started at 14.00 h and the loading procedure was determined by a sequence of random numbers.

The solution used for intubation consisted of 1.8% saline (2N NaCl: 580 mOsmol/l), and was mixed with a 0.04% solution of phenol red in a ratio of 10 parts saline : 1 part phenol red. The rats were lightly etherised and loaded with 5 ml of this mixture through an intra-gastric polyethylene tube. The time interval between loading and removal of the stomachs was 10 minutes. At 8 minutes the rats were placed in an ether jar. Two minutes later the abdomen was opened, the gut clamped at the cardia and pylorus, and the stomach removed. The contents were immediately placed into glass tubes and centrifuged at 10,000 revs/min for 60 minutes to remove stray food particles.

The volume and dye concentration of the supernatant were then determined. One ml of the contents was mixed with 9 ml of 0.05 M NaOH, titrating the phenol red to alkalinity. The resulting deep red solution was read through a 540 nm filter on a UNICAM SP 600 spectrophotometer, to determine the change in optical density from the original solution. The volume emptied from the stomach was computed according to the equations described by Snowdon (1970). The volume injected into the stomach and the volume recovered are known. The volume of fluid secreted by the stomach is determined by the change in dye concentration:

Therefore,

(a) (Dye concentration injected x volume injected) = (Dye concentration recovered x volume recovered) + (Dye concentration emptied x volume emptied).

... Eq. (2.1)

where,

Dye concentration emptied $\simeq$ (Dye concentration injected + Dye concentration recovered)/2

(b) Volume injected + volume secreted = Volume recovered + volume emptied.

... Eq. (2.2)

2.3.2 Results

Figure 2.3 shows the volume secreted by the stomach of ovariectomized rats treated with oil only or 5 µg oestradiol benzoate. Rats treated with oestradiol benzoate secreted significantly more fluid into the stomach than controls (t-test: $n = 22$; with $df. = 20$; $t = 2.027$; $p < 0.05$). These findings contrast with those reported by Omole (1972) who found that the oestrous state, whether natural or induced, exerted an inhibitory effect on secretion in the female rat stomach. Omole used anaesthetised rats and induced ovariectomized females into oestrus by injections of oestradiol benzoate plus progesterone. The use of anaesthetised animals in experiments

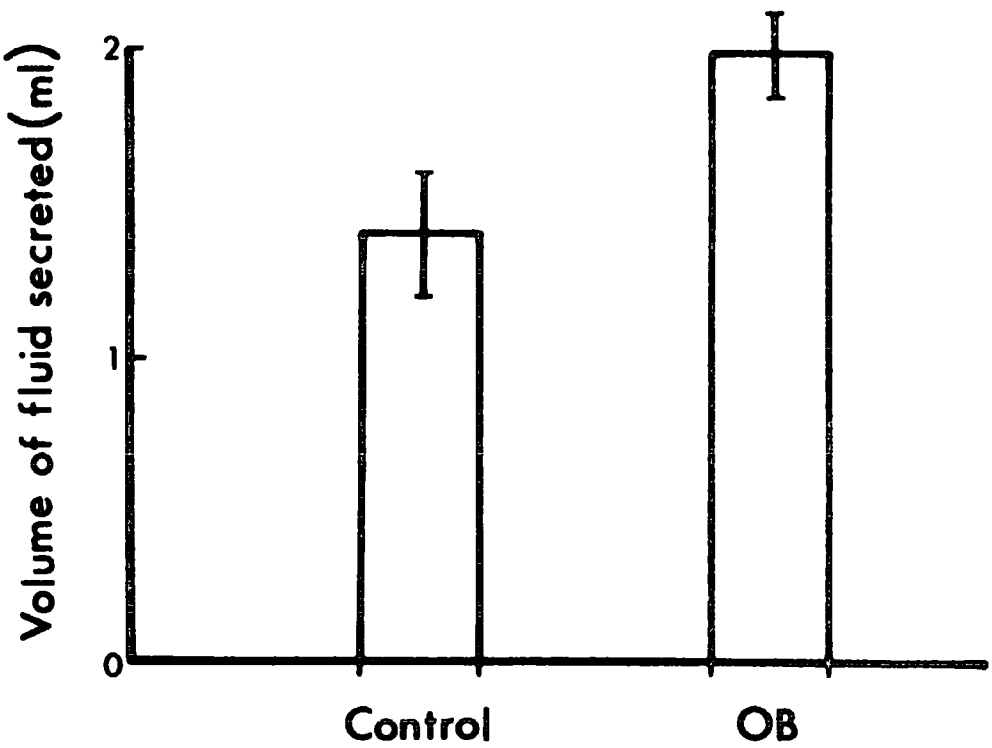


Figure 2.3

Experiment 2. Mean volume of fluid (± standard error) secreted into the stomach, in ovariectomized rats treated with oil only (control) or oestradiol benzoate (OB).

of this kind has already been criticised (Chapter 1).

Figure 2.4(a) presents the data of the per cent of stomach contents emptied - that is, the total volume emptied divided by the volume injected plus the volume secreted into the stomach - and Figure 2.4(b), the mean volume of saline emptied (Total volume emptied x dye concentration emptied/ dye concentration injected). There was no difference in either of these measures between rats treated with oestradiol and untreated rats.

2.4 Experiment 3 : The effect of oestradiol benzoate on the stomach emptying of glucose in the female rat

Rats eat less after stomach loads of glucose than after non-nutritive loads. This inhibition cannot be attributed to osmotic factors, except for a short period after loading. For example, the unmetabolised glucose analogue 3-O-methylglucose does not match the inhibition produced by an equimolar dose of glucose. Furthermore, the total decrease in food intake is proportional to the amount of glucose administered, and approximately equivalent in content of utilizable energy, suggesting a satiating effect arising from the metabolism of absorbed glucose (Booth, 1972). This experiment investigated whether oestradiol alters the emptying of glucose from the stomachs of female rats.

2.4.1 Method

Experimental animals

The animals used in this experiment were thirty-six Wistar female rats, about 120 days of age and weighing 267 $\pm$ 16 g. They were kept in a reversed light room (12 h light-dark cycle), and given food and water ad libitum. All the rats were ovariectomized one month before the start of the experiment.

Experimental procedure

The rats were randomly allocated to two groups, one received oestradiol benzoate (5 μg in 0.1 ml arachis oil/rat) and the other, a control injection of 0.1 ml arachis oil/rat only.

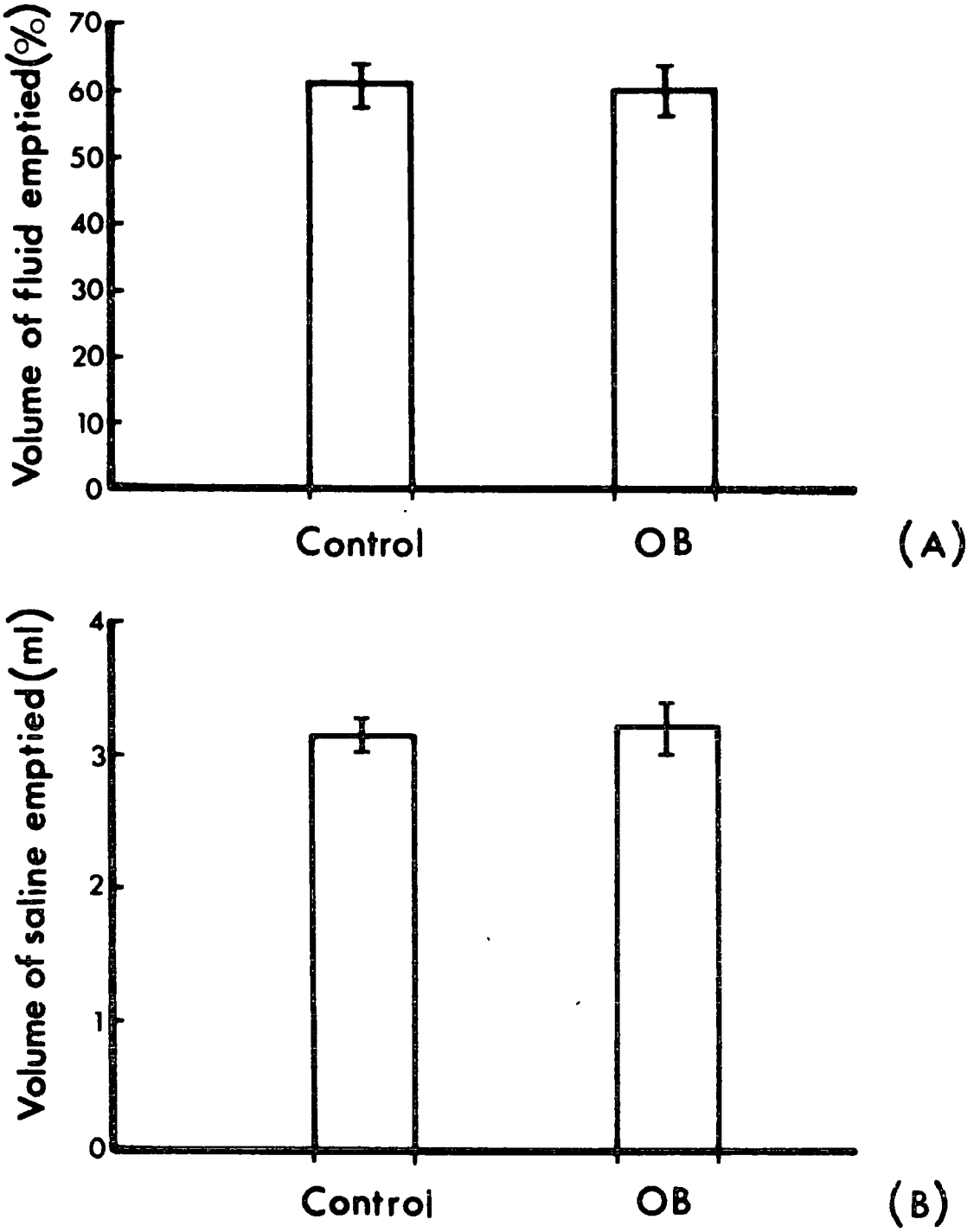


Figure 2.4

Experiment 2. (A) Mean volume of fluid (P/O ± standard error) emptied from the stomach, in ovariectomized rats treated with either oil only (control) or oestradiol benzoate (OB).

(B) Mean volume of saline (± standard error) emptied from the stomach, in ovariectomized controls or rats treated with OB.

In order to maximize any effects oestradiol may have on stomach emptying, it was decided to inject the rats on three consecutive days rather than two days (as was the case in Experiment 1, 2 and 4). The deprivation schedule also differed from the one previously employed, and was based on data on the clearance of ingested foodstuffs from the stomach published by Booth and Jarman (1976). They found the stomachs to be empty of food, in rats from which maintenance diet had been witheld for 3 hours in the dark period. Consequently, oestradiol benzoate or oil placebo injections were administered 48 h, 24 h, and 3 hours before deprivation. Food was removed at 12.00 h on the onset of the dark period and loading commenced three hours later, at 15.00 h.

Each injected load consisted of a 40% glucose solution (2 g of anhydrous d-Dextrose in 5 ml of distilled water; 2223.5 mOsmol; BDH Chemicals, Poole). This concentration was used for the following reasons:

(1) The oxidation energy of glucose (3.74 kcal/g) approximately corresponds to the digestible energy content of maintenance diet (3.25 kcal/g - as supplied by BP Nutrition, Witham).

(2) The amount of glucose administered (2 g) is approximately equivalent to amounts that are yielded by the digestion of the carbohydrate content of a meal of the size typical for the freely fed rat (about 2 to 3 g; Le Magnen and Tallon, 1966; Levitsky, 1970).

The glucose solution was prepared in distilled water two hours before gastric administration and kept at room temperature. A 0.04% solution of phenol red was mixed with the glucose in a ratio of 10 parts glucose : 1 part phenol red. The animals were killed after 15, 30, or 60 minutes. The oestradiol treated or untreated rats were allotted these time periods and loaded according to a paired sequence of random numbers. The animals were loaded from 15.00 h onwards, and were killed with ether according to this schedule. The procedures for stomach removal and optical density readings

were the same as those used in Experiment 2. Similarly, equations (2.1) and (2.2) were employed in calculating the volume secreted, percentage of fluid emptied from the stomach and absolute volume of glucose emptied from the stomach.

2.4.2 Results

Analyses of variance (two-between, 0-within factors; Tables 2.1 to 2.3) showed that neither the volume of fluid emptied nor the absolute volume of glucose emptied was significantly changed by oestradiol benzoate treatment (Figure 2.5(a) and (b)). However, there was a significant difference in the volume of fluid secreted over time, resulting in a significant treatment x time period interaction ($F = 5.14$; with $df. = 2, 30$; $p < 0.05$, Figure 2.6). These results suggest, then, that a rapid change in gastric secretion occurs soon after the ingestion of a meal, and that this change takes place earlier in the oestradiol treated rat.

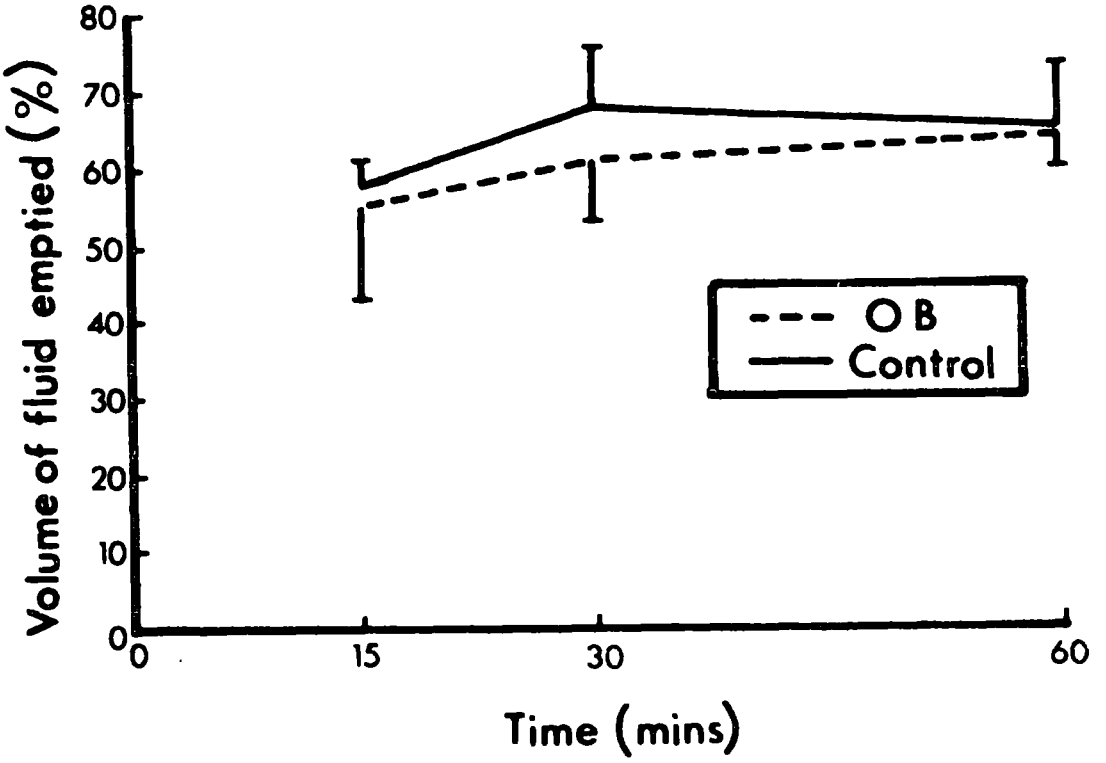
2.5 Experiment 4 : The effect of oestradiol benzoate on the stomach emptying of fats in the female rat

Triglycerides injected into the duodenum inhibit the motility of a transplanted fundic pouch (Farrell and Ivy, 1926), and it is generally held that a hormone, probably enterogastrone, mediates the slowing of stomach emptying by fats (Hunt and Knox, 1964). More recently Knoebel (1976) suggested that the emptying of fats, protein and digestion products from the stomach was under endocrine control, and also involving other hormones such as cholecystokinin and secretin. The following experiment investigated the effect of oestradiol benzoate on the emptying of glycerol trioleate (an unsaturated fatty acid) from the stomach, in female rats.

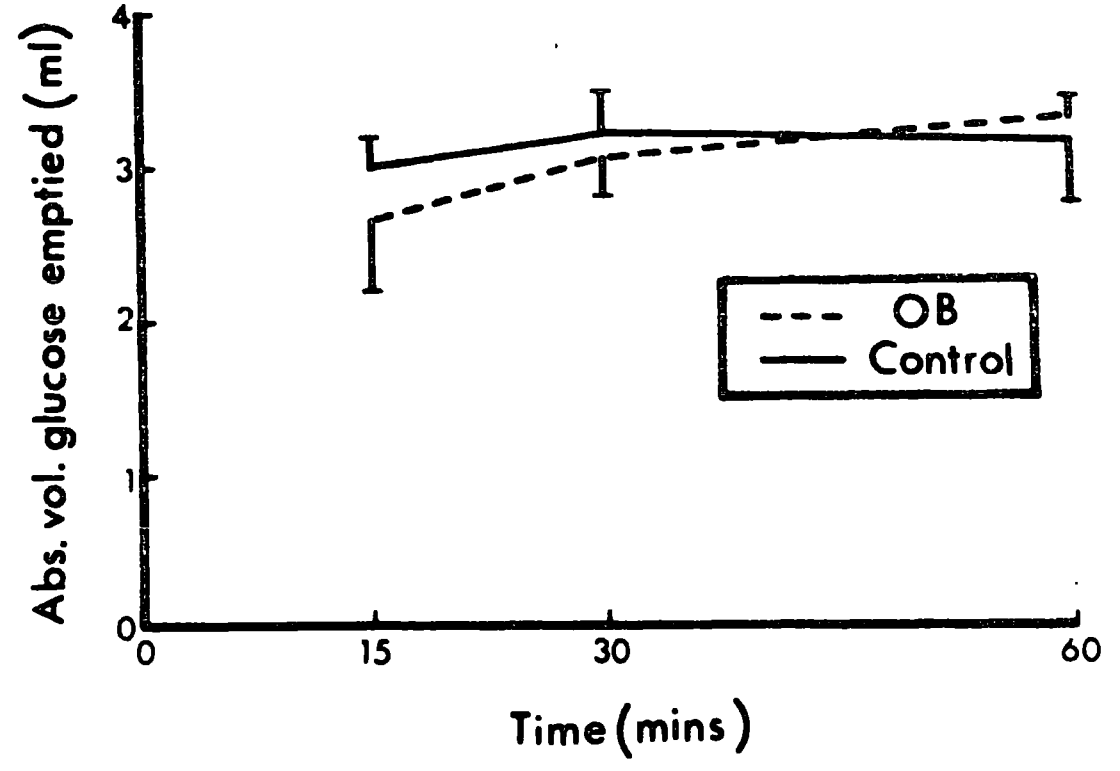
2.5.1 Method

Experimental animals

Thirty-two young (120 day old) hooded female rats, and weighing between 230 and 263 g, were kept in a reversed light room and given food and water



(A)



(B)

Figure 2.5

Experiment 3. (A) Mean volume of fluid (± standard error) emptied from the stomach, in ovariectomized rats treated with oil only (control) or oestradiol benzoate (OB), after 15, 30 or 60 minutes.

(B) Absolute volume of glucose (± standard error) emptied from the stomach, in ovariectomized controls or rats treated with OB, after 15, 30 or 60 minutes.

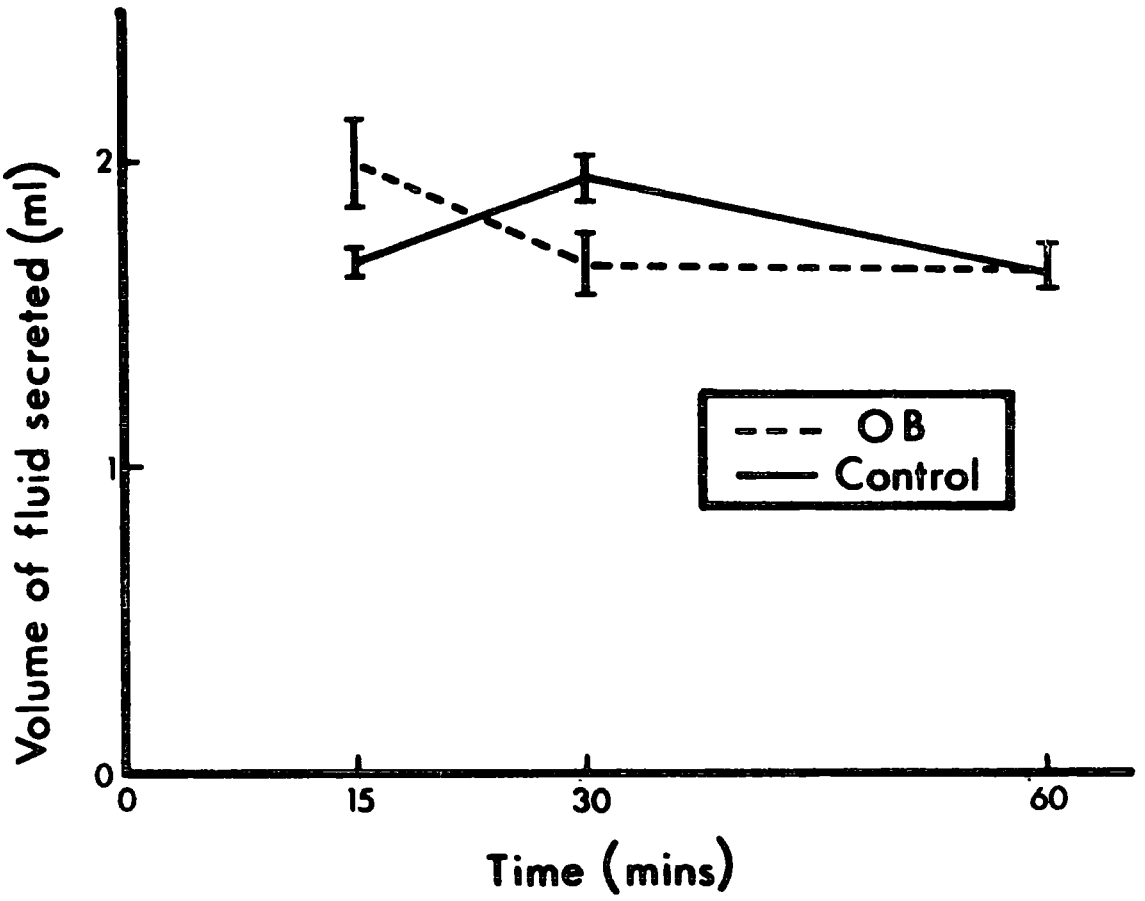


Figure 2.6

Experiment 3. Mean volume of fluid (± standard error) secreted into the stomach, in ovariectomized rats treated with oil only (control) or oestradiol benzoate (OB), after 15, 30 or 60 minutes.

Table 2.1

Experiment 3. Effect of oestradiol benzoate on gastric secretion in the female rat: Glucose load. Summary of analysis of variance

Source	df	SS.	MS.	F-ratio	P
Subjects	35	2.4964			
Treatment	1	0.0003	0.0003	< 1	ns.
Time period	2	0.2606	0.1303	2.35	ns.
Treatment x Period	2	0.5706	0.2853	5.14	< 0.05
Error	30	1.6650	0.0555		
Within cell	0	0.0			

Table 2.2

Experiment 3. Effect of oestradiol benzoate on gastric emptying in the female rat: Glucose load. Summary of analysis of variance

Source	df	SS.	MS.	F-ratio	P
Subjects	35	11074.37			
Treatment	1	89.62	89.62	< 1	ns.
Time period	2	539.66	269.83	< 1	ns.
Treatment x Period	2	45.32	22.66	< 1	ns.
Error	30	10399.78	346.66		
Within cell	0	0.0			

Table 2.3

Experiment 3. Effect of oestradiol benzoate on absolute volume of glucose emptied. Summary of analysis of variance

Source	df	SS.	MS.	F-ratio	P
Subjects	35	16.95			
Treatment	1	0.12	0.12	< 1	ns.
Time period	2	0.96	0.48	< 1	ns.
Period x Treatment	2	0.25	0.12	< 1	ns.
Error	30	15.62	0.52		
Within cell	0	0.0			

ad lib. They were ovariectomized under ether anaesthesia one month before the start of the experiment.

Experimental procedure

The rats were randomly allocated to two groups, which received either 5 μg oestradiol benzoate in 0.1 ml arachis oil or a control injection of 0.1 ml arachis oil only. All the injections were administered subcutaneously. The animals were food deprived and injected according to the schedule in Experiment 1. Stomach loading began two hours after the start of the dark period, at 14.00 h, and 24 hours after the food was removed. The loading procedure was determined by a sequence of random numbers.

The rats were lightly etherised and each rat was loaded with 5 ml of glycerol trioleate (BDH Chemicals, Poole) through an intra-gastric polyethylene tube. The time interval between loading and stomach removal was 10 minutes. The rats were killed by ether. The stomachs were quickly removed as described in Experiment 2, and emptied. There was no aqueous phase in the recovered fluid. The volume of fat recovered was measured with a micropipette.

2.5.2 Results

Figure 2.7 shows the volume of fat emptied by the stomach in rats treated with oestradiol benzoate or the oil vector only. A t-test showed that the difference between the means for the two groups is significant ($n = 36$; with $df. = 34$; $t = 2.398$; $p < 0.05$). There was no secreted fluid recovered. This suggests two alternatives (1) either the fat load inhibited secretion or (2) the secreted fluid bypassed the fat load and passed through into the duodenum. An obvious interpretation of the result of this experiment is that the fat content of a meal affects stomach emptying in the oestradiol treated rat, thereby inhibiting food intake and enhancing satiation.

2.6 General Discussion

The experiments presented in this chapter investigated the possibility

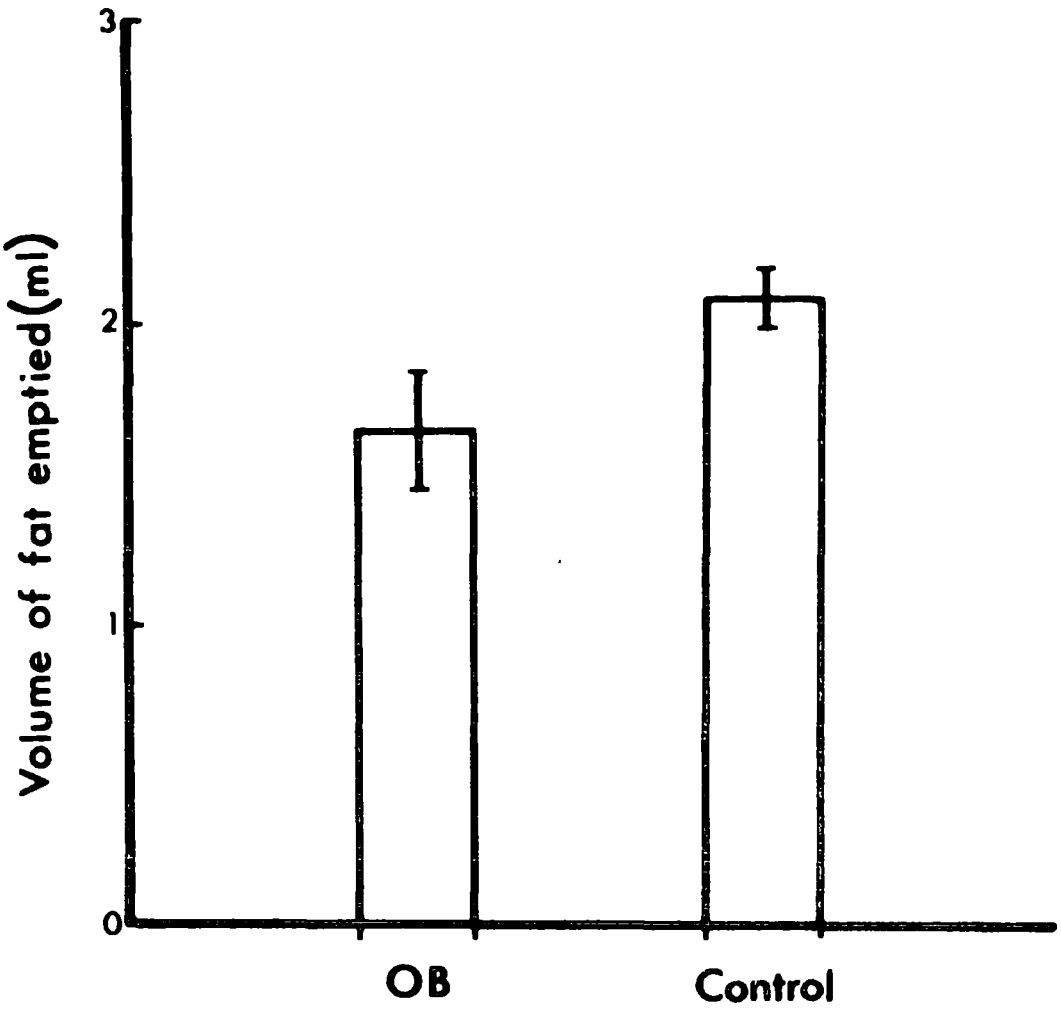


Figure 2.7

Experiment 4. Mean volume of fat (± standard error) emptied from the stomach, in ovariectomized rats treated with oil only (control) or oestradiol benzoate (OB).

that oestradiol altered stomach emptying in the female rat, thereby giving rise to a decrease in meal size.

Gastric retention was not significantly affected by oestradiol benzoate when a 1.8% saline (Experiment 2) or a 40% glucose load (Experiment 3) was used. However, a difference was observed in the volume of fluid secreted into the stomach. A larger volume of fluid was secreted earlier in the hormone treated rats. The amounts agree well for both 2N saline and glucose loads. These findings suggest that there are rapid changes in the gastric environment which possibly cause satiation, in the oestradiol treated rat.

Two explanations present themselves. First, oestradiol caused a larger volume of fluid to be secreted earlier after the ingestion of a meal. This would lead to a fuller stomach and also, possibly, increase gastric distension. This factor is known to be an important satiety signal; and inert bulk in the stomach is as effective as food in producing short-term inhibition of eating (Janowitz and Grossman, 1949). Second, the composition of the secreted fluid may have been different. Thus it is possible that substances which would normally increase satiation and mediate the termination of a meal, were secreted earlier into the stomachs of rats treated with oestradiol benzoate. This would give rise to smaller meals. As yet there is no evidence for a gastric inhibition of this type.

Experiment 4, in which an unsaturated fatty acid was injected into the stomach, produced a significant increase in gastric retention in rats treated with oestradiol benzoate. It is possible that oestradiol induced a neurally mediated change in the sensitivity of duodenal receptors to fats. An alternative possibility is that oestradiol mediates a change in motility of the stomach, through altering the secretion or activity of a hormone (or hormones) known to participate in the regulation of stomach emptying, such as enterogastrone (Hunt and Knox, 1968; Thomas and Baldwin, 1968) or

cholecystokinin (Antin, Gibbs and Smith, 1978). Enterogastrone normally inhibits gastric emptying, and is released in the duodenum whenever either the volume or osmotic pressure of a bolus of gastric contents is very large (Thomas and Baldwin, 1968). It has recently been shown that cholecystokinin mimics the satiety effect of food in the intestine on sham feeding (Antin et al., 1978).

CHAPTER 3

OESTRADIOL, SEXUAL MOTIVATION AND REWARD IN THE FEMALE RAT

3.1 Introduction: Studies on sexual motivation in the female rat

In the original study on animal drives by Moss (1924), the "drive" was measured by the frequency with which the animals (rats) crossed an electrified grid to reach a reward. Subsequently, C. J. Warden's group adopted the same method as Moss', using the Columbia Obstruction Apparatus, and used it to measure "sex drive" in both male and female rats. The intensity of the drive was measured by the number of times the animal crossed an electrified grid to reach the incentive animal during a period of 20 minutes (Jenkins, Warner and Warden, 1926; Warner, Warden and Nissen, 1928).

Warner (1927) showed that females in oestrus crossed the electrified grid ten times as often as females in dioestrus. Nissen (1929), using the same apparatus, showed that the females' willingness to cross the electric grid to reach a male declined after ovariectomy, but could be restored by extracts from cow placenta. Jenkins (1928), also using the Columbia Obstruction Apparatus, measured the effects of sex segregation upon the sexual drive of female rats. He found that segregation at 30 days of age decreased the number of grid crossings in intact oestrous rats when the incentive was a male; but increased, when compared to non-segregated females, when the incentive was a dioestrous female.

More recently, a wide variety of runway and maze techniques have been used in the study of sexual motivation in male and female rats (Sheffield, 1951; Beach and Jordan, 1956; Bolles, Rapp and White, 1968; Drewett, 1973c; Hill and Thomas, 1973; Meyerson and Lindström, 1973; Gilman and Westbrook, 1978). One of the more common measures used is the time taken to run down a runway, and this method was the one used by Bolles et al.

(1968). They found that oestrous females ran faster than their dioestrous controls, but females did not run any faster to a sexually active as opposed to a passive male. It should be noted, however, that the sexually passive males were not castrates. This suggests that an intact male may have other rewarding characteristics to a female rat apart from copulatory behaviour.

Using a runway-choice technique, Meyerson and Lindström (1973) showed that when a sexually active male and an oestrous female were used as incentive animals, oil-treated controls chose the active male in the majority of the test sessions, and after treatment with oestradiol-17β there was a clearcut increase in preference for the males, and this persisted for several days. The choice time for the two incentive animals was not changed by oestradiol replacement. There was no difference in running times between trials in which an active male was chosen and trials in which a female rat was chosen, which is in agreement with the findings of Bolles et al. (1968). However, after oestradiol treatment running times were much shorter than for the oil treated animals, but only when the male was chosen.

Male rats behave differently towards oestrous females than to diestrous females. In some of the experiments a difference in the learning of the females in the different conditions was observed (e.g. Warner, 1927; Bermant, 1961; Bermant and Westbrook, 1966, using the bar-press method). This therefore may have been due to differences in the incentive value of the males' behaviour, rather than differences in the motivational state of the females. The experiments by Meyerson and Lindström (1970, 1973) and Eliasson and Meyerson (1975) using several different techniques but similar designs to the previous studies, are to some extent open to the same criticisms. Their males were kept behind a grid in order to avoid contact with the females; but although the animals were not allowed to mate, there was still no guarantee that the behaviour of the males towards the oestradiol-treated and untreated females was identical.

In order to illustrate the point further, experiments by Carr, Loeb and Dissinger (1965) showed that receptive female rats preferred the odour of normal males over that of castrates. This would suggest that the oestrous females in the open-field, increasing barrier or runway choice tests (as used by Meyerson and Lindström, 1970, 1973, and other experiments cited earlier and using similar techniques) might have selected active or intact male rats over castrates independent of their copulatory behaviour, on the basis of their odour.

Using a similar runway-choice technique, B. Gorzalka (unpublished data, 1969, cited in Hardy and DeBold, 1972), used two intact potent male rats and prevented one male from achieving intromission (and ejaculation) by applying lidocaine anaesthetic ointment to the penis. Receptive female rats preferred this rat to the normal male which was capable of intromission and ejaculation. But the possibility that the female rats also preferred the "novel" odour of the anaesthetic cannot be discounted. However, there is indeed a strong indication that the behaviour of receptive females towards males (i.e. approaching the male, orienting behaviour and lordosis) may be reinforced by the stimulus qualities of the males (for example odour or pre-copulatory behaviours, such as ano-genital sniffing and licking) and not just by intromission and ejaculation.

Although there is abundant evidence showing that copulation can reinforce instrumental learning in the male rat (Beach and Jordan, 1956; Kagan, 1955; Kaufmann, 1953), the role of reinforcement in the sexual behaviour of the female laboratory rat remains unclear. More specifically, we do not know the reinforcing value of the different components of sexual behaviour of the male rat such as mounting, intromission and ejaculation.

In studies by Bermant (1961) and Bermant and Westbrook (1966), oestrous females trained to press a bar in order to achieve contact with a male rat, showed longer intervals between responses (bar-presses) with increasing

sexual stimulation. For example, females were slower to respond, or showed greater latencies, after intromission and ejaculation than after mounting. Although the most likely inference from these two studies is that contact with a male and subsequent copulation are reinforcing, appropriate control conditions were not run to show conclusively whether mating per se was reinforcing: the opportunity to engage in social or exploratory behaviour could have been the source of the small amount of reinforcement shown in these studies.

In an attempt to clarify the issue, Hill and Thomas (1973) ran a similar experiment to Bolles', in order to examine the role of reinforcement in the control of sexual behaviour, and under more controlled conditions. In contrast to Bolles' findings, Hill and Thomas found that females ran faster to approach active as opposed to passive (non-castrate) males, but that both types of males reinforced learning. Furthermore, Hill and Thomas showed that there was no difference in the runway performance of female rats in natural oestrus, and those in induced oestrus. However, the odour of the passive males could have been different from that of active males, although their results indicate that females could indeed be running faster because of a mating reward.

Using a T-maze, Drewett (1973c) examined the choice of an individual female rat for a potent (copulating) against an impotent (castrate) male rat. He found that the absence of behavioural oestrus neither reduced the females' preference for the potent males nor increased the latency of the choice between the potent males and the castrates. Females chose the arm of the maze which housed a potent male over that which housed an impotent male; and they took twice as long to relearn the task during reversal training. This experiment showed that a potent male is rewarding to a female rat, but did not specify the nature of the reward.

3.2 Experiment 5(a) : Reassessing sexual motivation in the female rat

The experiments reported here were designed to investigate first, the relationship between running performance of receptive females in a straight alley, and the gonadal state of the male (whether sexually active or castrate). Second, using either sexually receptive or unreceptive females and active or castrate males, it was hoped to determine whether oestrus causes a female rat to seek sexual contact with a male, as proposed by Meyerson and Lindström (1973; Experiment 5(b)).

3.2.1 Method

Animals

All the animals used in the following study were experimentally naive rats (MRC: derived from the Piebald VIROL-GLAXO strain), bred in the animal wing of this department. They were weaned and the two sexes separated at 21 days of age, and kept in group cages in batches of 10 animals per cage under a 12 hr light-dark cycle, until they were used for experimentation at about four months of age. Food (Rat and Mouse maintenance diet No. 1 - BP Nutrition, Witham) and water were given ad lib. If not otherwise stated, the animals were ovariectomized or castrated when sexually mature and used in the experiments no earlier than three weeks after surgery.

Experimental subjects

Sixteen female rats about 120 days of age were housed in two group cages under a 12 hr light-dark reversed cycle. The animals were transferred to the reversed-light room at least one month before the start of any surgical or experimental procedures, in order to allow for habituation. The temperature was kept constant at 21^{o}C. All the rats were ovariectomized, under either anaesthesia, through a single dorsal mid-line incision three weeks prior to the start of testing for mating behaviour.

Incentive animals

Twelve male rats were selected for sexual vigour, and housed in two

group cages. After the open-field testing with the ovariectomized females, six of these males were selected randomly and castrated. These served as the sexually inactive males, and were not used experimentally until at least two months after castration. The active males maintained their sexual vigour by mating with oestrous females at least once a week in the "open-field". The active and castrate males were kept separately in two group cages for the rest of the experiment.

Hormone injections

Oestradiol benzoate and progesterone were dissolved in benzyl alcohol and arachis oil (in a ratio of 1:10) and injected in a volume of 0.1 ml. The arachis oil was injected as the oil blank solution. Unless otherwise stated, the quantities of hormone injected were as follows:

(1) Oestradiol benzoate: 5 μg in 0.1 ml of oil.

(2) Progesterone: 500 μg in 0.1 ml of oil.

All injections were given subcutaneously, and were administered about 2 hours before the start of the dark period, at 10.00 h.

Testing room

The experiments were performed in a screened room, separate from the animal house in which all the rats used in this study were housed. The room was windowless and quiet. Light came from two 60 watt red lights, sufficient to permit the experimenter to make his records. The experiments started about two hours after the start of the dark period, at 14.00 hrs. Experimental and incentive animals were transferred to the testing room at least half an hour before the start of the experiment. Care was taken not to keep the male and female cages in the immediate vicinity of each other. The temperature in the testing room was the same as in the maintenance room, thermostatically controlled at 21°C.

Test apparatus

A diagrammatic representation of the runway is given in Figure 3.1.

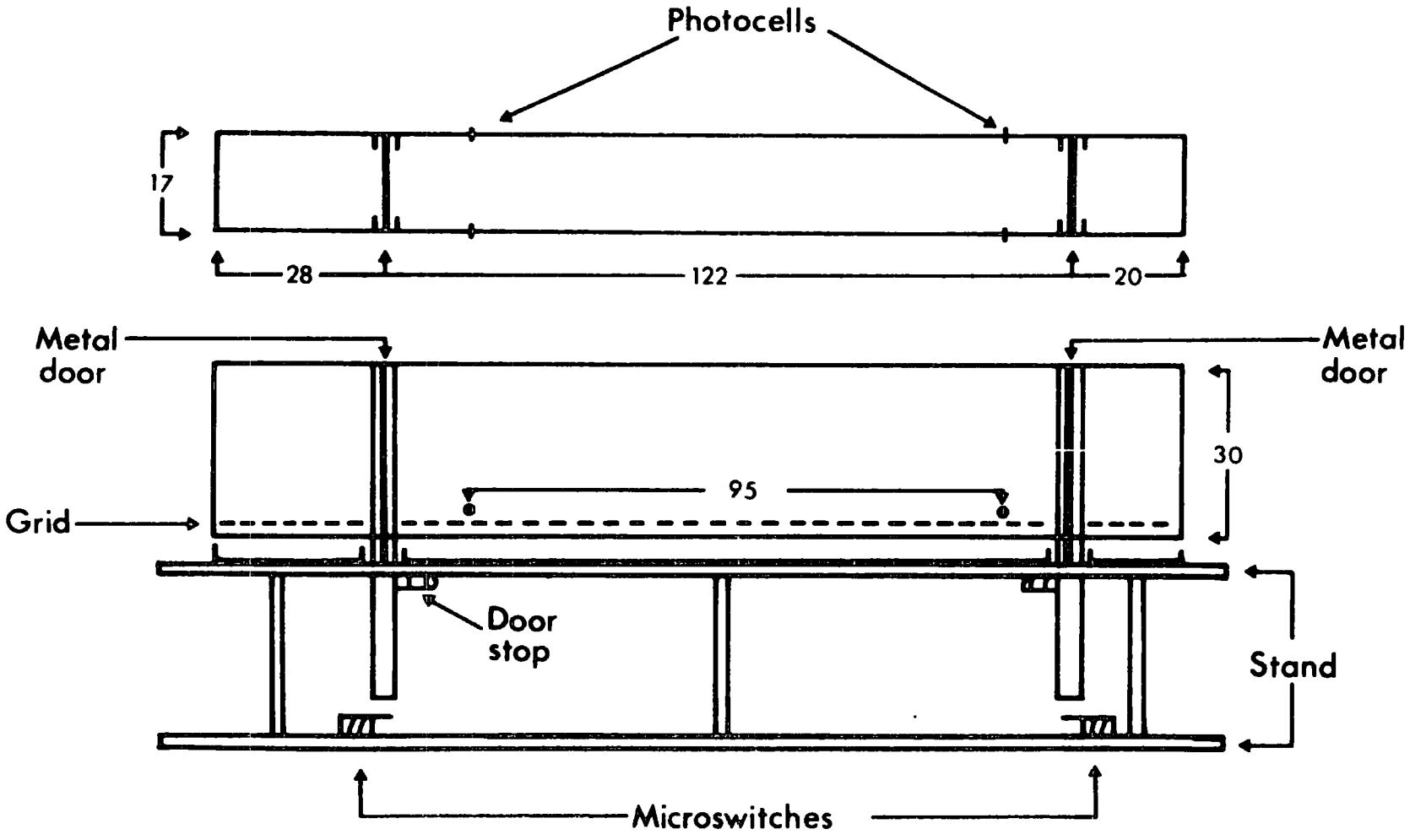


Figure 3.1

Experiment 5(a). The Runway: Plan (top), and Front Elevation. Measurements are given in centimetres.

The main components of the apparatus were: a start box or female holding box, a straight runway and a goal box or male holding box. The start box measured 20 x 17 x 30 cm, and was covered by a plexiglass lid. The runway measured 122 x 17 x 30 cm, while the male holding box, which was also covered by a perspex lid, measured 28 x 17 x 30 cm. Metal sliding doors, operated by levers, sectioned the apparatus into three parts. The female rats were timed by electric timers operated by microswitches on the doors and photocells in the runway (see Figure 3.1), to the nearest 0.01 seconds.

Testing for mating behaviour

Gerall and Dunlap (1973) have shown that female rats show increased receptivity if they are given prior experience with both hormone injections and sexual behaviour tests. Although habituation to the hormone injection itself might not be the only reason for increased receptivity, this procedure is necessary in order to establish responsiveness to the hormone in ovariectomized animals. Beach and Orndoff (1974) showed that female rats are less responsive to oestradiol treatment after oestrogen deprivation (in ovariectomized subjects) as indicated by the lordosis response. Earlier studies, although using different experimental designs, also showed some indication of this effect (Damassa and Davidscn, 1973; Whalen and Nakayama, 1965). Therefore this procedure was established to give the subjects mating experience as well as a similar, controlled amount of sexual behaviour for each individual. This procedure also establishes responsiveness in the animals to oestradiol treatment.

There were two mating tests which took place every week. All the females were brought into oestrus with two subcutaneous injections of oestradiol benzoate (5 μg/0.1 ml arachis oil) 48 and 24 hours before testing, and 500 μg progesterone in 0.1 ml oil, 6 hours before testing. Each animal was tested about 6 hours after progesterone treatment, and was subjected

during each session to 6 mounts by a sexually vigorous male.

The testing took place in a circular arena measuring 90 cm in diameter, having a 30 cm high circular wall. The arena was painted black, and sawdust was spread on the floor of the cage. Illumination consisted of a dim, 60 watt red light bulb which was placed directly above the open field. By the second mating session, all the animals displayed a clearcut lordosis response.

Pretesting

This consisted of three mating experiences in the test runway with all the doors open, during which each female received six mounts by a sexually vigorous male. Each mating test took place every four days, the females being brought into oestrus as described in the previous section. In addition, prior to the test-mating experiences, both males and females were given two fifteen minutes exploration/habituation periods in the apparatus with all doors open.

Training

All the females were injected subcutaneously with 5 µg oestradiol benzoate in 0.1 ml arachis oil daily, until receptivity was established. These daily injections continued during the training period in order to maintain receptivity. Females were brought into oestrus by one week of oestradiol injections.

The sixteen female rats were divided randomly into two groups of eight, one group running to potent males the other to castrate males. The females were trained over ten days and each female was given four consecutive trials daily for the first eight days and then six consecutive trials on each of the last two days. Each trial ran as follows: a female was placed in the starting box and after a short delay of a few seconds the door was opened and the female was allowed to run to the male holding box. If the female did not leave the start box within 120 seconds, the trial was terminated; and if the female did not

traverse the runway within 60 seconds after leaving the start box, the trial was terminated. The next trial began immediately.

When the female arrived at the male holding box, the door was opened and the female allowed to enter. The active males were allowed two intromissions in each test trial. If no copulation occurred within 60 seconds of the male holding box door being opened, the trial was terminated, and the female returned to the starting box. Generally, however, it was found that this time period was adequate for the required number of intromissions to occur. To avoid the ejaculatory plug, each active male was removed after eight intromissions, and a fresh male was substituted for the next trial. The castrate males were randomly yoked to an active male, so that each castrate received as much time with a female as one of the potent males.

During training, records were kept of (1) the time taken by a female to traverse the runway from the start box to the goal box, (2) the time taken for the required number of intromissions to take place; and (3) the number of males ejaculating.

3.2.2 Results

Because each rat was given four trials on the first eight days and six trials on the last two days (Days 9 and 10), the data for Days 1 to 8 and Days 9 and 10 were analysed separately using analysis of variance (one-between, two-within factors). Summaries of the relevant analyses are presented in Table 3.1 (a and b). Figure 3.2 shows that female groups ran faster over time (Days 1 to 8: $F = 28.73$; with df. = 7, 98; $p < 0.001$), although initially the running times for the two groups were comparable (first trial on Day 1: Active group: 35.1 $\pm$ 16.4 seconds; Castrate group: 44.9 $\pm$ 21.3 seconds (mean $\pm$ standard error). See also Appendix A, Table A1, for daily trial means. There was no difference in the overall running times between Days 9 and 10 (Table 3.1 (b)). Females ran faster to active males than to castrates (Days 9 and 10: $F = 9.56$; with df. = 1, 14; $p < 0.01$).

Table 3.1(a)

Experiment 5(a). Oestrous females running to sexually active or castrate males: Days 1 to 8. Summary of analysis of variance

Source	df.	SS	MS	F-ratio	P
Subjects	15	277446.85			
Potency	1	92227.34	92227.34	6.97	< 0.05
Error	14	185219.50	13229.96		
Days	7	672455.18	96065.03	28.73	< 0.001
Days x Potency	7	35119.10	5017.01	1.50	ns.
Error	98	327709.83	3343.98		
Trials	3	168316.92	56105.64	35.66	< 0.001
Trials x Potency	3	12537.84	4179.28	2.66	$0.06 > p > 0.05$
Error	42	66084.87	1573.45		
Days x Trials	21	103488.22	4928.01	3.70	< 0.001
Days x Trials x Potency	21	17704.76	843.08	< 1	ns.
Error	294	391563.19	1331.85		
Within cell	496	1794979.91			

Table 3.1(b)

Experiment 5(a). Oestrous females running to sexually active or castrate males: Days 9 and 10. Summary of analysis of variance

Source	df.	SS	MM	F-ratio	P
Subjects	15	20624.92			
Potency	1	8367.74	8367.74	9.56	< 0.01
Error	14	12257.18	875.51		
Days	1	52.23	52.23	< 1	ns.
Days x Potency	1	21.47	21.47	< 1	ns.
Error	14	2346.93	167.64		
Trials	5	4534.56	906.91	2.58	< 0.05
Trials x Potency	5	2006.82	401.36	1.14	ns.
Error	70	24626.67	351.81		
Days x Trials	5	2970.47	594.09	1.23	ns.
Days x Trials x Potency	5	2558.24	511.65	1.06	ns.
Error	70	33896.61	484.24		
Within cell	176	73014.00			

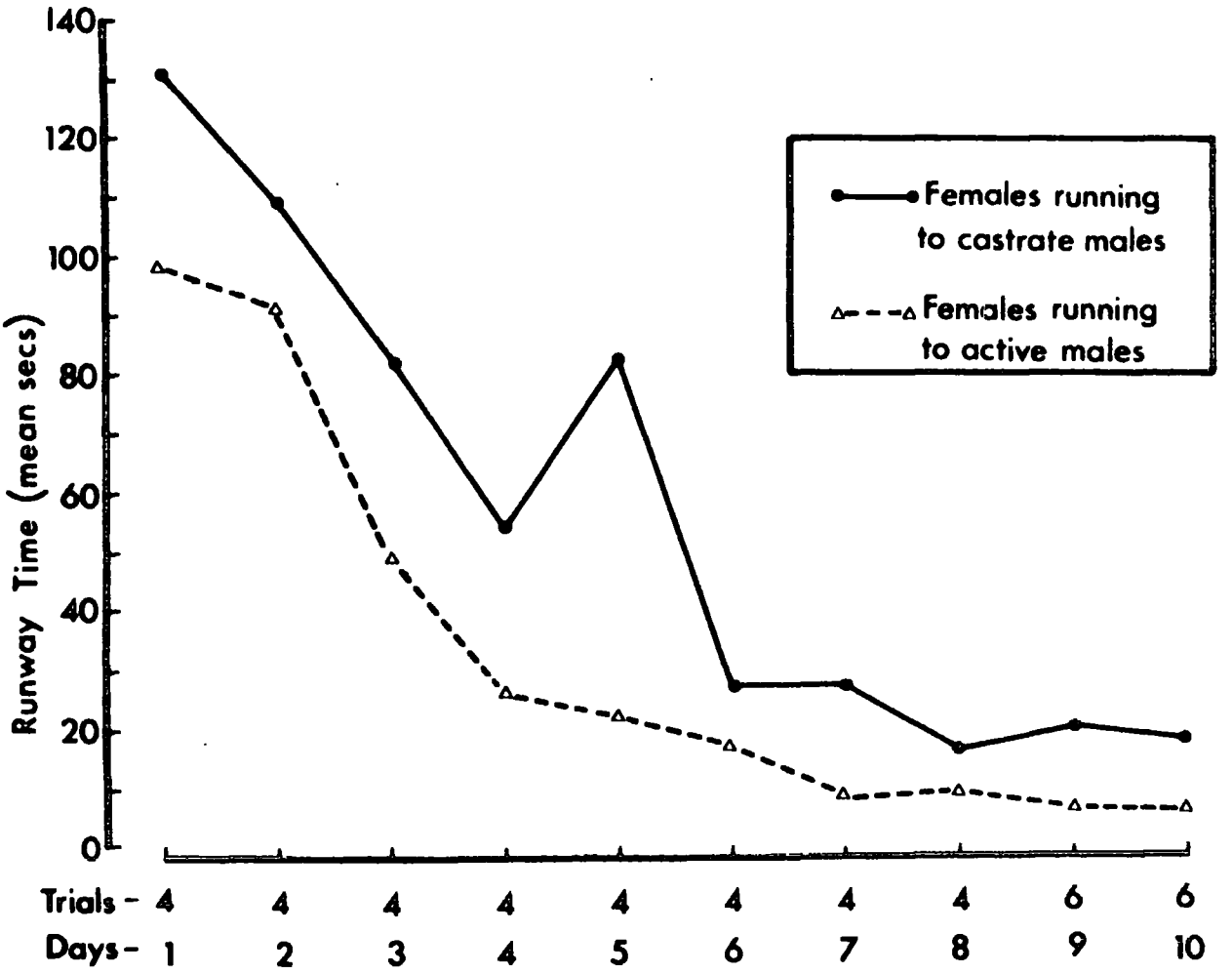


Figure 3.2

Experiment 5(a). Mean runway time (in seconds) of oestrous females running to either sexually active or castrate males.

Generally females ran significantly slower over trials (see Table 3.1 (a) and (b)). Five ejaculations by males were recorded throughout the training period. Discussion of these results will follow after the next experiments.

3.3 Experiment 5(b) : Sexual motivation in the oestrous or anoestrous rat

This experiment investigated the runway performance of oestrous and anoestrous rats running to active or castrate males, and followed on directly from Experiment 5(a).

3.3.1 Method

The two groups of female rats, which ran to either active or to castrate males in Experiment 5(a), were used in this experiment; and the females were kept in the same, previously allocated groups. This was necessary because of the nature of the experimental design. One week after the termination of training in Experiment 5(a), a "re-test" was run in order to maintain running performance in all the females. Oestrus was reinstated in all the females with injections of oestradiol benzoate (5 μg in 0.1 ml arachis oil/rat) given 48 and 24 hours before the test session, and progesterone (500 μg in 0.1 ml oil/rat) 6 hours before the test. Otherwise, the same experimental procedure as the one used in the previous experiment was employed. Each rat was given six trials, and all the females ran well within the criterion levels imposed (see Method section of Experiment 5(a) for details). The experimental sessions consisted of two tests separated by a period of four days. A counterbalanced design was employed in which the oestrous condition of the females and the gonadal state of the males were varied systematically. This was achieved in the following way: after the re-test, the females in both groups were further divided into two groups of four: one group in the oestrous condition and the other in the anoestrous condition. Subsequently, for the second test, the oestrous condition was reversed for each group, so that the oestrous animals in the first test now became the anoestrous animals and vice-versa. For each test, oestrus was reinstated

in an identical manner to that employed in the re-test. The anoestrous animals received a placebo injection of 0.1 ml arachis oil per rat. All the injections were administered subcutaneously. The experimental procedure was identical to the procedure described in Experiment 5(a), with the following exceptions:

1. Instead of having two groups of receptive rats running to either active males or castrates, females were either receptive (oestrous condition) or unreceptive (anoestrous condition), and ran to either active or castrate males.

2. Each female was given eight trials per test.

During the experimental testing, the following were recorded: (1) time taken to traverse the runway from the start box to the male holding box and (2) occurrence of copulation. Each experimental session started at 14.00 hours, two hours after the start of the dark cycle.

3.3.2 Results

Only two ejaculations were observed throughout testing. Further, it was noticed that females ran much slower after a male had ejaculated. A summary of the analysis of variance (two-between, two-within factors) is presented in Table 3.3. All the females ran faster to active males than to castrate males ($F = 4.78$; with $df. = 1, 12$; $p < 0.05$; Figure 3.3). This replicates the findings of the previous experiment, using a different method for reinstating oestrus in the females, and extends them to include anoestrous females. Females in oestrus also ran faster than anoestrous females ($F = 8.95$; with $df. = 1, 12$; $p < 0.05$; Figure 3.4). Again, as in Experiment 5(a), females ran significantly slower over trials. The interaction between trials and oestrous condition is represented in Table 3.2. This shows that over trials females in oestrus ran faster than anoestrous females, and that this difference is significant ($F = 3.34$; with $df. = 7, 84$; $p < 0.01$). This manifests itself particularly over the last two trials.

Table 3.2

Experiment 5(b). Mean runway times (in seconds) for oestrous and anoestrous females over trials. The interaction is significant at the 1% level

Trials	Oestrous Females	Anoestrous Females
1	5.4	7.2
2	15.3	8.6
3	27.7	11.1
4	13.7	31.0
5	13.5	24.4
6	24.5	21.8
7	19.8	49.4
8	17.6	60.9

Table 3.3

Experiment 5(b). Oestrous or Anoestrous females running to sexually active or castrate males. Summary of analysis of variance

Source	df	SS	MS	F-ratio	P
Subjects	15	64055.70			
B1	1	16213.88	16213.88	4.78	< 0.05
B2	1	3687.83	3687.83	1.09	ns.
B12	1	3442.17	3442.17	1.01	ns.
Error	12	40711.82	3392.65		
W1	1	5937.67	5937.67	8.95	< 0.05
W1B1	1	33.68	33.68	< 1	ns.
W1B2	1	1816.68	1816.68	2.74	ns.
W1B12	1	21.18	21.18	< 1	ns.
Error	12	7964.69	663.72		
W2	7	26300.83	3757.26	4.69	< 0.001
W2B1	7	5934.34	847.76	1.06	ns.
W2B2	7	4241.28	605.90	< 1	ns.
W2B12	7	2923.51	417.64	< 1	ns.
Error	84	67281.20	800.97		
W12	7	22098.20	3156.89	3.34	< 0.01
W12B1	7	4842.37	691.77	< 1	ns.
W12B2	7	2838.47	405.50	< 1	ns.
W12B12	7	3338.23	476.89	< 1	ns.
Error	84	79369.08	944.87		

B : Between Group;

W : Within Group.

B1 : Active/Castrate;

B2 : Group;

W1 : Oestrus/Anoestrus;

N2 : Trials

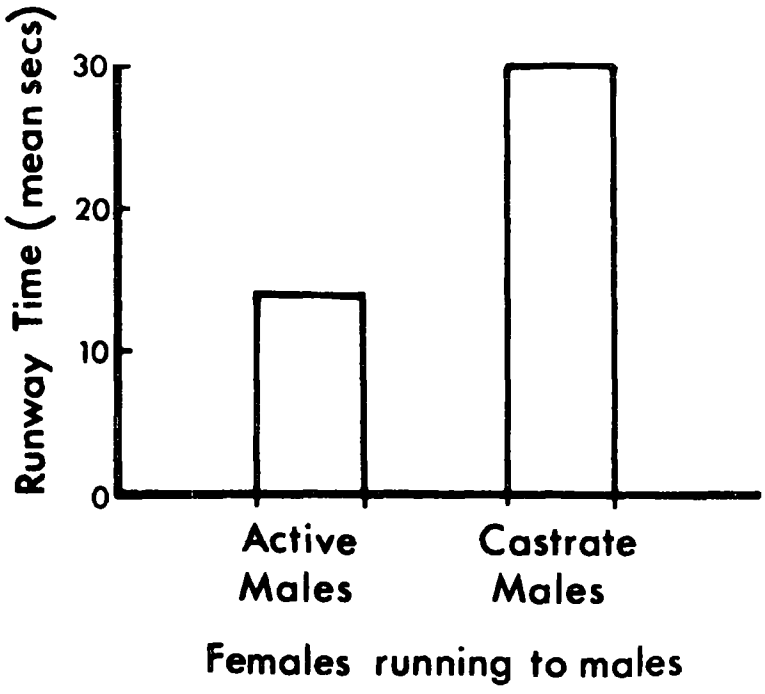


Figure 3.3

Experiment 5(b). Mean runway time (in seconds) of females running to active or castrate males.

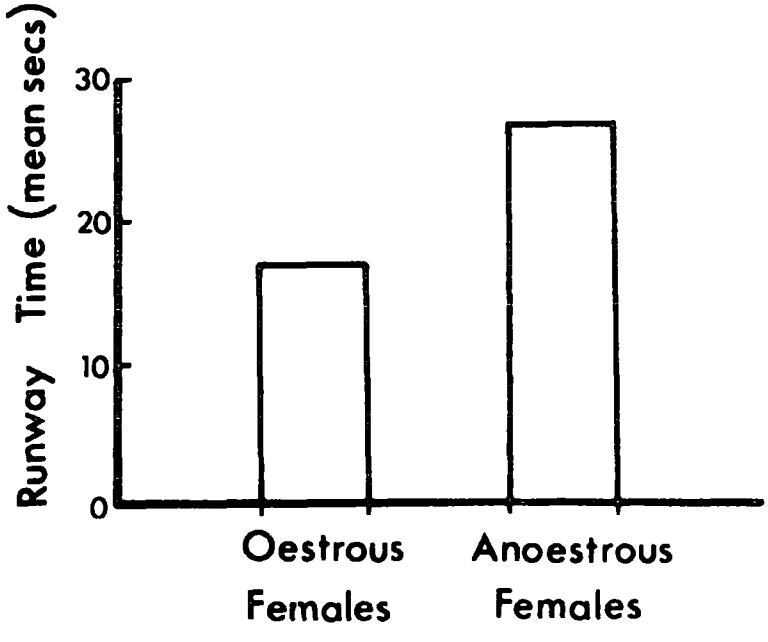


Figure 3.4

Experiment 5(b). Mean runway time (in seconds) of oestrous and anoestrous females running to males.

However, there was no significant interaction between gonadal condition of the males (that is, active or castrate) and the receptive state of the females (oestrus or anoestrus). That is to say, the mean difference in runway times between oestrous and anoestrous females running to active males (8.9 seconds) and oestrous and anoestrous females running to castrate males (10.3 seconds) was not significant. The result of this experiment can thus be adequately summarised in Figure 3.5, which shows the mean runway times for females in the different receptive conditions running to the different incentive males. There were no other significant interactions (see also Appendix A, Table A2).

3.4 Experiment 6 : Oestrus and food reward in the female rat

Drewett (1974) argues that the hyperactivity shown by the oestrous rat might be an example of deprivation-induced activity, attributable to the anorexic properties of oestradiol (the female rat eats less at oestrus), and not to the stimulating effect of this oestrogen on sexual behaviour. Thus, if the rat's sensitivity to the resulting energy deficit were not reduced, signs of a hunger drive might appear at oestrus, even though its expression in increased food intake were suppressed by the simultaneously enhanced satiation. The following experiment investigated the runway performance of ovariectomized female rats, brought into oestrus by injections of oestradiol benzoate and progesterone, running to a food reward. This experiment served as a control for Experiment 5(b).

3.4.1 Method

Experimental animals

Ten hooded female rats, about 120 days of age, served. All the rats were ovariectomized six weeks before the start of the experiment. Their mean weight at the start of behavioural testing was 253 $\pm$ 16 g.

Apparatus

The test apparatus consisted of the perspex runway used in Experiment 5

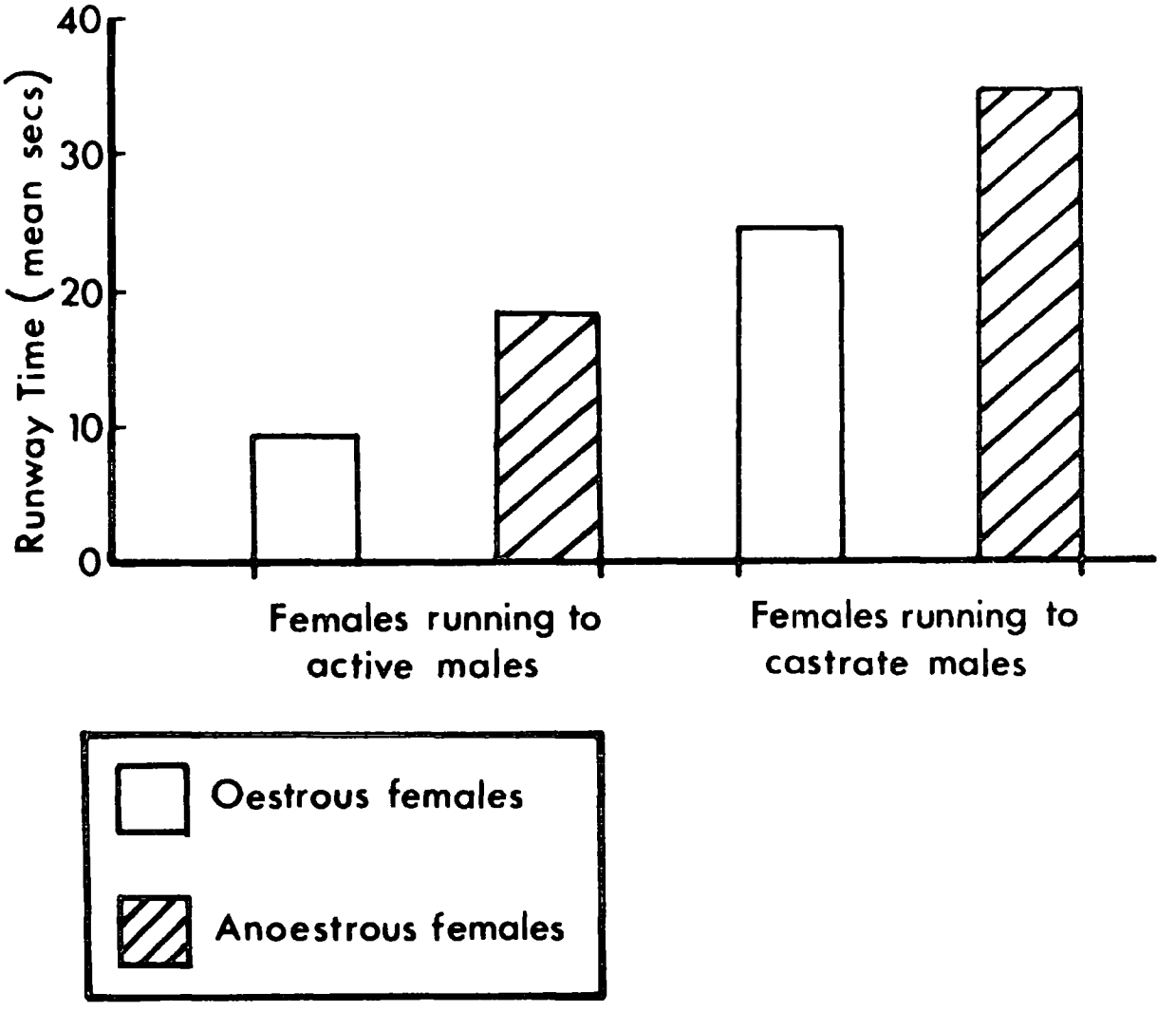


Figure 3.5

Experiment 5(b). Mean runway time (in seconds) of oestrous and anoestrous females running to sexually active or castrate males.

(Figure 3.1), with some slight modification. A removable food cup was placed at the end of the goal box, and a photocell was attached to the wall of the runway, 10 cm in front of the food cup. Thus microswitches, and the breaking of successive photobeams after opening the start box door registered the time taken by a female to traverse the runway from the start box to the food cup, to the nearest 0.01 second. Each rat received a habituation period of six ten-minute periods prior to training, with all the doors open.

Training

The deprivation schedule employed during training proceeded as follows. The rats were deprived of food 24 hours before training. They were then trained to run to a food reward in the runway, and fed again at least half an hour after the completion of training. The reward consisted of two 45 mg Noyes food pellets, and the rats were rewarded on every trial (a continuous reinforcement schedule). Food was removed again 72 hours (on the third day) after the preceding deprivation. The running order of the animals was determined by a sequence of random numbers. A new sequence was generated for each training session. There were ten trials in each session, which lasted about thirty minutes with an inter-trial interval of ten seconds. This period was spent in the start box. Training continued according to this schedule: a rat was placed in the start box and after 10 seconds delay, the door was opened and the rat allowed to run to the goal box. The second door, which was kept open during the start of the trial, was shut after the rat had entered the goal box. After 60 seconds had elapsed from entry into the goal box, the rat was replaced into the start box and the procedure was repeated. The rats were considered trained once the following criteria were met: (1) a rat left the start box within 120 seconds, (2) ran along the runway within 60 seconds and (3) ate the food pellets within 60 seconds, (4) on ten consecutive trials. A trial was terminated if any of these time

periods were exceded, and the rat was replaced in the start box. The rats ate the food pellets well within the allocated time and the rest of the time was spent in the goal box. It proved unusually difficult to train these ovariectomized rats to criterion, so that training took a total of thirty consecutive sessions, over a period of 90 days.

Experimental testing

After training the rats were randomly allocated to two groups, five rats in each group. One group served as the experimental group (injected with oestradiol benzoate and progesterone), the other as control (injected with arachis oil only). At this time, the rats were re-caged so that like-condition animals were caged together. Seven days after the end of training, the rats were deprived according to the deprivation schedule already outlined, and each rat was given a preparatory or reacquisition session of ten trials in the runway. During these sessions the rats always ran to criterion. Oestrus was reinstated in rats in the following way. After two days of ad libitum feeding, each rat in the experimental group was injected with 5 µg of oestradiol benzoate in 0.1 ml arachis oil. The rats in the control group were injected with 0.1 ml arachis oil only. The food was removed 24 hours later, at 14.00 h, and both groups of rats were given the same treatment as above. On the day following food removal, and 6 hours before testing began, each experimental rat was injected with 500 µg progesterone in 0.1 ml arachis oil; the control rats received another injection of the oil blank solution only. During testing a record was kept of the time taken by a female to traverse the runway, from the start box to the food cup. After testing, the rats were fed on normal maintenance diet for a week. Following this period of ad lib. feeding, the group treatments were reversed and the testing procedure was repeated.

3.4.2 Results

The results of this experiment are presented in Figure 3.6. The runway

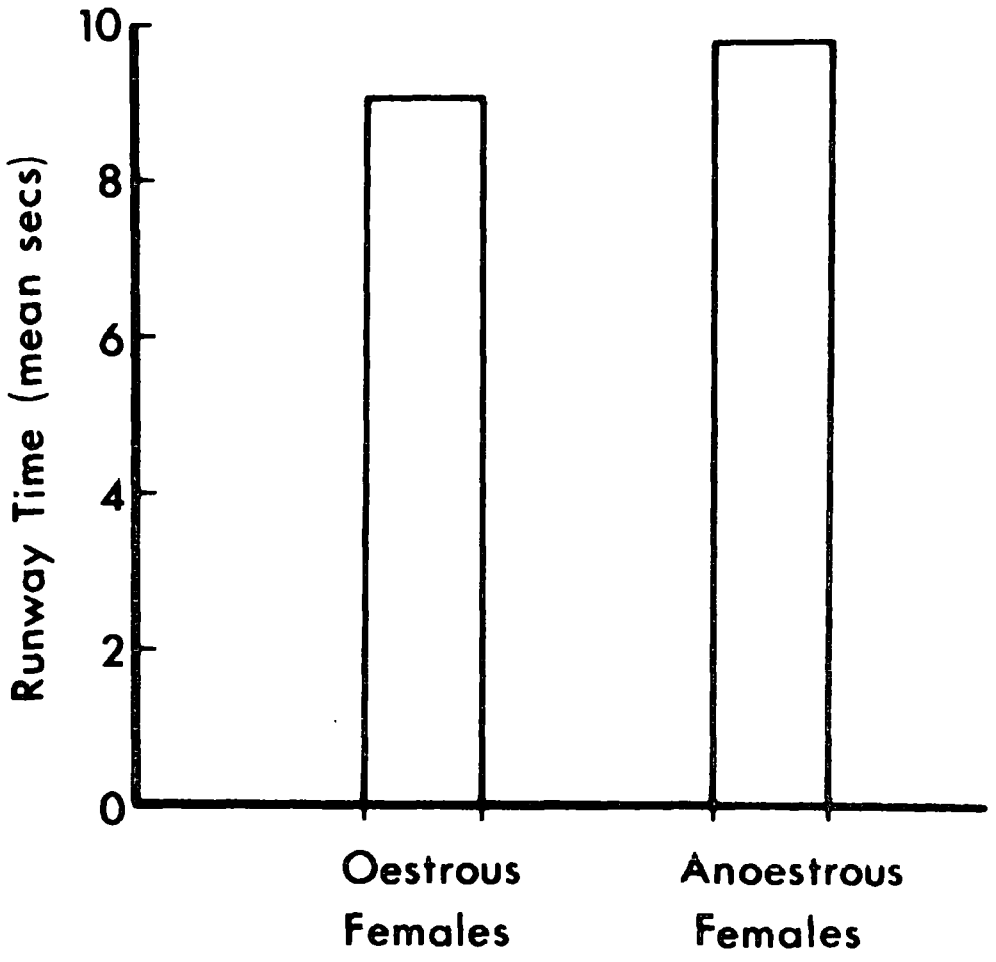


Figure 3.6

Experiment 6. Mean runway time (in seconds) of oestrous and anoestrous females running to a food reward.

times for the experimental and control groups were analysed with analysis of variance, a summary of which is presented in Table 3.4. There was no evidence that treatment with oestradiol benzoate in combination with progesterone affected in any way the females' runway performance; and there were no significant interactions. Therefore, oestrus does not alter a female's runway performance when food is used as a reward, but only when a male is.

3.5 General Discussion

In Experiment 5(a) receptive female rats were allowed to run to active or castrate male rats. As shown in Figure 3.2 females ran faster to an active male. These results are consistent with the observations of Drewett (1973c), Meyerson and Lindström (1973), Hill and Thomas (1973) and Eliasson and Meyerson (1975). The results of this experiment, however, contrast with the findings of Beach (1958), Bolles et al. (1968) and Williams and Drewett (unpublished observations, cited in Drewett, 1973c), all of which demonstrated that females did not run any faster to sexually active than to sexually passive males. The study by Bolles et al. was not very adequate, since by the eighth and final trial all the females were running rapidly whether to a potent or impotent male - the possibility of ceiling effects, although unlikely, cannot be discounted. Further, it is possible that the females did not run as fast after an ejaculation, as after a mount with intromission (this was noticed in the present study). No details are given by Bolles et al. and Beach as to the number of ejaculations during the experimental tests.

The results of Experiment 5(b) showed that an active male was a more effective incentive than a castrate, irrespective of whether the females were sexually receptive or unreceptive. This experiment also demonstrated that females in oestrus ran faster than females in anoestrus when a male rat was used as an incentive (and irrespective of the gonadal state or sexual activity of the male). When food was used as an incentive, in Experiment 6,

Table 3.4

Experiment 6. Oestrous and anoestrous females running to a food reward. Summary of analysis of variance

Source	df.	SS	MS	F-ratio	P
Subjects	9	19317.70			
Groups	1	202.63	202.63	< 1	ns.
Error	8	19115.07	2389.38		
Oestrous/Anoestrous	1	25.78	25.78	< 1	ns.
Oestrous state x Group	1	722.11	722.11	< 1	ns.
Error	8	9826.31	1228.29		
Trials	9	4570.02	507.78	1.68	ns.
Trials x Group	9	3111.99	345.78	1.13	ns.
Error	72	21946.87	304.82		
Oestrous state x Trials	9	1926.63	214.07	< 1	ns.
Oestrous state x Trials x Group	9	1287.04	143.00	< 1	ns.
Error	72	16140.35	224.17		
Within cell	190	59557.11			

oestrous females did not run faster than anoestrous females. Further, in the studies by Meyerson and Lindström (1973) female rats treated with oestradiol benzoate had shorter running times than after oil blank treatment; but this effect was significant only when a male was chosen. In a recent study, Gilman and Westbrook (1978) found that females did not run significantly faster when sexually receptive than when unreceptive; but they preferred sexually active males significantly on more trials when in a sexually receptive condition. Gilman and Westbrook used a biweekly interval between testing sessions. Furthermore they tested the females three times (without prior training), twice in the oestrous condition separated by a test in the anoestrous condition. Thus, procedural differences could account for the absence of a significant difference in running times between oestrous and anoestrous rats.

Taken together, the experimental results presented in this chapter, and other studies also cited here, suggest that the opportunity for a female to seek contact with a male conspecific is reinforcing. This indicates that social factors are also present as a possible source of reward. However, in the present study, sexual components are obviously evident, since receptive females ran significantly faster to sexually active males than to castrate males. Had social and exploratory factors been the primary motivation, as suggested by Bolles et al. (1968), female rats should have run as fast in both sexually receptive and unreceptive conditions. What these experiments do not specify is the nature of the reward; that is whether it is behavioural (such as mounting and intromission) or olfactory or both.

In summary, then, the performance in an instrumental task by a female rat when a male is used as an incentive is dependent upon both the hormonal condition (sexual receptivity) of the female and the gonadal condition (sexual activity) of the male; and these are independent and additive. They

do not interact in such a way that the effect of a given variation in incentive (using sexually active or castrate males) is itself dependent upon the current level of drive (using sexually receptive or unreceptive females). In the next chapter, the reward characteristics of male sexual behaviour are considered.

CHAPTER 4

OESTRADIOL, SEXUAL MOTIVATION AND REWARD IN THE FEMALE RAT (continued)

4.1 Introduction

Female rats learn to approach an intact and sexually potent male in preference to a castrated (i.e. sexually inactive) male if given the choice in a T-maze (Drewett, 1973) or a Y-maze (Gilman and Westbrook, 1978). The experiments described in Chapter 4 showed that females also run faster to an active male than to a castrate, when tested in a straight runway. These experiments show that a sexually active male is different, from a castrate male, in its reward value for a female rat but do not specify the nature of the difference.

Hill and Thomas (1973) found that female rats in a straight alley ran faster to a sexually active male than to a male that is intact but sexually inactive. One might infer from this study that the important difference is in the behaviour of the males, since uncastrated sexually passive males would presumably smell similar to sexually active males. However, one cannot be sure on this point. It is possible that the males' sexual inertness is due to hormonal deficiencies.

On the other hand, Meyerson and Lindström (1973) found that females chose an intact, active male over an oestrous female, even when direct sexual contact was prevented by a grid between the animals. This suggests that the odour of the intact male might be rewarding even when differences in sexual activity are eliminated. That a female rat is able to discriminate between the odour of a sexually active and a castrate male is in no doubt. Carr and Caul (1962) demonstrated that both normal and ovariectomized female rats are capable of discriminating between the odours of sexually active and castrated males. Carr, Loeb and Dissinger (1965) showed that, regardless of their oestrous state at the time of testing, sexually

experienced female rats spent significantly more time investigating the odour(s) from sexually active males than from castrates.

The following experiments independently vary the odour and sexual activity of the male rat. The feasibility of such an investigation is made possible by studies on the various behavioural and physiological properties of the androgen, dihydrotestosterone, and its combined action with oestradiol. It is to these studies that we now turn.

4.2 Behavioural and physiological effects of dihydrotestosterone in the male rat

The active form of testosterone in peripheral target tissues is the 5α - reduced metabolite, dihydrotestosterone (Bruchovsky and Wilson, 1968; Wilson and Gloyna, 1970). While this compound is very active peripherally, it appears to exert no effect on the neural mechanisms controlling sexual behaviour in the male rat.

In studies using long term castrated male rats, several workers have shown that dihydrotestosterone does not restore mounting, intromission or ejaculatory behaviour (McDonald, Beyer, Newton, Brien, Baker, Tan, Sampson, Kitching, Greenhill and Pritchard, 1970; Feder, 1971). Other studies (Brown-Grant, Munck, Naftolin and Sherwood, 1971; McDonald, 1970; McDonald et al., 1970) demonstrated that dihydrotestosterone does not produce sexual (hypothalamic) differentiation. However, the potent effect of this androgen in stimulating accessory sex structure growth, such as seminal vesicles, preputial, prostate and coagulating glands, and penile papillae, is in no doubt (Wilson and Gloyna, 1970; Parrott, 1975).

On the other hand, oestradiol treatment stimulates sexual behaviour in castrated male rats (Pfaff, 1970; Larsson, Södersten and Beyer, 1973) without stimulating accessory sex structure growth (Larrson et al., 1973) and function (Price and Williams-Ashman, 1961). When oestradiol is combined with dihydrotestosterone, sexual behaviour, as well as accessory sex

structure growth and function, are stimulated to levels comparable with the testosterone treated castrate or intact male (Baum and Vreeburg, 1973; Larsson et al., 1973; Feder, Naftolin and Ryan, 1974; Larsson, Södersten, Beyer, Morali and Pérez-Palacios, 1976).

4.3 Experiment 7 : Attractant qualities of urine from castrated male rats treated with dihydrotestosterone and oestradiol benzoate

Le Magnen (1952) found that intact male rats preferred the arm of a T-maze which contained the odour from a female in oestrus to the arm which contained the odour from a female in dioestrus or a male rat.

Carr et al. (1965) showed that sexually experienced male and female rats prefer the odour from sexually active (intact) to that from the inactive (castrate or ovariectomized) members of the opposite sex. Among sexually naive rats, however, only females in oestrus (natural or induced) prefer the odour of active males; sexually naive male rats show no preference for either female odour. Thus in males, the preference for the odour from sexually receptive females over that from non-receptive females requires both the presence of gonadal hormones and previous sexual experience (although mounting experience is sufficient (Stern, 1970)); in females the preference of odour from intact males over that of castrates requires either the presence of ovarian hormones or previous sexual experience. This preference is dependent on circulating androgens or oestrogens, because it disappears after castration or ovariectomy and can be restored by testosterone or oestradiol injections (Carr and Caul, 1962; Carr et al, 1965; Stern, 1970).

More recently, accessory sex glands of the male and female rat have been implicated in the production of sex pheromone(s). (Orsulak and Gawienowski, 1972; Gawienowski, Orsulak, Stacewicz-Sapuntzakis and Joseph, 1975.)

Since there are no differences in the peripheral, stimulatory effects of dihydrotestosterone, when injected alone or in combination with

oestradiol, on the accessory sex structures of castrated male rats (e.g. Larsson et al., 1973), one would not expect to find differences in odours from castrates treated with dihydrotestosterone only or in combination with oestradiol. The following experiment checked this supposition by investigating the responses of female rats to sawdust impregnated with urine from castrated male rats treated with either oil placebo, or dihydrotestosterone, or dihydrotestosterone in combination with oestradiol benzoate.

4.3.1 Method

Experimental animals

Six experimentally and sexually naive female Wistar rats, about 150 days of age and weighing 261 $\pm$ 20 g, were housed in a group cage. They were ovariectomized 25 days before the start of the experiment.

Male rats

Eighteen experimentally and sexually naive male rats, about 180 days of age and weighing 373 $\pm$ 18 g, were housed in three group cages. They were castrated approximately 12 weeks before the start of the experiment.

Testing room

The experiments were performed in a windowless, air conditioned, quiet room, lit by one 60 watt dim red light.

Olfactory choice box

A diagram of the olfactory choice box is presented in Figure 4.1. It consisted of a wooden enclosure (60 x 60 x 30 cm) with a removable wire mesh floor fitted 10 cm from the base. A metal tray containing deodourising litter (Kat-Lit) beneath the cage floor. One side of the cage consisted of a clear perspex observation window. Three wooden olfaction-boxes were attached to the remaining sides. Each box measured 20 x 20 x 30 cm and was separated from the main enclosure by a tight-fitting sliding door. A hole, 3.5 cm in diameter and 2 cm from the bottom edge of each door, allowed a rat to insert its head into the box and, with some difficulty, enter it. The

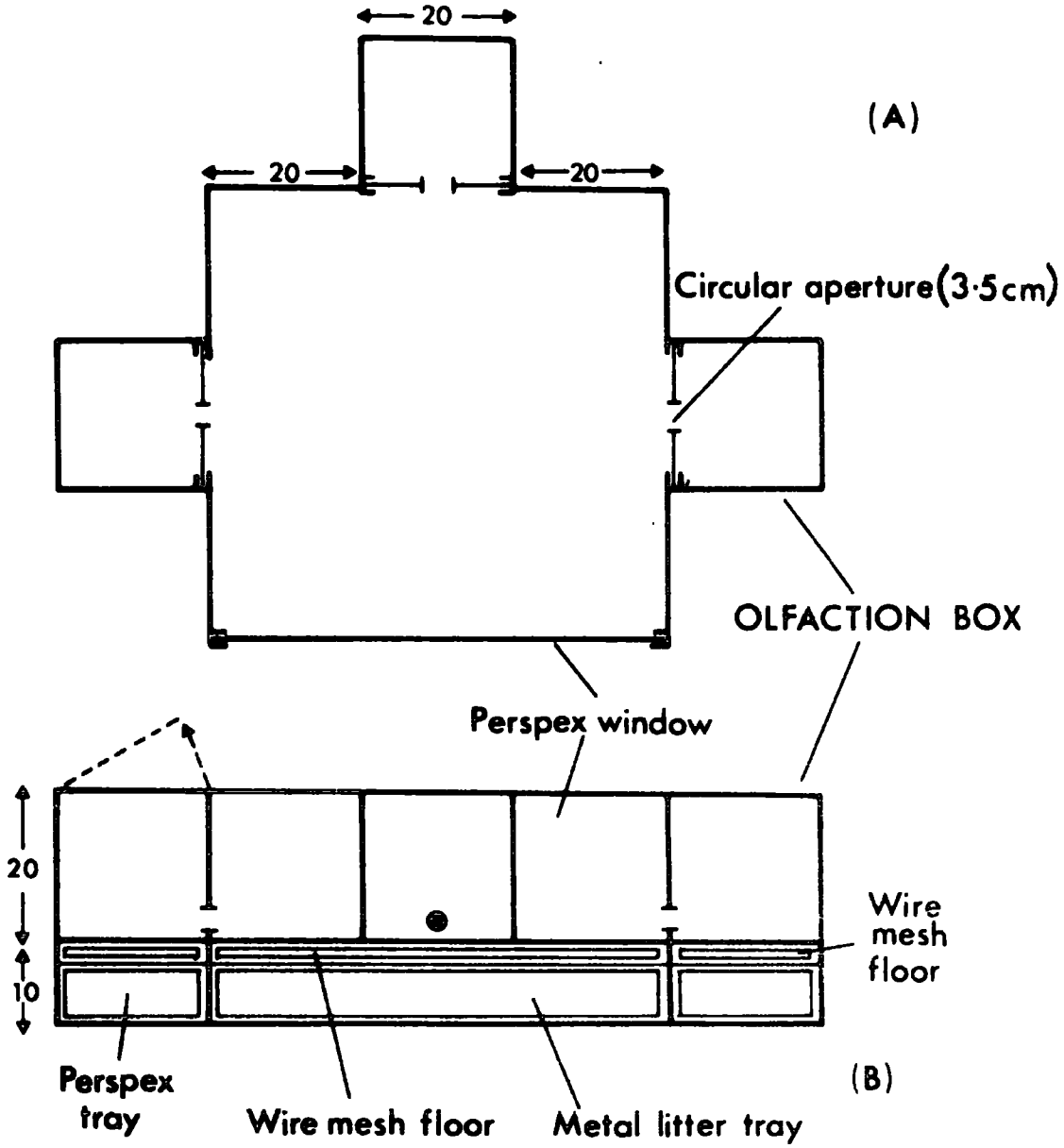


Figure 4.1

Experiment 7. Olfactory choice box: (A) Plan. (B) Front elevation. Measurements are given in centimetres.

boxes had removable wire mesh floors, 10 cm from the base, which separated a test animal from perspex sawdust containers. Each of these containers measured 20 x 20 x 10 cm, and could be removed with ease. Three electric timers, operated by on/off keys by the experimenter, were used to record the time the rat spent investigating each box.

Experimental schedule

All the animals were housed singly in plastic cages, 15 days before testing began. The males were allotted to three groups of 6 rats at random and injected for 15 days prior to the start of experimental testing, and during the testing period. They were injected with either (1) arachis oil, 0.1 ml/rat/day only or (2) with dihydrotestosterone (DHT), 1 mg in 0.1 ml oil or (3) with 1 mg DHT and 5 µg oestradiol benzoate (OB). The females were allocated three males each, one from each group, at random.

For the testing sessions, oestrus was reinstated in all the females with injections of oestradiol benzoate (5 µg in 0.1 ml arachis oil/day, daily for seven days before and then throughout the testing period). The experimental females were considered in oestrus when the four ovariectomized "test" females, of comparable age and body weight to the experimental females, showed lordosis to 8 out of 10 mounts by "stud" males. This occurred on the seventh day of injections, so testing began on Day 8 (Experimental day 1). The females were first tested with clean sawdust placed in all 3 containers (Days 1 to 3), and then with sawdust from the males' cages (Days 4 to 6; inexperienced condition). For the next three days, the females were again tested with clean sawdust in the containers (Days 7 to 9). But, in addition, after each testing session, the females were placed with their allocated males for a period of 15 minutes with each male. The order in which females were placed with the males was counterbalanced across groups (6 possible combinations over three days). After this period, the females were tested with urine-treated sawdust in the containers (Days 10 to 12; experienced

condition). Finally, (Days 13 to 15), the females were tested with clean sawdust again. A time schedule for the experimental procedure is given in Table 4.1.

Testing sessions started about two hours after the start of the dark period, at 14.00 h. To control for positional preferences by the rats, the position of the sawdust containers for each rat was determined by a 3 x 3 counterbalanced latin square design (Winer, 1971, p. 690); so that each container appeared in each possible position in the olfaction box only once for each rat, during the three days. Because six rats were used, the design allowed two replicates of each possible position per day. The same perspex containers were kept for each group of male rats, and each container was wiped clean with warm water after each test. Fresh sawdust was placed in all the cages after each daily testing session. There were six tests in each session (one test per rat), and each test lasted for five minutes.

A female was placed in the middle of the mesh floor facing the observation window, and the stop-watch was started. Investigation time was recorded when a female inserted her snout through the circular aperture in the door, or entered the box. At the end of five minutes, the female was removed from the enclosure and returned to her cage. During testing the following were recorded: (1) the first choice box investigated by a female; (2) the investigation time (in seconds) of each box; and (3) the number of times a female entered a particular box. At the end of the experiment all the males were killed, and their seminal vesicles were removed and weighed intact.

4.3.2 Results

All the females mated when they were placed with sexually active males (i.e. males treated with DHT and OB. On the other hand, males treated with DHT or oil only did not exhibit any sexual behaviour. The total investigation time (that is, the investigation time for each three day period) by each

Table 4.1

Experiment 7. Time schedule for experimental procedure

Sexual Experience	Sexually Naive							Sexually Experienced							
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sawdust															
Control	X	X	X				X	X	X				X	X	X
Urine-treated				X	X	X				X	X	X			

female of urine impregnated sawdust, was analysed with analysis of variance (two-between, 0-within factors). A summary of this analysis is presented in Table 4.2.

Figure 4.2 shows that a rat investigated urine impregnated sawdust more than clean sawdust, during a test session. Throughout the three control sessions, the rats were seen to enter the boxes on only five occasions (the same rat was observed on four of these); whereas rats entered the boxes on 43 instances when urine-impregnated sawdust was placed in the containers. These control sessions reliably demonstrate that (1) oestradiol benzoate treatment did not induce a general increase in investigatory or exploratory behaviour by females, during the course of the experiment; (2) the increase in the time spent investigating urine-impregnated sawdust after sexual experience could not be attributed to habituation to the apparatus; (3) sexual experience did not increase exploration when clean sawdust was placed in the containers.

Figure 4.3 shows that females investigated urine-impregnated sawdust of males treated with either DHT only, or in combination with OB, more than sawdust from rats treated with oil only. The overall difference in mean investigation time is significant ($f = 9.83$; with $df. = 2, 25$; $p < 0.01$). Individual post hoc comparisons using the Least Significant Difference test (Keppel, 1973), showed that females investigated the sawdust from cages of males treated with DHT and OB more than from cages of males treated with oil only ($f = 9.37$; with $df. = 1, 25$; $p < 0.01$). However, females did not spend more time investigating sawdust of males treated with DHT than of males treated with DHT and OB ($f = 1.56$; with $df. = 1, 25$; $p =$ ns.).

There was no significant interaction between socio-sexual experience and investigation time of the various urine impregnated sawdust of males (Table 4.3). Females entered boxes which contained sawdust from cages of males treated with either DHT alone or in combination with OB on a similar

Table 4.2

Experiment 7. Urine odour preference and socio-sexual experience: Summary of analysis of variance

Source	df.	SS	MS	F-ratio	p
Subjects	5	70566.92			
Experience	1	32100.69	32100.69	5.87	< 0.05
Treatment	2	107525.17	53762.58	9.83	< 0.01
Treatment x Experience	2	2997.39	1498.69	< 1	ns.
Error	25	25(5468.10)	5468.10		
Within cell	30	279325.83			

Table 4.3

Experiment 7. Mean investigation times (± standard error) of sexually naive and experienced females investigating sawdust impregnated with urine from castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), OR DHT in combination with oestradiol benzoate (DHT-OB)

	OIL	DHT	DHT-OB
Sexually Naive	69.17 ± 27.92	221.00 ± 58.38	168.00 ± 39.39
Sexually Experienced	147.67 ± 21.13	256.00 ± 21.67	233.67 ± 15.92

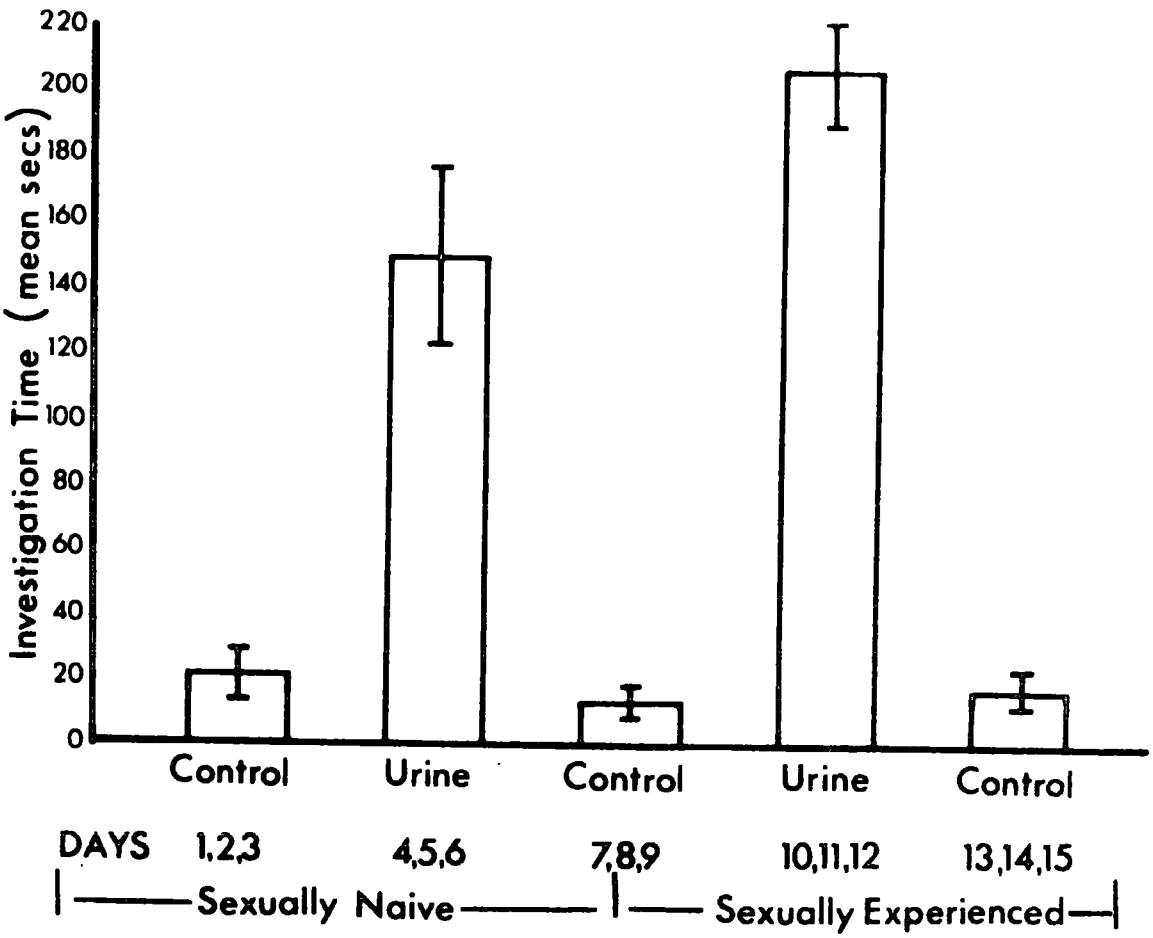


Figure 4.2

Experiment 7. Mean investigation time (± standard error) of receptive females investigating untreated sawdust, or sawdust impregnated with male urine. The days in the figure correspond to the experimental time schedule.

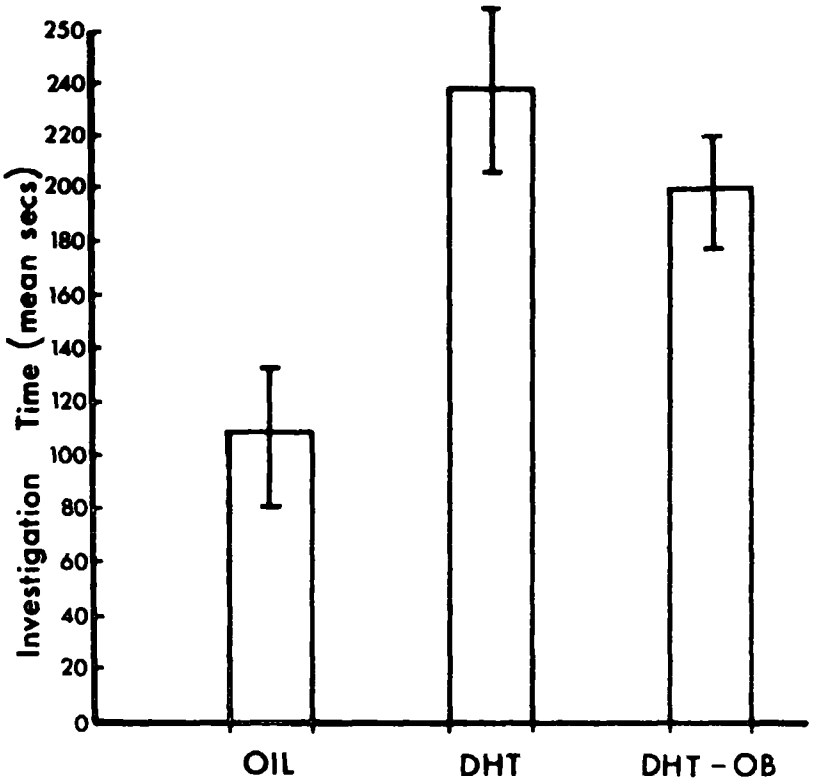


Fig. 4.3

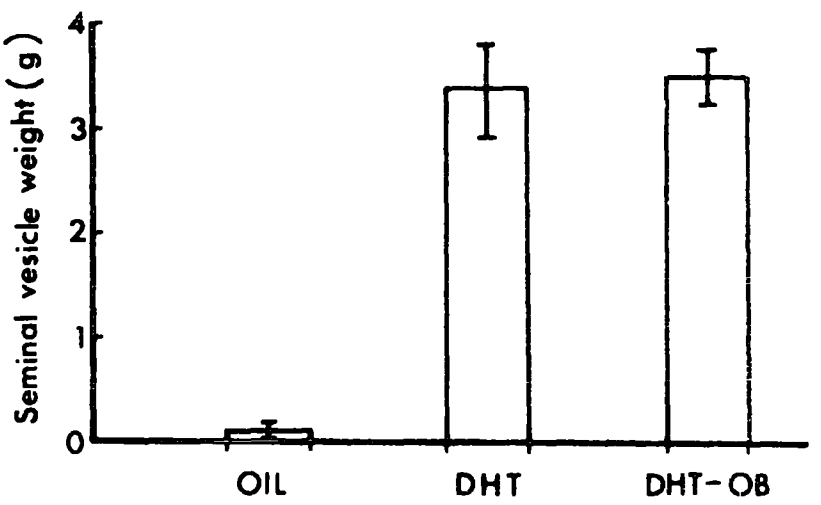


Fig. 4.4

Figure 4.3

Experiment 7. Mean investigation time (± standard error) of receptive females investigating sawdust impregnated with urine from castrate males treated with either the oil vehicle only (OIL), or with dihydrotestosterone (DHT), or with DHT in combination with oestradiol benzoate (DHT-OB).

Figure 4.4

Experiment 7. Seminal vesicle weight of castrate male rats treated with either oil only (OIL), or dihydrotestosterone (DHT) or DHT in combination with oestradiol benzoate (DHT-OB).

number of occasions, when sexually naive or experienced (naive: 7 vs 6 times; experienced 13 vs 12 times respectively). Sexually naive females entered boxes with sawdust from cages of oil-treated castrates on three occasions, and sexually experienced females on two occasions.

Finally, a reliable indication of the peripheral effects of the hormones is given by seminal vesicle weight. Figure 4.4 shows that this was high and equal in the DHT and DHT-OB groups, and low in the oil-treated castrates.

4.3.3 Discussion

The results of this experiment show that urine of castrated male rats treated with DHT or DHT and OB has an odour which is more attractive to female rats than that from urine of those males treated with oil alone. This does not depend on the female having previous sexual experience, and agrees *in toto* with the findings of Carr et al. (1965). In the study by Carr et al. (1965), the sexually experienced, receptive females spent much more time investigating intact male odours than they did investigating castrated male odours, but this was not statistically significant. However, Carr (1974) presents more detailed analysis of this study, and showed that a preference for intact male odours did exist.

To summarise the findings of this experiment; dihydrotestosterone stimulates the secretion of substances into the urine of male rats, which attract female rats. The nature of this secretion does not seem to be impaired by the additional administration of oestradiol benzoate (eliciting similar investigation times in females, in either case). These findings strongly suggest that odour production in the male rat requires the presence of dihydrotestosterone. Further, castrated male rats treated with dihydrotestosterone have the same odour, but lack the sexual activity of castrated males treated with dihydrotestosterone in combination with oestradiol benzoate.

In the following experiments, the odour and the sexual activity of the male rat are varied independently, with a view to determining whether one or both are effective sources of incentive to a female rat.

4.4 Experiment 8 : The sexual attractiveness of male rats: An endocrinological dissection

Meyerson and Lindstrom (1973) showed that the odour of an intact male rat is rewarding to a female rat; whereas Hill and Thomas (1973) suggested that the difference was in the behaviour of a sexually active male towards a female.

This experiment investigated the approach of receptive female rats to (1) castrated males, which neither smell nor behave like intact males, (2) castrated males treated with dihydrotestosterone, which smell like intact males but behave like castrate males and (3) castrated males treated with dihydrotestosterone and oestradiol benzoate, which both smell and behave like intact males.

4.4.1 Method

Experimental animals

The animals used were twenty-four 120 day old Wistar females and twenty-four 180 day old Wistar males. They were kept in group cages, 8 animals in each cage.

All started experimentally naive and sexually inexperienced, and were given a controlled amount of sexual experience and habituated to the runway as follows. The females were ovariectomized under either anaesthesia, and two weeks later oestrus was reinstated with injections of OB (5 µg in 0.1 ml arachis oil, administered subcutaneously 48 h and 24 h before the test) and progesterone (500 µg in 0.1 ml arachis oil, 6 hours before testing). Each male and female then received controlled amounts of sexual experience before the experiment proper: ten mounts per rat, repeated three times, twice in an open field (circular testing arena) and once in the runway with

all the doors open.

The males were then castrated and allowed a two-month recovery period before the injections began. During the recovery period both males and females received controlled amounts of habituation/exploration in the runway, separately, with all the doors open: three fifteen-minute periods per rat, on three separate days. Each period was separated by an interval of three days. The males were tested on separate days from the females, and after each session the runway was cleared with warm water, and the sawdust in the litter trays changed. Training took place in a perspex runway, details of which have already been given in Chapter 4.

Experimental schedule

Before the training sessions began, each female was randomly allocated to a male (all the animals were ear clipped for identification). The males were allotted to three groups at random, and injected for 15 days prior to the start of training, and then throughout the training period. One group of male rats was injected with 0.1 ml of arachis oil/day, a second group with 1 mg DHT in 0.1 ml oil/day; and a third group received 1 mg of DHT in 0.1 ml oil and 5 μg of OB in 0.1 ml of oil/day.

Each female was given 8 trials daily for a period of 3 days. Each trial ran as follows: a male was placed in the goal box and its allocated female in the start box, and after a delay of a few seconds the door was opened and the female was allowed to run to the male holding box. If the female did not leave the start box within 120 seconds, or traverse the runway within 60 seconds of leaving the start box, the trial was terminated. When the female arrived at the male holding box, the door releasing the male was opened. The trial was terminated after one mount or intromission, or if none occurred after 60 seconds. The female was then replaced in the start box. Since the grasping of a female by the experimenter's hand may imitate a mount by a male particular care was taken not to handle the females in an erotic

manner: all females were picked up by the tail. Each female was then replaced in the start box. The interval between trials was about 10 seconds. During the experiment the following were recorded: (1) running time from the start box to the goal box and (2) occurrence of copulation. The animals were run in their respective groups, and the running order of the groups was determined by a sequence of random numbers. A new sequence was generated for each day of training. There was a half-hour interval between the testing of the groups, during which the runway was wiped clean with warm water, and fresh sawdust placed in the litter tray.

At the end of the experiment the males were killed and their seminal vesicles removed and weighed to the nearest 0.1 g.

4.4.2 Results and Discussion

The results were analysed with analysis of variance (one-between, two-within factors) and appropriate post-hoc comparisons. A summary of the analysis of variance is presented in Table 4.4.

The mean runway time for the first trial on Day 1 was similar for all three groups of females (females running to oil treated males: 68.7 $\pm$ 23.1 (standard error) seconds; for females running to DHT treated males: 48.9 $\pm$ 10.3 seconds; and for females running to DHT-OB treated males: 52.3 $\pm$ 7.2 seconds. See also Appendix A, Table A3, for daily trial means). This shows that all the females started with the same level of performance. All female groups ran faster over time ($F = 20.28$; with df. = 2, 42; $p < 0.001$; Figure 4.5). There was no significant interaction between treatment and days. The three groups, however, were clearly different, as shown in Figure 4.6. The females ran faster to the DHT group than to the control group, and faster to the DHT-OB group than the DHT group. The overall difference was significant ($F = 15.50$; with df. = 2, 21; $p < 0.001$), and both individual Least Significant Difference tests (Keppel, 1973) were also significant (Control vs DHT: $F = 8.97$; with df. = 1, 21; $p < 0.01$; and DHT vs DHT-OB groups:

Table 4.4

Experiment 8. The sexual attractiveness of male rats: An endocrinological dissection. Summary of analysis of variance

Source	df.	SS	MS	F-ratio	P
Subjects	23	795025.28			
Treatment	2	473915.58	236957.79	15.50	< 0.001
Error	21	321109.70	15290.94		
Days	2	265013.78	132506.89	20.28	< 0.001
Days x Treatment	4	61289.57	15322.39	2.34	ns.
Error	42	274481.69	6535.28		
Trials	7	107281.29	15325.90	8.98	< 0.001
Trials x Treatment	14	67850.75	4846.48	2.84	< 0.001
Error	147	250913.42	1706.89		
Trials x Days	14	25761.35	1840.10	1.33	ns.
Trials x Days x Treatment	28	59822.34	2136.50	1.54	< 0.05
Error	294	407480.76	1385.99		
Within cell	552	1519894.94			

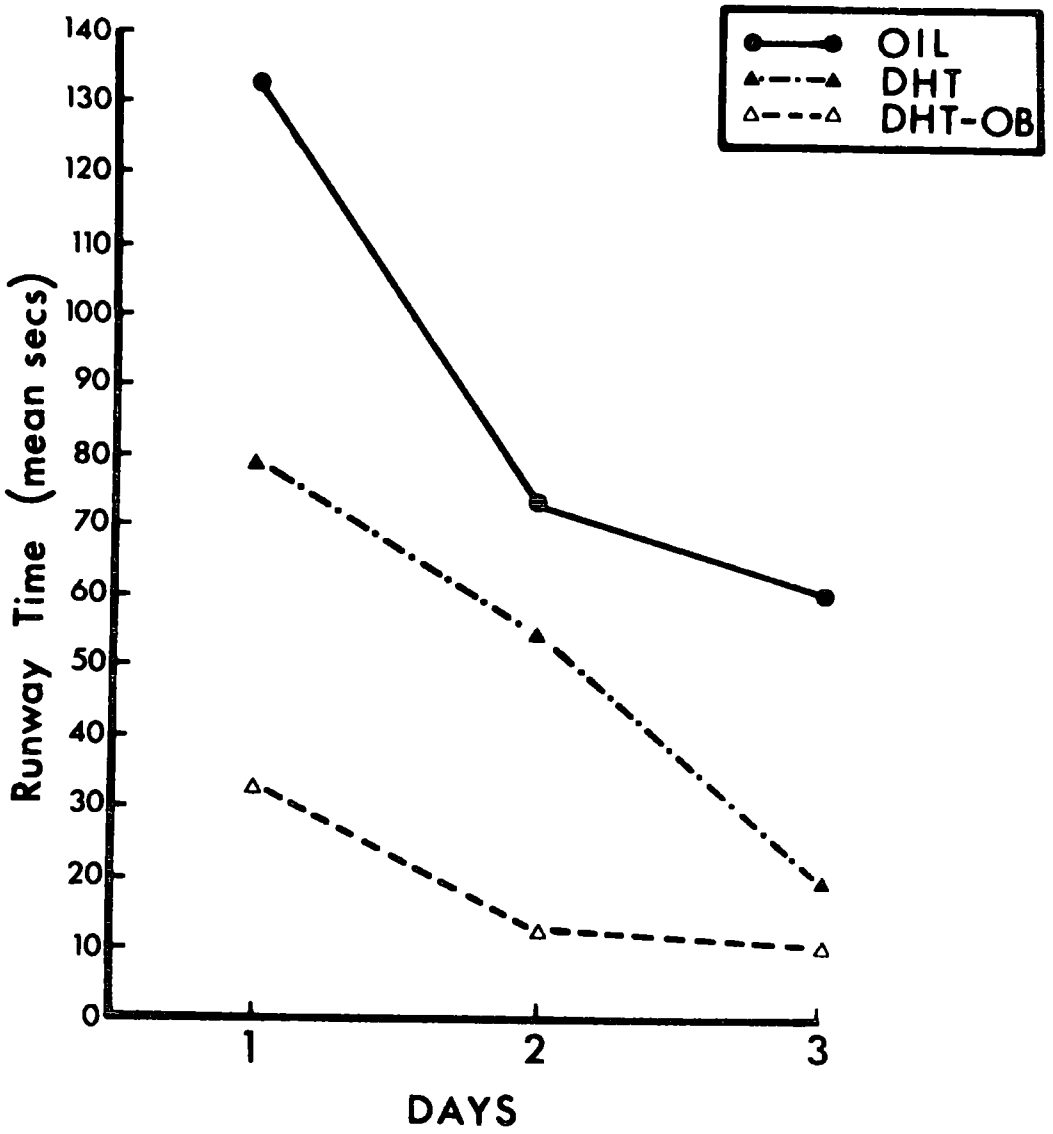


Figure 4.5

Experiment 8. Mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB).

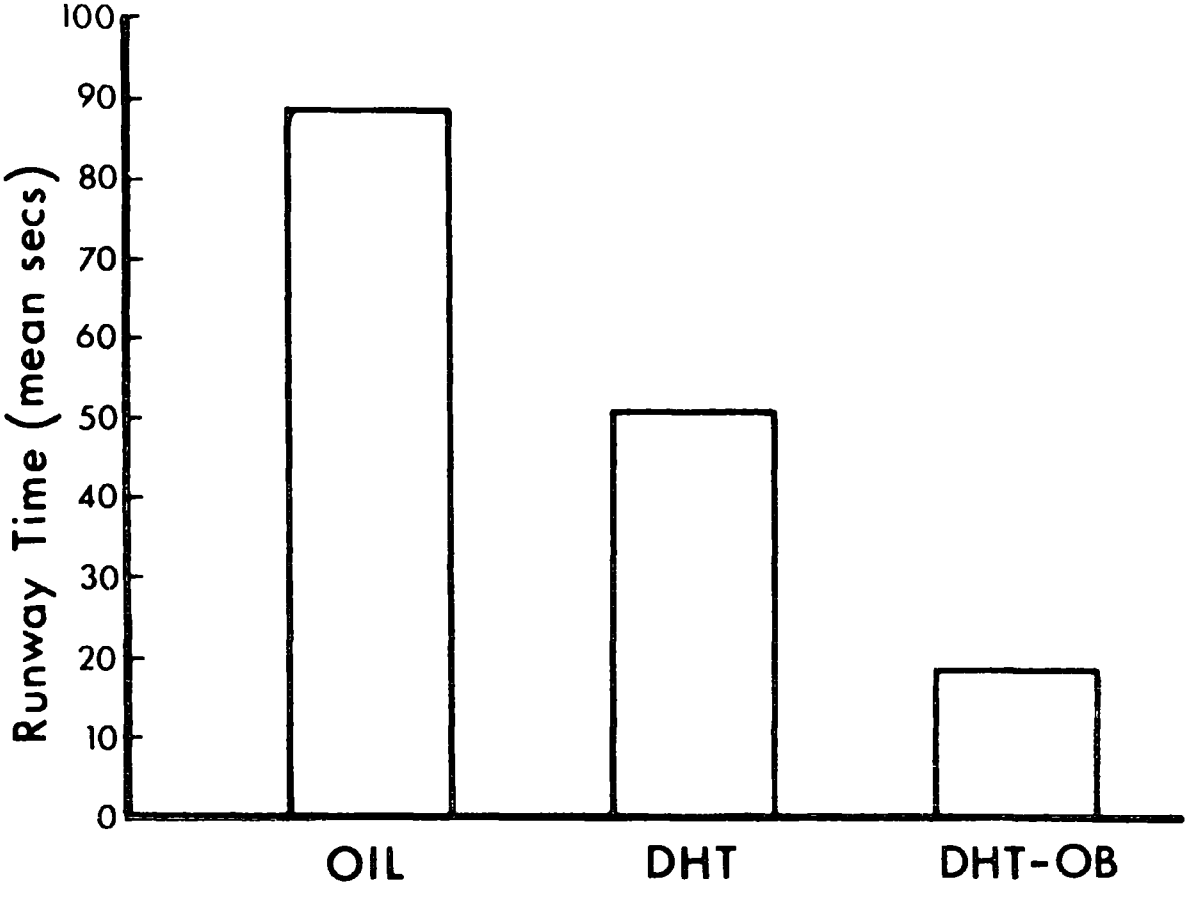


Figure 4.6

Experiment 8. Mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB), averaged across the 3 experimental days.

F = 6.59; with df. = 1, 21; $p < 0.05$).

Figure 4.7 shows that females running to DHT-OB treated males maintained their running performance over trials; whereas females running to DHT males started the same but did not maintain their performance over trials. The interaction was significant (F = 2.84; with df. = 14, 147; $p < 0.001$). This result suggests that copulatory behaviour may have a proactive effect on the runway performance of female rats. There was a significant trials x days x treatment interaction (Table 4.4); but this did not lend itself readily to meaningful interpretation.

Seminal vesicle weight for the DHT-OB treated males was significantly greater than for the DHT treated animals; but both were much higher than those of placebo treated rats, as shown in Figure 4.8(A). One DHT treated rat mounted on 4.9% of the trials, whereas the males treated with DHT and OB mounted on nearly all the trials (Figure 4.8(B)). The first result was somewhat surprising in view of published evidence, and suggests that OB may also act peripherally. It seems, then, that the hormones did not have the expected effect.

4.5 Experiment 9 : The effects of dihydrotestosterone alone, and in combination with oestradiol benzoate, on seminal vesicle weight in the male rat

This experiment tested the effects of DHT administered alone or in combination with OB on seminal vesicle weight of castrated male rats, in order to establish whether or not the effects obtained in Experiment 8 were due to chance alone.

4.5.1 Method

Experimental animals

Twenty-four sexually inexperienced male Wistar rats, about 150 days of age and weighing 443 $\pm$ 13 g, were housed in two group cages. The animals were transferred to the reversed light room at least one month before the start of any surgical or experimental procedures.

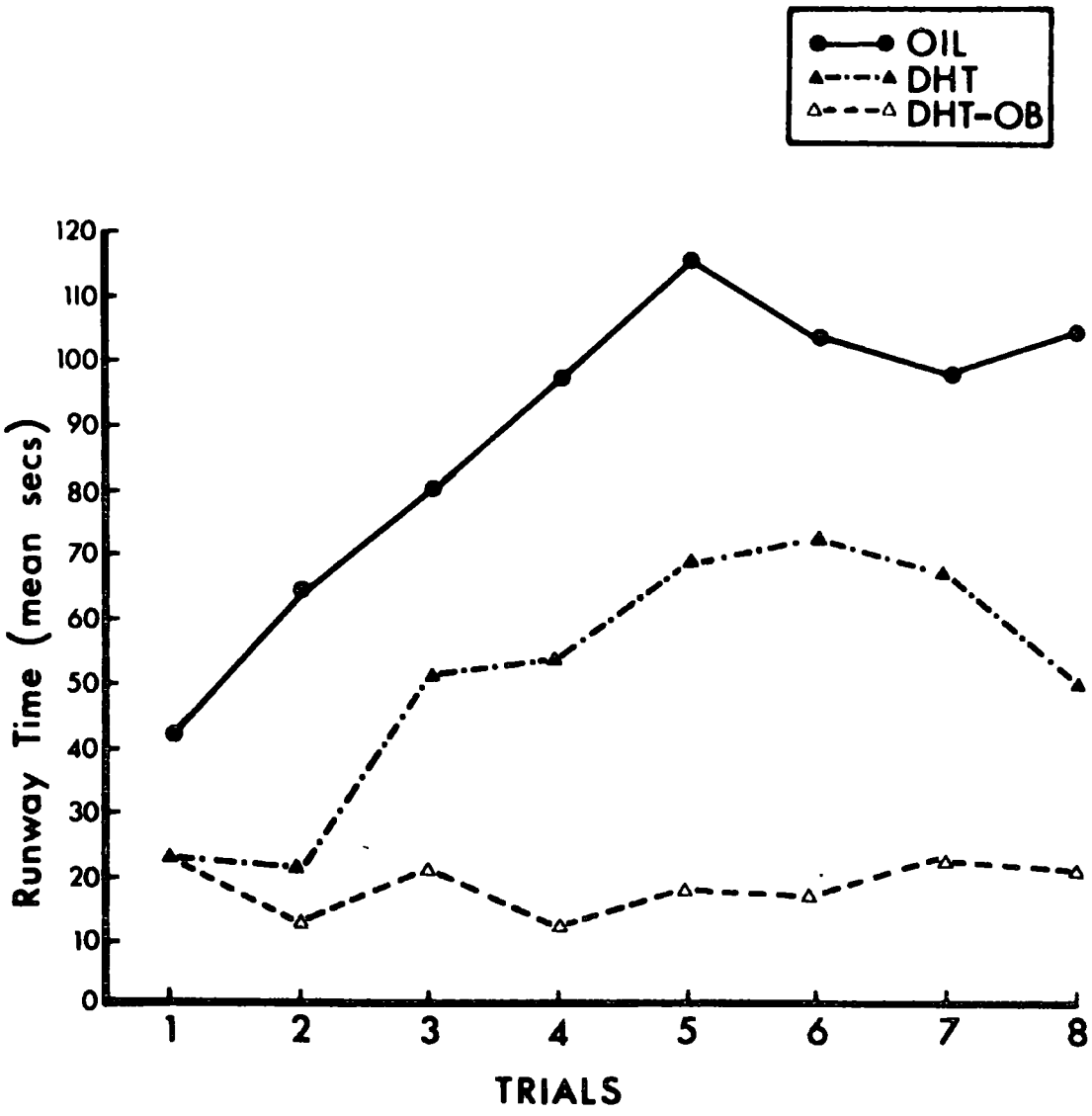


Figure 4.7

Experiment 8. Mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB) as a function of trials (averaged over 3 days).

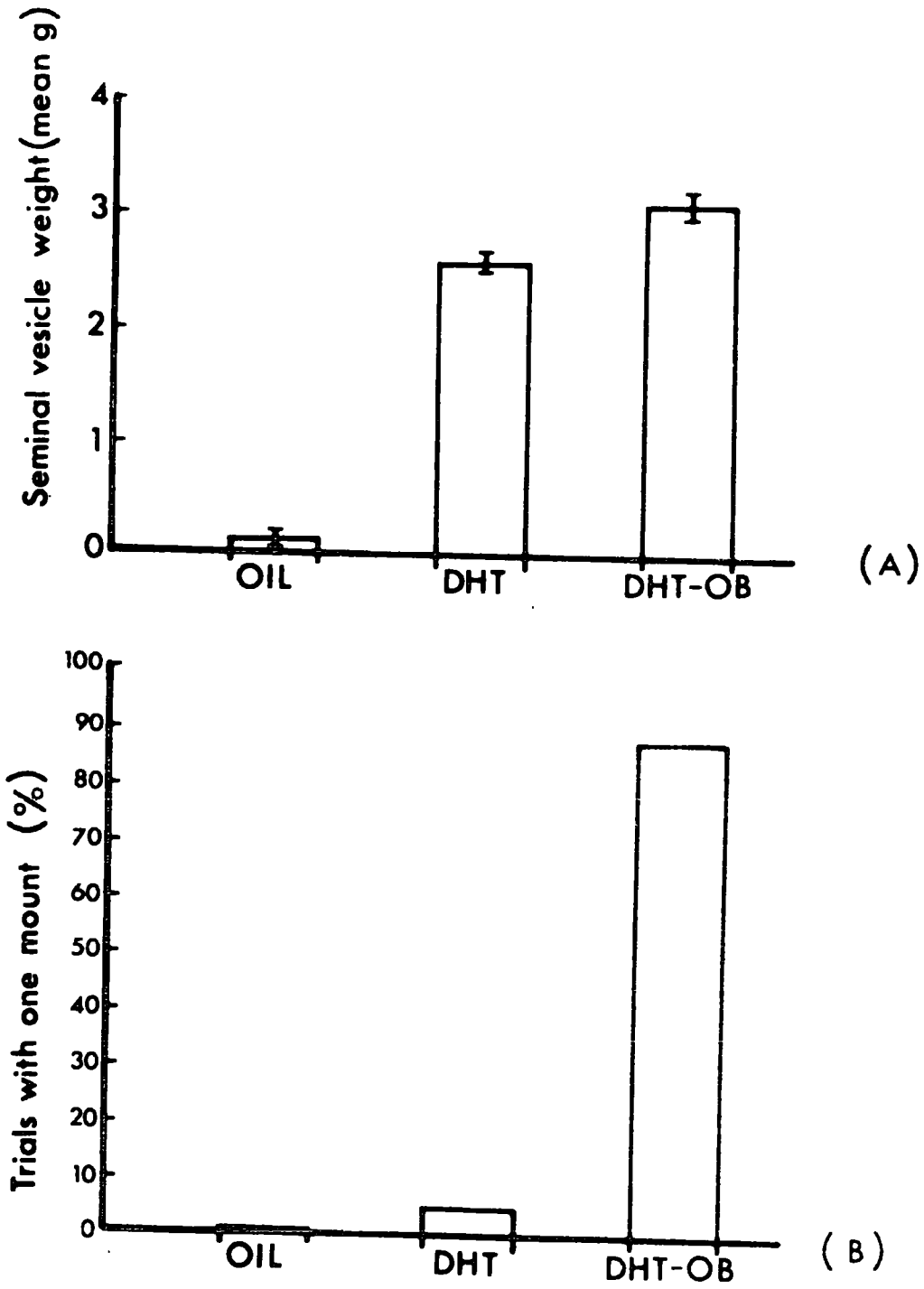


Figure 4.8

Experiment 8. (A) Mean seminal vesicle weight (± standard error) of castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT) or DHT in combination with oestradiol benzoate (DHT-OB).

(B) Trials with one mount (%) for castrate males treated with either oil only or DHT, or DHT-OB.

The males were castrated and allowed a two month recovery period before injections began. The males were allotted to two groups at random and injected for 21 days. One group was injected with 1 mg DHT in 0.1 ml arachis oil/rat/day; and the second group with DHT, 1 mg in 0.1 ml oil, in combination with OB, 5 μg in 0.1 ml arachis oil/day. All the injections were administered subcutaneously. Body weight was measured every third day to the nearest 1.0 g at 11.00 h, one hour before the start of the dark period. All the rats were killed, and their seminal vesicles removed and weighed intact and with seminal fluid expressed, 21 days after the start of injections. The order in which the seminal vesicles of rats from the two treatment groups were dissected out was determined by the toss of a coin. The seminal vesicles were weighed intact. The seminal fluid was then expressed, the vesicles washed in physiological saline, dried between paper towelling, and weighed again on a Mettler balance to the nearest 0.1 g.

4.5.2 Results

Figure 4.9 shows mean seminal vesicle weight intact, and with fluid expressed. A Student's t-test testing the difference between the means for seminal vesicle weight, with and without seminal fluid, was carried out on the data. There was no significant difference between the two groups (intact weights: $t = 0.3496$; with $df. = 22$; $p =$ ns.; with seminal fuid expressed: $t = 0.8154$; with $df. = 22$; $p =$ ns.).

4.6 Experiment 10 : A second study on the sexual attractiveness of male rats: Olfactory and behavioural components

The findings of Experiment 9, that injections of dihydrotestosterone alone or in combination with oestradiol benzoate do not have differential effects on seminal vesicle weight, in agreement with published data, suggested that the result obtained in Experiment 8 was in fact due to chance. Experiment 8 was therefore repeated.

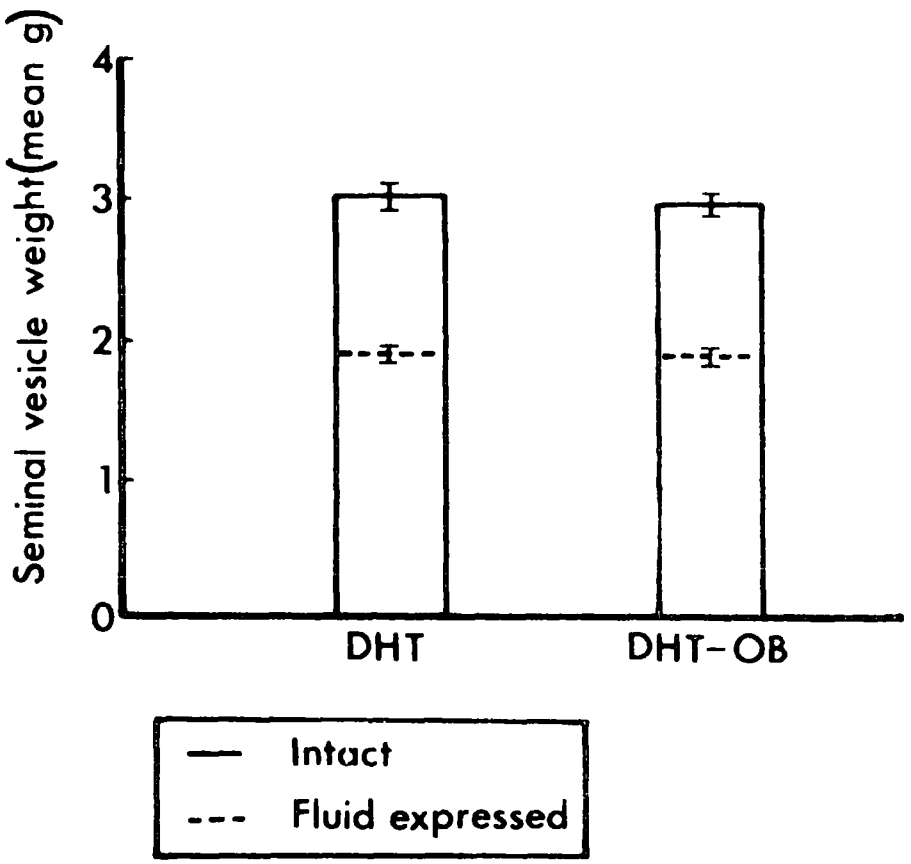


Figure 4.9

Experiment 9. Mean seminal vesicle weight (± standard error), intact and with fluid expressed, of castrate males treated with either dihydrotestosterone (DHT) or DHT in combination with oestradiol benzoate (DHT-OB).

4.6.1 Method

Experimental animals

Twenty-four 120 day old females and twenty-four 180 day old males were used. All the animals were experimentally naive and sexually inexperienced. They were given controlled amounts of sexual experience and habituated to the runway, as follows. The females were ovariectomized under ether anaesthesia and oestrus was reinstated with injections of OB and progesterone as in Experiment 8. Each female was randomly allocated to a male and the same pairing was kept throughout the experiment proper (during training). Sexual experience consisted of 10 mounts per rat, repeated 3 times, twice in an open field and once in the runway with all the doors open. The males were then castrated under ether anaesthesia, and allowed a three-month recovery period before injections began. During the recovery period, all the animals received controlled amounts of habituation/exploration in the runway as described in the 'Method' section of Experiment 8.

Experimental schedule

This was the same as in Experiment 8. The running order of the individual animals was determined by a sequence of random numbers, without a break in continuity of testing. At the end of the experiment all the males were killed and their seminal vesicles removed and weighed.

4.6.2 Results and Discussion

The results were analysed using analysis of variance (one-between, two-within factors; Table 4.5), and appropriate post hoc comparisons. Figure 4.10 shows that males treated with DHT and OB mounted on almost all the trials, while the males treated with DHT alone, like the placebo treated controls, did not mount at all. Seminal vesicle weight however - a measure of the peripheral effect of the hormones - was equal and high in the DHT and DHT-OB groups and low in the control group. The hormones, then, had the expected effect.

Table 4.5

Experiment 10. The sexual attractiveness of male rats: Olfactory and behavioural components. Summary of analysis of variance

Source	df.	SS	MS	F-ratio	p
Subjects	23	655632.49			
Treatment	2	402099.81	201049.90	16.65	< 0.001
Error	21	253532.69	12072.99		
Days	2	118298.34	59149.17	19.44	< 0.001
Days x Treatment	4	13436.83	3359.21	1.10	ns.
Error	42	127783.30	3042.46		
Trials	7	92676.99	13239.57	8.43	< 0.001
Trials x Treatment	14	59992.45	4285.18	2.73	< 0.001
Error	147	230905.27	1570.78		
Trials x Days	14	40766.59	2911.90	1.52	ns.
Trials x Days x Treatments	28	71092.07	2539.00	1.33	ns.
Error	294	562471.22	1913.17		
Within cell	552	1317423.06			

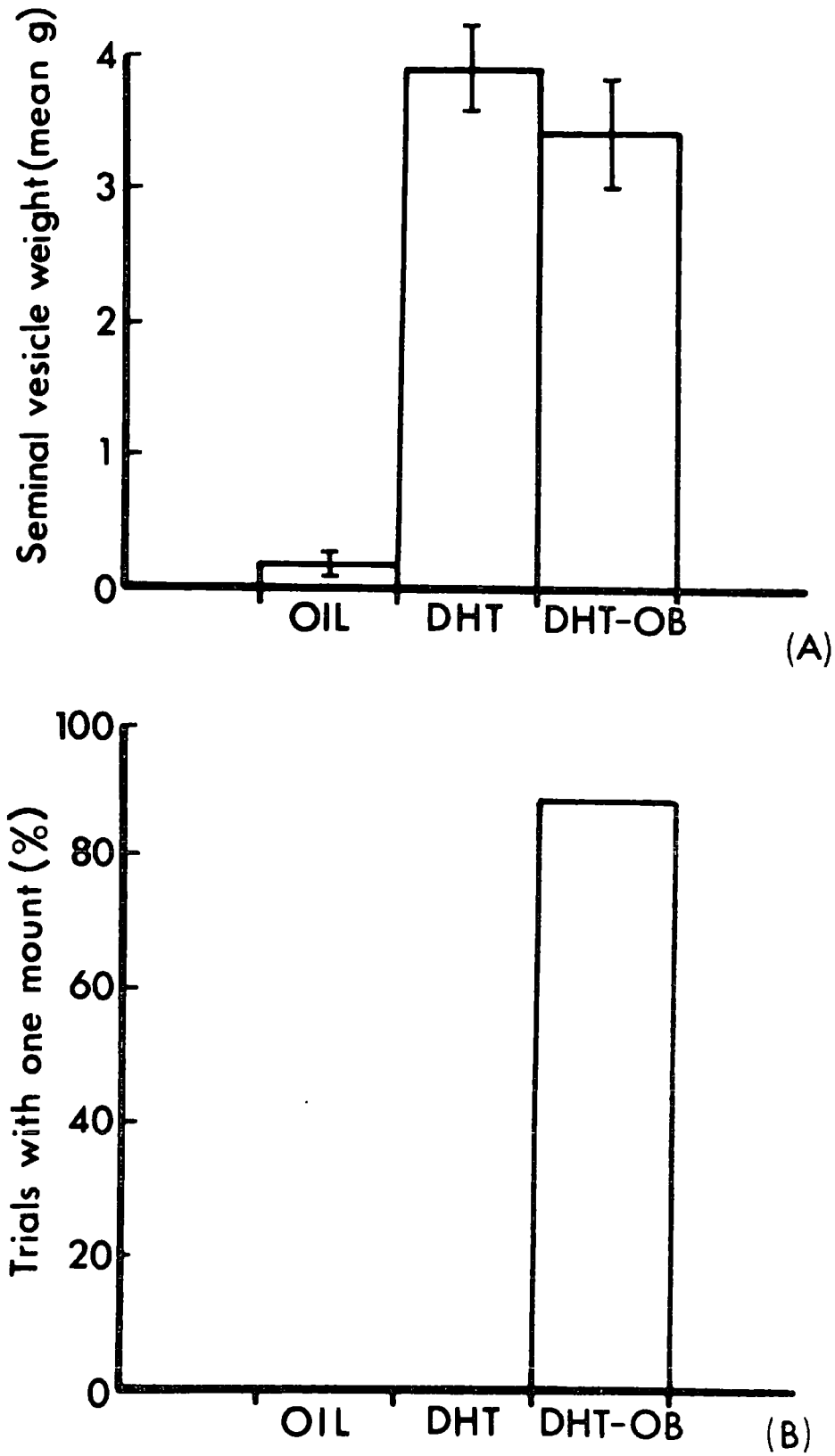


Figure 4.10

Experiment 10. (A) Mean seminal vesicle weight (± standard error) of castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB).

(B) Trials with one mount (%) for castrate males treated with either oil only, or DHT or DHT-OB.

As regards running speed, all the female groups started at the same level of performance (first trial means for Day 1: oil group: 27.7 $\pm$ 6.2 s; DHT group: 24.6 $\pm$ 3.3 s; DHT-OB group: 27.9 $\pm$ 5.8 s. See also Appendix A, Table A4, for daily trial means), and ran faster over time ($F = 19.23$; with df. = 2, 42; $p < 0.001$; Figure 4.11). There was no significant interaction between treatment and days. The three groups, however, were different in their level of performance. As in Experiment 8, the females ran faster to the DHT than to the placebo-treated males; and faster to the DHT-OB males than to the DHT males. The overall difference was significant ($F = 16.65$, with df. = 2, 21; $p < 0.001$; Figure 4.12). Least Significant Difference tests (Keppel, 1973) between individual groups were also significant (oil vs DHT: $F = 13.04$; with df. = 1, 21; $p < 0.01$; DHT vs DHT-OB: $F = 4.38$; with df. = 1, 21; $p < 0.05$; two-tailed). The interaction between treatment and trials was also significant ($F = 2.73$; with df. = 14, 147; $p < 0.001$), and is shown in Figure 4.13. This demonstrates that females running to DHT treated males ran as fast as females to the DHT-OB treated males on every first trial; but subsequently showed a decrement in performance as a function of trials. The oil group also showed a similar decrement. However, the females running to the active males (DHT-OB) maintained their initial performance over trials. As in Experiment 8, this suggests that copulatory behaviour may have a facilitatory or proactive effect on the runway performance of female rats.

Experiment 8 and Experiment 10 : Reliability of Results

It was decided a posteriori to test the robust nature of these findings with an analysis of variance on the treatment means of the two experiments. A summary of such an analysis is presented in Table 4.6. There was no overall difference between experiments. However, the effect due to treatment was very large. In summary, then, and in view of the previous experimental findings, comparisons of the treatment means suggest that treatment effect

Table 4.6

Reliability of effects obtained in Experiments 8 and 10. Summary of analysis of variance

Source	df.	SS	MS		P
Experiment (E)	1	80.60	80.60	3.84	ns.
Treatment (T)	2	4544.22	2272.11	108.56	< 0.01
Error (T x E)	2	41.86	20.93		
Total	5	4666.68			

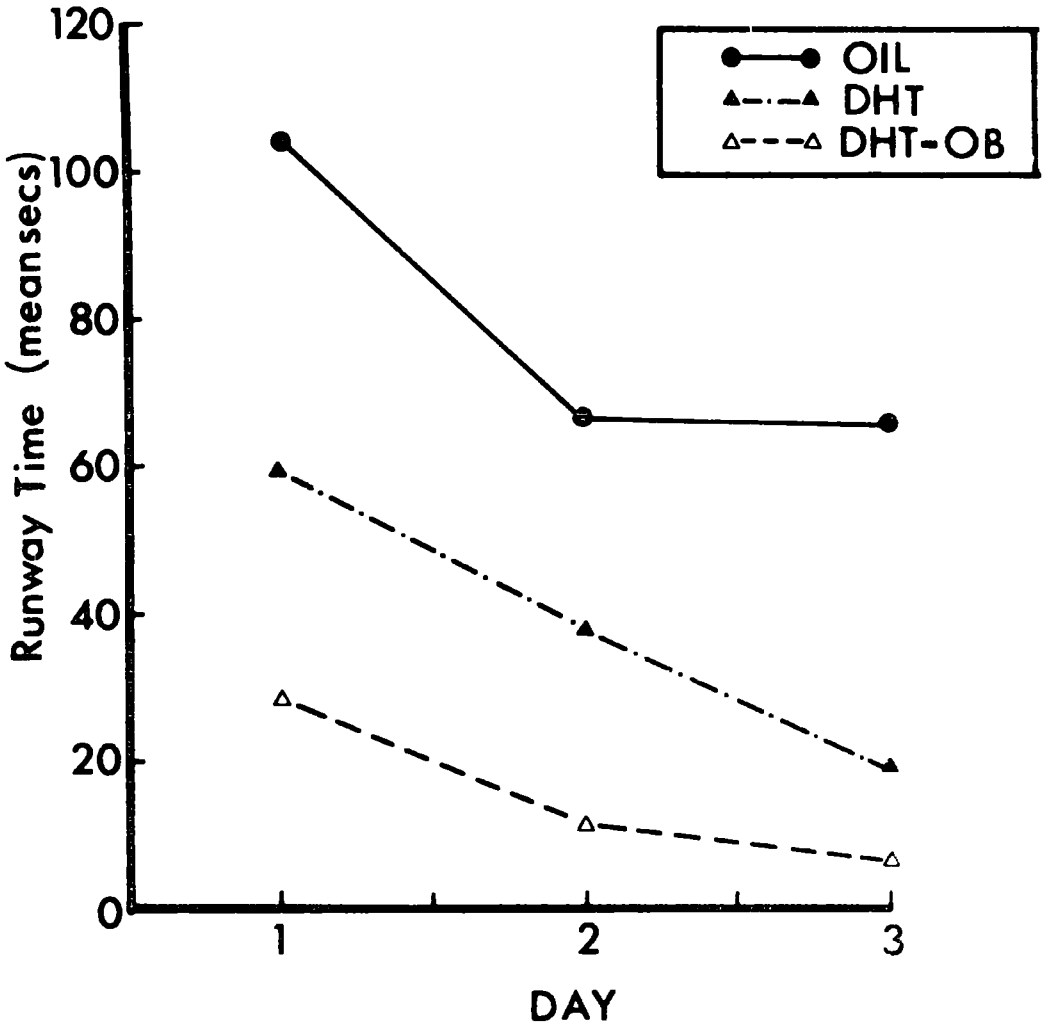


Figure 4.11

Experiment 10. Daily mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB).

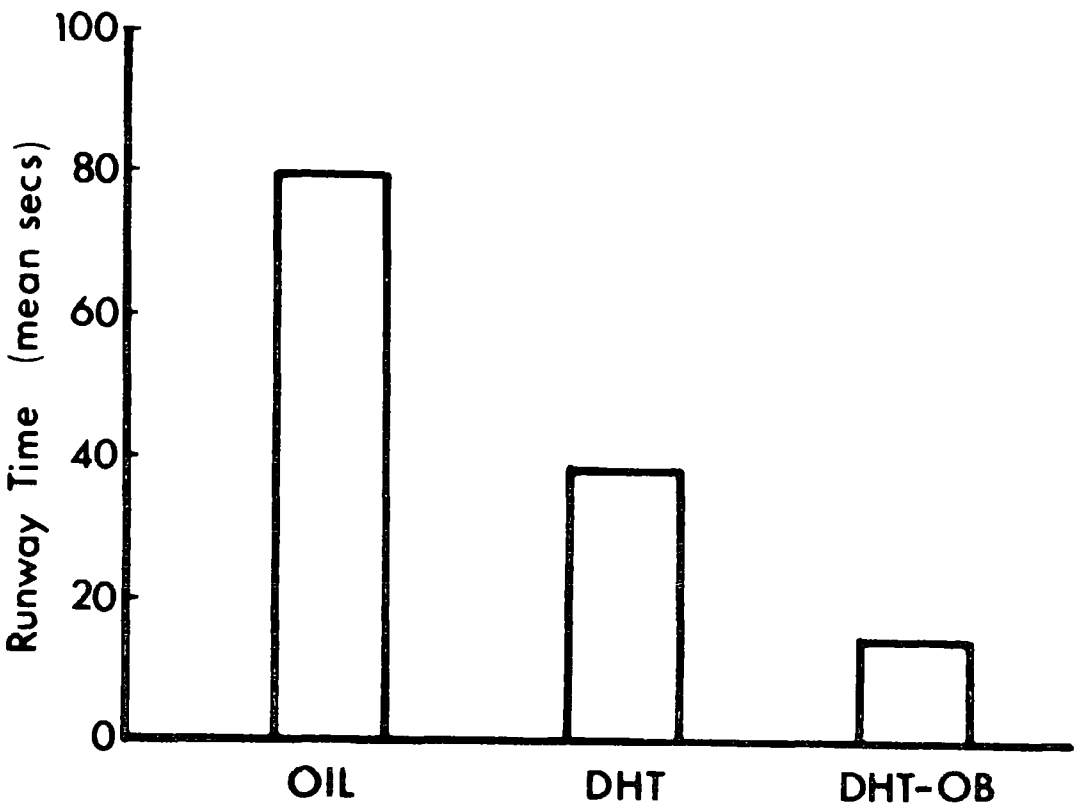


Figure 4.12

Experiment 10. Mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB), averaged across the 3 experimental days.

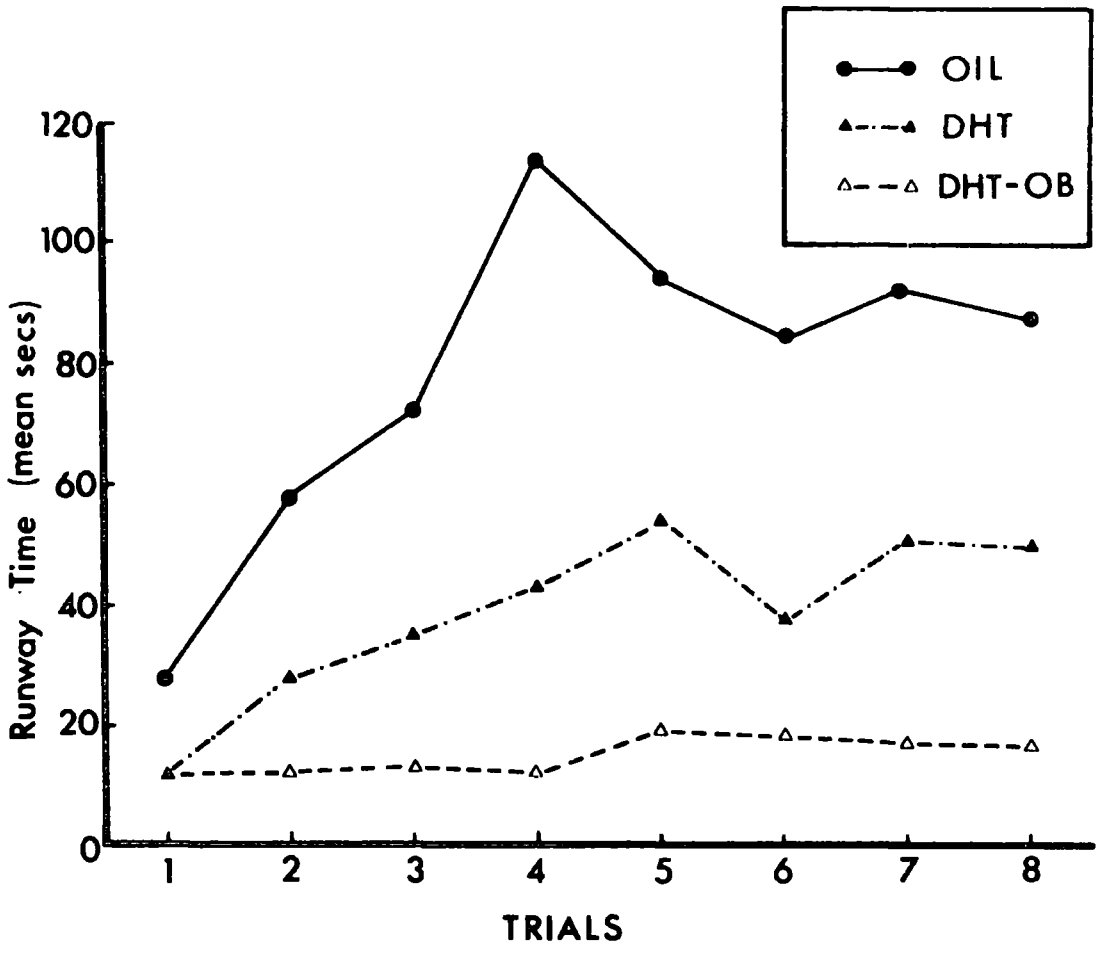


Figure 4.13

Experiment 10. Mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB) as a function of trials (averaged over 3 days).

can be explained in the following way: there is a significant difference in the performance of females running to hormone treated males than to the placebo treated controls. Females ran faster to the hormone treated animals ($F = 179.79$; with df. = 2, 2; $p < 0.01$). In addition, females ran faster to the sexually active males (DHT-OB) than to the castrates treated with DHT only ($F = 37.32$; with df. = 2, 2; $p < 0.05$).

These results show, then, that the rewarding characteristics of the testosterone treated male can be split into somatic and behavioural components; and that both are effective. The behavioural component must include mounting and intromission. The somatic component is likely to be olfactory in nature, since accessory sex glands in the male rat release an odour which is attractive to the female rat (Gawienowski et al., 1975).

4.7 Experiment 11 : Sexual behaviour and proactivation in the female rat

The runway performance of females over trials in Experiment 8 and 10 suggests that mating may have a proactive effect (see also Figures 4.7 and 4.13). One way of investigating the role of proactive effects would be to test animals using discrete trials with a "rest period" in between successive trials.

In the following experiment, sexually receptive female rats were allowed to run to either (1) castrated males or (2) castrated males treated with DHT or (3) castrated males treated with DHT and OB (sexually active males), on one trial every day. This effectively introduced an inter-trial interval of approximately 24 hours. It was hoped that this experiment would (1) explain the role of proactivation in copulatory behaviour and (2) corroborate and further endorse the findings relating to the reward characteristics of a sexually active male rat.

4.7.1 Method

Twenty-four 120 day old females and twenty-four 180 day old males served as experimental animals. All the animals were experimentally and

sexually inexperienced. The procedures for giving the animals sexual experience and habituation to the runway were identical to the procedures used in Experiment 10. Each rat was given one trial on every day, and all the females were allowed 60 seconds of contact with their allocated males. Records were kept of the number of copulations during each trial, as well as the time taken by a female to traverse the runway from the start box to the male holding box.

4.7.2 Results and Discussion

Since it was anticipated that there would be variability in runway performance for the first few trials, it was decided a priori to analyse data which showed uniformity in runway time. Thus analysis of variance (one-between, one-within factor) with appropriate post hoc comparisons was carried out on the data obtained for trials (days) 9 to 17, Figure 4.14. After 17 days, the trials were discontinued, since it was noticed that one female was becoming increasingly aggressive to a DHT treated male. A summary of the analysis of variance is presented in Table 4.7.

The males treated with DHT and OB copulated on approximately 70% of the trials, whereas the males treated with DHT or oil only did not mount at all. There was a mean of 1 ($\pm$ 0.07) copulation per trial, and no ejaculations were observed.

All the females ran faster over trials ($F = 2.29$; with df. = 8, 168; $p < 0.05$). The three female groups, however, were different in their runway performance. As in Experiment 8 and 10, the females ran faster to males treated with DHT than to males treated with oil only; and faster to males treated with DHT-OB than to the DHT treated males (Figure 4.15). There was no interaction between trials and treatment over the last nine days.

The one trial paradigm was used because this effectively abolished possible immediate activational effects due to copulation. If the differences in runway performance over trials observed in the two previous experiments

Table 4.7

Experiment 11. Sexual behaviour and proactivation in the female rat. Summary of analysis of variance

Source	df.	SS	MS	F-ratio	P
Subjects	23	1641.58			
Treatment	2	801.08	400.54	10.01	< 0.01
Error	21	840.50	40.02		
Trials	8	189.08	23.63	2.29	< 0.05
Trials x Treatment	16	73.35	4.58	< 1	ns.
Error	168	1734.08	10.32		
Within cell	192	1996.51			

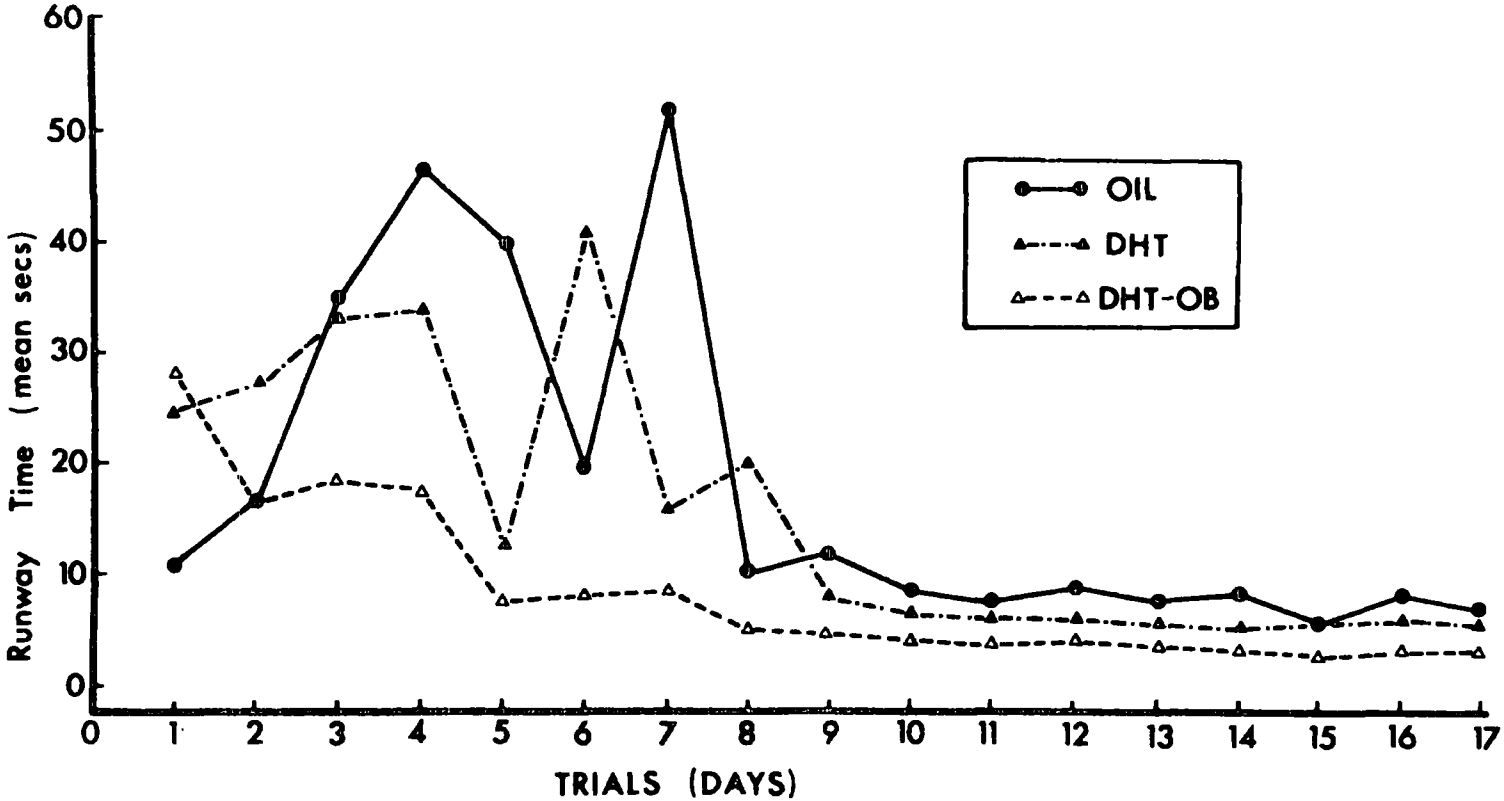
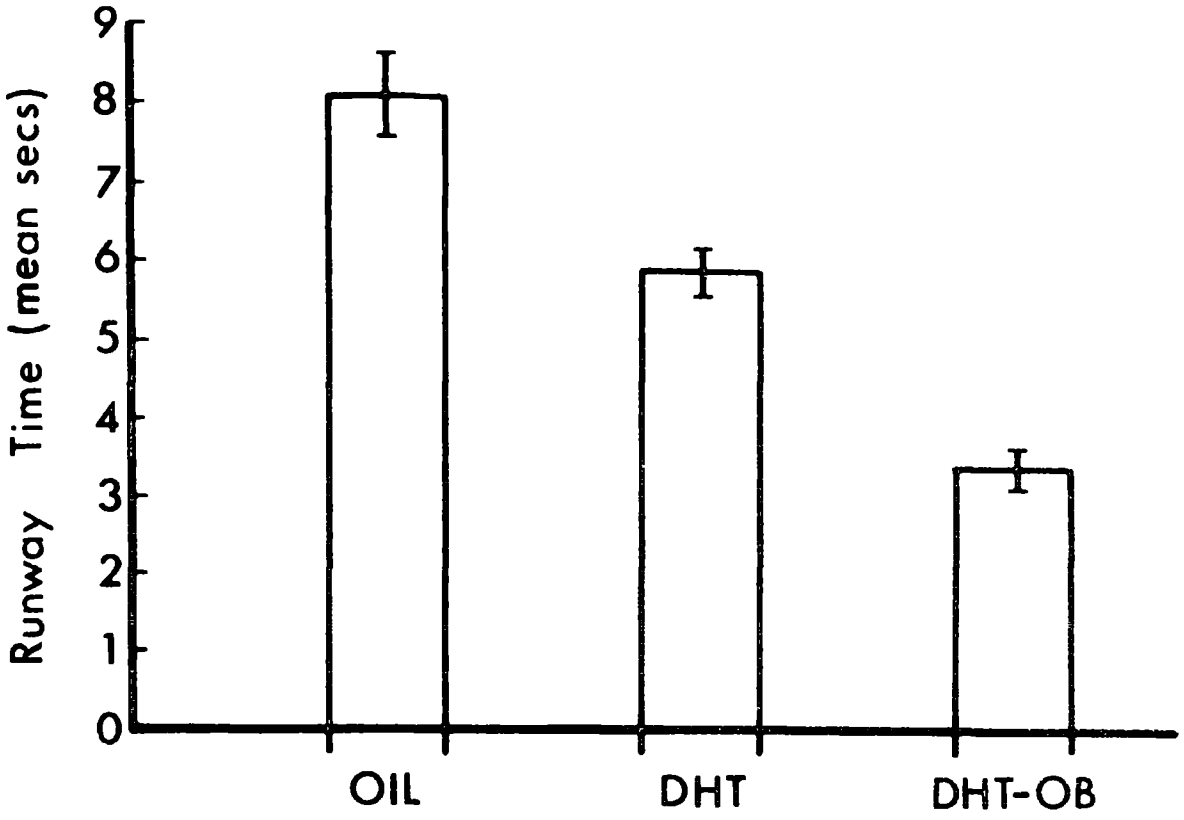


Figure 4.14

Experiment 11. Daily mean runway time of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB). Each female received one trial per day.



Figuro 4.15

Experiment 11. Mean runway time (± standard error) of receptive females running to castrate males treated with either oil only (OIL), or dihydrotestosterone (DHT), or DHT in combination with oestradiol benzoate (DHT-OB), averaged across Day 9 to Day 17.

were due to a proactive effect of copulatory behaviour, one would not have expected to find a difference between these two groups on a one trial a day basis. However, such a difference was observed, suggesting that proactive effects do not account for the differences in Experiments 8 and 10. These results agree unequivocally with those of Experiments 8 and 10, in that they show that non-copulatory (olfactory) as well as copulatory stimuli can reinforce and enhance an instrumental response of a sexually receptive female rat.

4.8 General Discussion

An experiment by Pfaff and Pfaffman (1969) supports the idea that urine odours elicit investigatory responses in rats. Pfaff and Pfaffman exposed male rats to two small bottles containing urine from oestrous and dioestrous females, and discuss their findings thus:

> "Some of the behavioural data raise the question of whether the differential behavioural responses by the normal males were more exploratory or more hedonic in nature. Normal males failed to show a selective response when only their time of sniffing at the most concentrated source of odor (the openings of the bottle tops) was measured, although they did respond selectively when times of sniffing along the sides of the bottle was included. This suggests that the normal males did not simply prefer the odor of receptive female urine, in the hedonic sense, but instead that the odor of receptive female urine enhanced the response of exploration and sniffing along the sides of the bottle."
>
> (pp. 264-265)

Brown (1978) found that both oestradiol and oestradiol plus progesterone failed to stimulate preferences for male odours and urine-marking in ovariectomized females. However, his testing procedures differed markedly from Experiment 7 and other published studies; and the results of Experiments 10 and 11 show that olfactory as well as behavioural components of male sexual behaviour are effective incentives. The studies of Orsulak and Gawienowski (1972) and Orsulak et al. (1975) clearly demonstrated that the preputial gland is responsible for the secretion of an arousal or attractant pheromone in both male and female rats. The role of this gland in the sexual behaviour

of the rat has been suspected for some time (Stanley and Powell, 1941). It also actively metabolises testosterone to dihydrotestosterone in the normal rat (Tveter and Aakvaag, 1969; Richardson and Axelrod, 1971), and this androgen in particular stimulates preputial gland growth, as well as that of other accessory sex structures, in castrated rats (Price and Williams-Ashman, 1961). (Seminal vesicle weights were recorded in this and other experiments because of their accessibility and size, as well as being a reliable measure of the peripheral action of dihydrotestosterone). According to Gawienowski et al. (1975), the nature of the pheromone appears to be a variety of volatile, lipid-soluble substances (aliphatic alcohols); but these authors do not exclude the possibility that other substances may also have attractant qualities, such as the more stable free fatty acids. Other candidates could include metabolites of gonadal steroids, already known to be the case in dogs (Kloek, 1961).

Krames and Mastromatteo (1973) suggest that olfactory stimuli from male partners may influence copulatory behaviour of the female rat at three stages: approach, initiation and maintenance. The idea that a variety of pheromones, of both a volatile and more stable nature, are produced serving the function of stimulating exploration and then eliciting sexual arousal in female rats, is given further support by some recent observations by Wiepkema (unpublished data, personal communication, 1978). He found that the longer the male groomed his flank, face and anogenital region, the more intense was the performance of ear vibration, darting and hopping by a female who could smell but not see the male. A possible explanation of this grooming behaviour is that the male may be stimulating odour-producing glands, or spreading genital secretions over his body by first licking his genitalia, and then grooming his flank and face. None of the soliciting behaviours observed by Wiepkema were observed in females during testing in Experiment 7.

Using a Y-maze, Gilman and Westbrook (1978) showed that sexually

receptive female rats demonstrated a preference for sexually active males over castrated males or females. However, in their experiments, the females could have been responding to the odour of a sexually active male at the choice point. In an elegant experiment, Mendelson, Chillag and Quadagno (1978) demonstrated that non-copulatory stimuli emanating from an intact male hamster reinforced an instrumental response, which consisted of making a correct choice for an intact sexually active male in a T-maze, of a female hamster in oestrus. The importance of odour in the initiation and maintenance of sexual behaviour in the hamster has been given great emphasis (see Johnston, 1977). Likewise, Krames and Mastromatteo (1973) have also emphasised the importance of olfactory stimuli, emanating from the male rat, in the performance of sexual behaviour by the female rat.

Several recent studies (Johnston and Zahorik, 1975; Johnston, Zahorik, Immler and Zakon, 1978, in the hamster; and Goldfoot, Kravetz, Goy and Freeman, 1976, in the rhesus monkey) have emphasised the possibility that, unlike insect pheromones, olfactory stimuli involved in mammalian sexual behaviour might owe some of their properties to learning processes. Obviously, in the case of the attraction of the female to the male, the odour of the male might become attractive as a result of its association with mating. The experiments presented in this chapter were not designed to answer such a question; but they provide a basis for doing so, since they illustrate a method for varying systematically the relationship between the odour of the male and his sexual activity.

CHAPTER 5

BEHAVIOURAL EFFECTS OF ETHYNYL OESTROGENS IN THE FEMALE RAT

5.1 Introduction

Ethynyl Oestradiol[1] and Mestranol[2] play a unique role, as far as oestrogens are concerned among the steroidal contraceptive agents. Ethynyl Oestradiol was first synthesised and developed as a potent, orally active oestrogen in the 1930s by Inhoffen, Logemann and Hohlweg and Serini (1938) and together with its 3-methyl ether, mestranol, has become an agent of major clinical importance. One or the other of these ethynyl oestrogens is an essential ingredient in nearly all oral contraceptive formulations in use at present, in combination with the progestagen component.

In view of the millions of women who use these orally potent contraceptive formulations the world over, a detailed knowledge of their pharmacological physiological and behavioural properties seems necessary. Knowledge of the pharmacological and physiological properties of ethynyl oestrogens is just beginning to accumulate, more than a decade after their clinical utilization became world-wide (Helton and Goldzieher, 1977). However, studies of the behavioural effects of these compounds in animals are non-existent. This lack of knowledge was the major stimulus for carrying out the series of experiments described in this chapter.

5.2 Pharmacological and Physiological properties of Ethynyl oestrogens in the female rat

5.2.1 Gastrointestinal absorption

The introduction of the 17α-ethynyl group (in the case of ethynyl oestradiol) and an additional 3-mythoxy group (in the case of mestranol)

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1 Ethynyl Oestradiol : 17α-ethynyl-1,3,5(10) estratrien 3, 17β-diol.

2 Mestranol : 17α-ethynyl-3-methoxy-1,3,5(10)-estratrien-17β-ol.

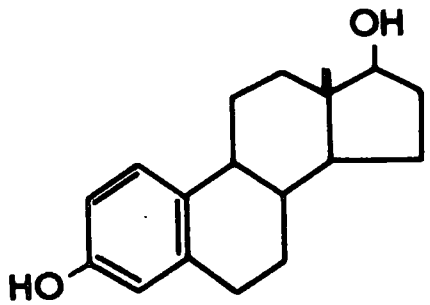
to the oestradiol molecule (Figure 5.1) makes the compound orally active. It causes a retardation of the breakdown of the steroid molecule by the liver.

Evidence of rapid gastric absorption of ethynyl oestrogens in the human female is provided by studies of plasma levels of ethynyl oestradiol after oral administration of commercially tableted formulations: peak values are usually observed within one hour after administration (de la Pena, Chenault and Goldzieher, 1975).

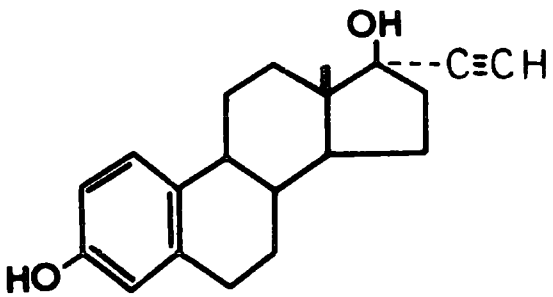
5.2.2 Tissue distribution

Studies of tissue distribution of ethynyl oestradiol and its 3-methyl ether mestranol have been performed in rodents particularly in rats. Steinetz, Meli, Giannina, Beach and Manning (1967) compared tissue distribution of oestradiol-17β to that of ethynyl oestradiol and quinestrol (3-cyclopentyl ether of ethynyl oestradiol) in the rat. Both oestradiol and ethynyl oestradiol accumulated in uterine tissue to a much greater extent than quinestrol, although the deposition of this oestrogen in fatty tissue by far exceeded that of either oestradiol or ethynyl oestradiol. The enhanced oral activity of quinestrol is attributed to its greater storage and release from body fat, and a similar mechanism is thought to be applicable in the greater volume distribution of mestranol as compared to oestradiol or ethynyl oestradiol (Meli, Wolff and Honrath, 1963). Bolt and Remmer (1972) suggest that mestranol is taken up by the body fat in considerable amounts, and by redistribution it is translocated slowly from fatty tissues into the liver where it is metabolised.

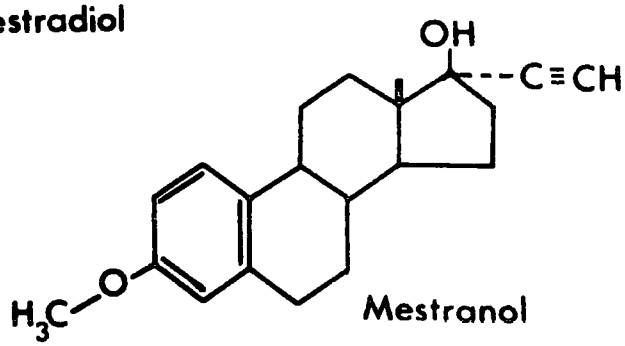
Kappus, Bolt and Remmer (1972) showed that four hours after the intravenous injection of radiolabelled oestrogens, there was 200 to 300 per cent more mestranol in brain and adrenal tissue than oestradiol. The study also put forward the possibility that the steroid nucleus of mestranol was being degraded, and this would also account for its low urinary



Oestradiol - 17β



Ethynyl oestradiol



Mestranol

Figure 5.1

Structural formulae for Oestradiol-17β, Ethynyl Oestradiol and Mestranol

excretion. The authors suggest that mestranol binds irreversably to tissue and that this phenomenon might account for its prolonged retention in the body. In mice, however, ethynylated oestrogens disappear more rapidly than oestradiol, i.e. they are bound more rapidly by the liver (Bolt and Remmer, 1972). The above studies indicate that ethynyl oestrogens accumulate in tissues to a two or three-fold greater extent than oestradiol (a natural oestrogen), and that ethynyl oestradiol but particularly mestranol may form different metabolites to oestradiol, which may account for their greater irreversible binding.

5.2.3 Conversion of Mestranol to Ethynyl Oestradiol

An important aspect of the metabolism of mestranol is its conversion to ethynyl oestradiol. Brown (1962) and Breuer, Knuppen, Gross and Mitternmayer (1964) demonstrated that methylated oestrogens are demethylated in vivo. Jensen, Jacobson, Flescher, Saha, Gupta, Smith, Colucci, Shiplacoff, Neumann, De Sombre and Jungblut (1966) showed that demethylation dramatically increases uterine oestrogen receptor binding, and that mestranol is demethylated to ethynyl oestradiol. They postulated that this step is necessary for mestranol to become hormonally active. A similar mechanism may operate in brain tissue, since brain and uterine oestrogen cytosol receptors are similar. Other studies by Korenman (1969) supported this theory by demonstrating that ethynyl oestradiol was highly competitive with oestradiol for binding with the oestrogen receptor whereas mestranol was not as competitive. Studies using rabbits and mice showed that mestranol could compete with and effectively inhibit oestradiol-17β in binding to uterine cytosol receptors: the concentration of mestranol used was five times that of oestradiol-17β (Hahn, McGuire, Greenslade and Turner, 1971). These studies suggest that mestranol has some "oestrogenic character", but more important is the suggestion that demethylation enhances oestrogenicity. Kappus, Bolt and Remmer (1973) studied the problem of demethylation

further. They demonstrated that the progestogens ethynodiol diacetate, norethynodrel and progesterone also inhibited the demethylation of mestranol to ethynyl oestradiol, but added that the amount of progestogens in oral contraceptives is not high enough to affect demethylation *in vivo*.

5.2.4 Excretion

The lipophilic properties of mestranol lead to an excretion pattern different from that of ethynyl oestradiol. Twenty-four hours after administration of radiolabelled mestranol to rats, 19% of the radioactivity was retained in fatty tissue and then released gradually until the tenth day after administration. However, the amount of radioactivity recovered after intravenous administration of mestranol or ethynyl oestradiol was 57% in ten days, whereas for oestradiol the recovery amounted to 75% within three days. No further significant amounts of radioactivity from mestranol or ethynyl oestradiol were excreted after ten days (Bolt and Remmer, 1972a,b). However, the method of administration of these hormones, whether by an intravenous, intraperitoneal or subcutaneous route, leads to different metabolic and excretory rates, and there is still a paucity of such information (Bolt and Remmer, 1972b).

5.2.5 Anti-fertility properties of Ethynyl Oestrogens

The studies of Goldzieher and his co-workers (Gual, Becerra, Rice-Wray and Goldzieher, 1967) were the first which specifically investigated the anti-ovulatory potency of ethynyl oestrogens in primates and showed quite conclusively their high suppression of pituitary activity, such as gonadotrophin inhibition, relative to other oestrogenic effects. It is interesting to note, then, that anti-ovulatory assays in laboratory rodents failed to demonstrate, to such a large extent, the anti-ovulatory potency of these steroids observed in primates (Helton and Goldzieher, 1977). The anti-fertility activity of various steroids which were administered orally or subcutaneously, at different times during the oestrous cycle, using different doses, and their probable mode of action, have been reported for

different animal species (e.g. see Chang and Yanagimachi, 1965).

McCann and Taleisnik (1961) suggested that oestrogens could inhibit the release of both follicle stimulating hormone and luteinizing hormone from the pituitary, thereby inhibiting ovulation. Watnick, Gibson, Vinegra, and Tolksdorf (1964) found that ethynyl oestradiol inhibited pituitary gonadotrophin release but not ova transport or nidation. Treatment with this oestrogen also caused foetal resorption. Evidence that other factors besides inhibition of ovulation are involved comes from the studies by Kincl and Dorfman (1964, 1965). They established that the doses of oestradiol-17β and mestranol needed to produce anti-fertility in the rat or parabiotic rat were about one hundred times less than those needed for an anti-ovulatory effect in the rabbit. On the other hand, compounds having progestational activity, such as progesterone, 19-norprogesterone and norethindrone were considerably more active in the anti-ovulation test in the rabbit and only very weakly active as anti-fertility agents in the intact and parabiotic rat. Although such inter-specific comparisons are not strictly justified, this suggests that other factors besides inhibition of ovulation are involved. Using silastic tube implants of oestradiol-17β and ethynyl oestradiol, Casas and Chang (1970) demonstrated that the anti-fertility properties of these oestrogens may be due to a disturbance in the activity of the existing corpora lutea. Their results suggest that both oestradiol and ethynyl oestradiol could maintain the life span of the corpus luteum either by a direct effect or through the hypothalamo-pituitary axis, thereby causing a derangement not only of the ovulatory mechanism, but also of fertilisation, ova transport and implantation. They conclude, however, that the anti-fertility property of these oestrogens, as revealed in the study, was as a result of a disturbance in the mechanism of implantation.

Further studies by the Goldzieher group showed that in humans the pituitary-suppressing properties of ethynyl oestrogens was due to a

synergism between these oestrogens and the 19-norprogestogens; so that quantities of these steroids which by themselves were insufficient for the complete inhibition of ovulation, were highly effective when administered together (Goldzieher, de la Pena, Chenault and Cervantes, 1975). The effects of an oestrogen-progestogen formulation on hypothalamic and pituitary function in rats have been described by Minaguchi and Meites (1967). They used a norethynodrel (98.5%) - mestranol (1.5%) combination, which is widely used as an anti-fertility agent, and may be regarded as a prototype of other steroid combinations used for this purpose. Both norethynodrel and mestranol have been shown to inhibit ovulation in rabbits and the former also inhibited ovulation in rats (Kincl and Dorfman, 1964). Minaguchi and Meites found that this steroid formulation reduced pituitary luteinizing hormone and follicle stimulating hormone, but increased prolactin concentration. Furthermore, this treatment also stimulated uterine growth and mammary development in intact animals, but had no effect on the ovaries or mammary glands of hypophysectomized rats. These effects are thought to reflect the mode of action of norethynodrel-mestranol formulations on the pituitary. A major difference in the findings between the human and rat studies is the ability of oestrogen-progestogen combinations to extend luteal life in rats, presumably by the enhanced release of prolactin, which is luteotrophic in rats but not in women (Schally, Carter, Saito, Arimura and Bowers, 1968).

5.3 Experiment 12 : The effects of ethynyl oestrogens on food intake, water intake and body weight in the female rat.

An understanding of the effect of ethynyl oestrogens on behavioural changes could be of value for several reasons. This work may provide a valuable approach to the study of the effects of oestrogens on neural processes regulating feeding, drinking, sexual behaviour and body weight, since the pharmaco-kinetic properties (rates and mechanisms of uptake, metabolism, excretion etc. ...) of these oestrogens vary considerably.

Secondly, it should reveal new information about the behavioural effects of ethynyl oestrogens in the female rat. And thirdly, since there has been a great deal of emphasis recently on the possible involvement of contraceptive steroids in changes in human sexuality and sexual behaviour (Cullberg, 1974), this work could be of some practical importance. The effects of ethynyl oestrogens on sexual behaviour in the ovariectomized female rat are considered in Experiment 13 and Experiment 14.

This experiment investigates the effects of oestradiol-17β, and the ethynyl oestrogens, mestranol and ethynyl oestradiol on food intake, water intake and body weight in the ovariectomized female rat.

5.3.1 Method

Experimental animals

Twenty experimentally and sexually naive female Wistar rats, about 120 days of age, were used. Two of these rats served as spare animals. Ten days before they were ovariectomized, the rats were housed singly in plastic cages, which had been previously modified for the measurement of food intake as described in Chapter 2.

Body weight, food and water intake were measured to the nearest 1.0 g, 0.1 g and 0.1 ml respectively, on four consecutive days prior to ovariectomy, irrespective of the stage of the oestrous cycle. The rats were ovariectomized under ether anaesthesia during the course of one day. They were then returned to their respective cages and body weight, food and water consumption were monitored post-operatively in order to determine when nutrient intake and body weight had returned to, more or less, pre-operative levels. Because the dorsal wound of one animal did not heal satisfactorily and became inflamed, the animal was killed and substituted with one of the spare animals chosen at random. So the final number of animals eventually used in the experiment was 18, and, eight days after ovariectomy they had a mean post-operative body weight of 270 $\pm$ 13 g.

Hormone injections

Equimolar solutions of oestradiol-17β (mol. wt. = 272.4); ethynyl oestradiol (mol. wt. = 296.4) and mestranol (mol. wt. = 310.4; Sigma Chemical Co. - Poole) were prepared using a dose of 5 μg/0.1 ml of arachis oil/rat of oestradiol as a reference standard. The dose of ethynyl oestradiol used was 5.44 μg/0.1 ml oil/rat and that of mestranol, 5.69 μg/0.1 ml oil/rat. Using equimolar solutions of the hormones ensured that the doses of all the hormones were the same for all the animals. The hormones were kept in blackened bottles and maintained at room temperature to avoid decomposition. Fresh solutions of the hormones were prepared before each treatment period.

Experimental schedule

Rather than ramdomizing the treatment order for the eighteen rats, the order was counterbalanced across the animals so that there were three replications of the six possible orders. There were three "treatment periods" separated by ten "treatment-free" or "recovery" days, which allowed for body weight, food and water intake to return to near normal levels. Each treatment period consisted of four days during which the animals were given hormone injections. The animals were injected with one of the three hormones from Monday to Thursday of each treatment week. Food and water intake were measured from Monday to Friday of each week: the food hoppers and water bottles were filled on Sunday and 24 hours later the remainder, together with any spilt food was weighed, this procedure was repeated for the other week-days. The animals were weighed daily from Monday to Friday and all the measurements were made between 09.00 hr and 10.00 hr, 2 hours before lights-out.

On four days during the recovery period (from Tuesday to Friday) body weight, food and water intake were measured as for the treatment period. Thus records were kept for both treatment and recovery periods; the overall duration of the experiment was six weeks. A time schedule for

the experiment is presented in Table 5.1. The main features of the experimental design may be summarised as follows:

1. Each animal received each hormone treatment once, thus acting as its own control. The design, therefore allowed for economy in the number of animals used.

2. Each treatment period started with each rat at or around the same body weight. The rats were not obese and therefore this weight was comparable to normal body weight (pre-operative weight).

3. Food intake and water consumption were also comparable to pre-operative (or normal) levels.

4. The recovery to approximately normal levels of the above measures was achieved by allowing an extended treatment-free period (10 days) between treatments.

5.3.2 Results

Pre-experimental measures

Figure 5.2 shows food intake, water intake and body weight before and after ovariectomy. It can be seen that by Day 6 all the measures had returned to approximately pre-operative levels.

The mean food intake for the four days preceding ovariectomy (measured irrespective of the stage of the oestrous cycle) was 17.2 $\pm$ 0.3 g, and by Day 8 post-operatively it was 17.6 $\pm$ 0.6 g ($\pm$ standard error). The depression in food intake immediately after ovariectomy was probably due to post-surgical trauma. The mean pre-operative water consumption was 28.8 $\pm$ 2 ml, and by Day 8 post-operatively mean water intake was 29.3 $\pm$ 2 ml. Mean body weight before the start of the experiment was 270 $\pm$ 3 g. This compared well with the pre-operative level (on Day -1) of 267 $\pm$ 3 g.

So at the start of the experiment body weight, food and water intake were comparable to normal or pre-ovariectomy levels. Day 1 of the experiment proper corresponds to Day 9 after ovariectomy (see Table 5.1).

Table 5.1

Experiment 12. Time Schedule for the Experiment

Pre-Experimental Period:

Day	-4 Tu	-3 We	-2 Th	-1 Fr	0 Sa	1 Su	2 Mo	3 Tu	4 We	5 Th	6 Fr	7 Sa	8 Su
Ovariectomy					x								
Body weight	x	x	x	x		x	x	x	x	x	x	x	x
Food intake	x	x	x	x		x	x	x	x	x	x	x	x
Water intake	x	x	x	x		x	x	x	x	x	x	x	x

Experimental Period:

Day	1 Mo	2 Tu	3 We	4 Th	5 Fr	6 Sa	7 Su	8 Mo	9 Tu	10 We	11 Th	12 Fr	13 Sa	14 Su	15 Mo	16 Tu	17 We	18 Th	19 Fr	20 Sa	21 Su	22 Mo	23 Tu	24 We	25 Th	26 Fr	27 Sa	28 Su	29 Mo	30 Tu	31 We	32 Th	33 Fr	34 Sa	35 Su	36 Mo	37 Tu	38 We	39 Th	40 Fr	41 Sa	42 Su
Oestrogen	x	x	x	x											x	x	x	x											x	x	x	x											
Recovery					x	x	x	x	x	x	x	x	x	x					x	x	x	x	x	x	x	x	x	x					x	x	x	x	x	x	x	x	x	x
Body weight	x	x	x	x	x			x	x	x	x	x			x	x	x	x	x			x	x	x	x	x		x	x	x	x	x			x	x	x	x	x		
Food intake	x	x	x	x	x				x	x	x	x			x	x	x	x	x				x	x	x	x		x	x	x	x	x				x	x	x	x		
Water intake	x	x	x	x	x				x	x	x	x			x	x	x	x	x				x	x	x	x		x	x	x	x	x				x	x	x	x		

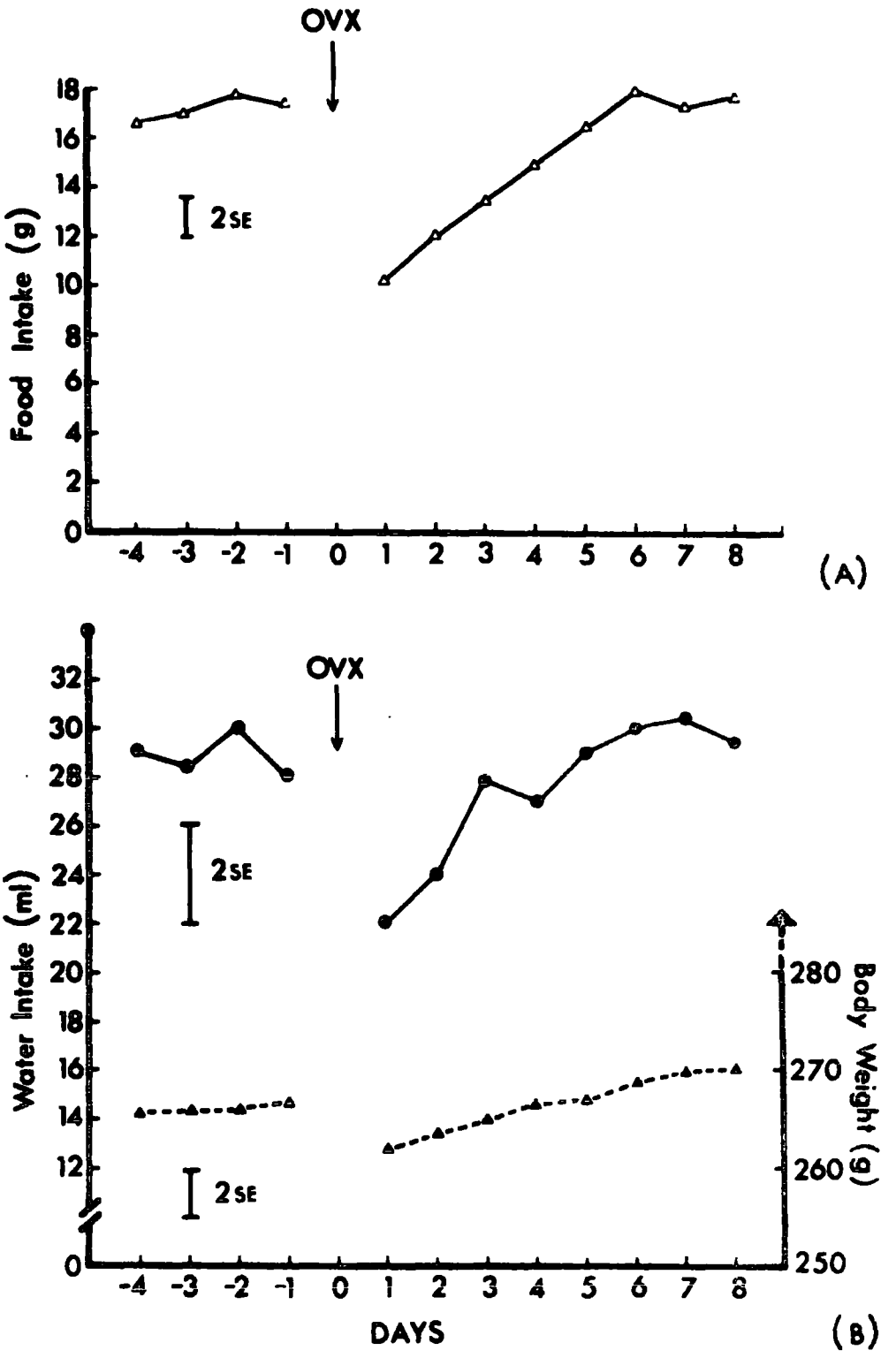


Figure 5.2

Experiment 12. (A) Mean food intake (in g).

(B) Mean water intake (in ml), and body weight (in g) of female rats before and after ovariectomy. Vertical bars represent two standard errors (2SE).

Experiment results

The results for the experimental and recovery periods were analysed using analysis of variance (0-between, two-within factors). The Least Significant Difference test was used for post hoc comparisons between means.

Food Intake

1. Experimental period:

Figure 5.3 shows the effect of oestrogen treatment on daily food intake. Table 5.2 is a summary table of the analysis of variance. As expected, oestrogen treatment depressed food intake (when compared with pre-experimental levels). Although oestradiol caused a drop in food intake, mestranol caused a greater drop and ethynyl oestradiol caused the greatest depression in food intake. The overall difference in food intake was significant ($F = 54.24$; with df. = 2, 34; $p < 0.001$), and both individual comparisons were also significant (oestradiol vs mestranol: $F = 15.17$; with df. = 1, 34; $p < 0.001$; mestranol vs ethynyl oestradiol: $F = 41.30$; with df. = 1, 34; $p < 0.001$). The difference in food intake between the first day before oestrogen treatment (Day 1: food intake was measured for the preceding 24 hours before injections were given), the fourth day of oestrogen treatment (Day 5) and the seventh day of the recovery period (Day 12: for details see Table 5.1) is shown in Figure 5.4. Food intake is equal and high on the first and twelfth days, and low on the fifth day. The difference between oestrogen treatments is noticeable (the values show the mean $\pm$ standard error).

2. Recovery period:

Figure 5.3 shows the mean daily food intake on four days during the recovery period (Days 9 to 12). Table 5.2 shows that food intake increased when oestrogen treatment was withdrawn ($F = 13.29$; with df. = 3, 51; $p < 0.001$); and the overall difference in food intake was still significant

Table 5.2

Experiment 12. Effect of Ethynyl Oestrogens on Food Intake in the Rat: Experimental Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	221.78			
Treatment	2	862.52	431.26	54.24	< 0.001
Error	34	270.28	7.95		
Days	3	640.13	213.38	59.73	< 0.001
Error	51	182.18	3.57		
Days x Treatment	6	19.71	3.28	< 1	ns
Error	102	359.49	3.52		
Within cell	198	2334.30			

Table 5.3

Experiment 12. Effect of Ethynyl Oestrogens on Food Intake in the Rat: Recovery Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	155.58			
Treatment	2	143.66	71.83	12.25	< 0.001
Error	34	199.33	5.86		
Days	3	60.67	20.22	13.29	< 0.001
Error	51	77.62	1.52		
Days x Treatment	6	25.14	4.19	2.67	< 0.05
Error	102	160.16	1.57		
Within cell	198	666.58			

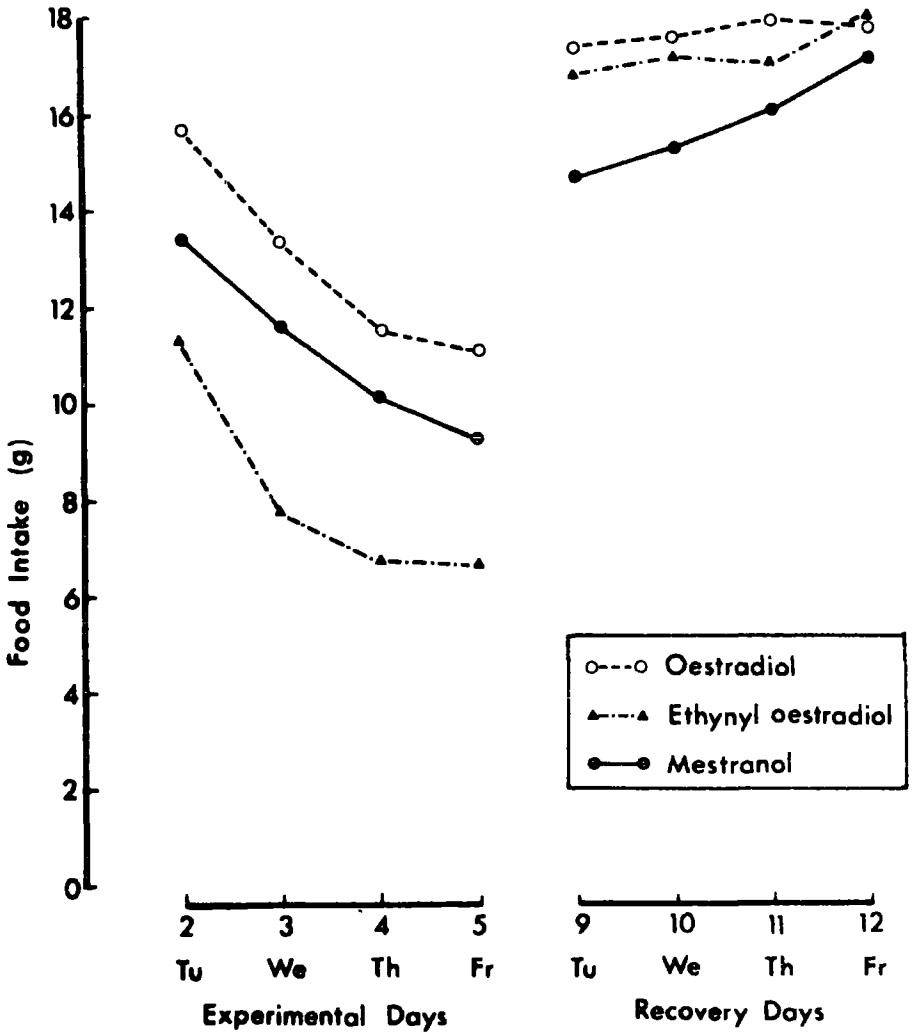


Figure 5.3

Experiment 12. Food intake from Day 2 to Day 5 of ovariectomized rats injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, and after injections were stopped (Day 9 to Day 12). The days in the figure correspond to the time schedule of the experiment.

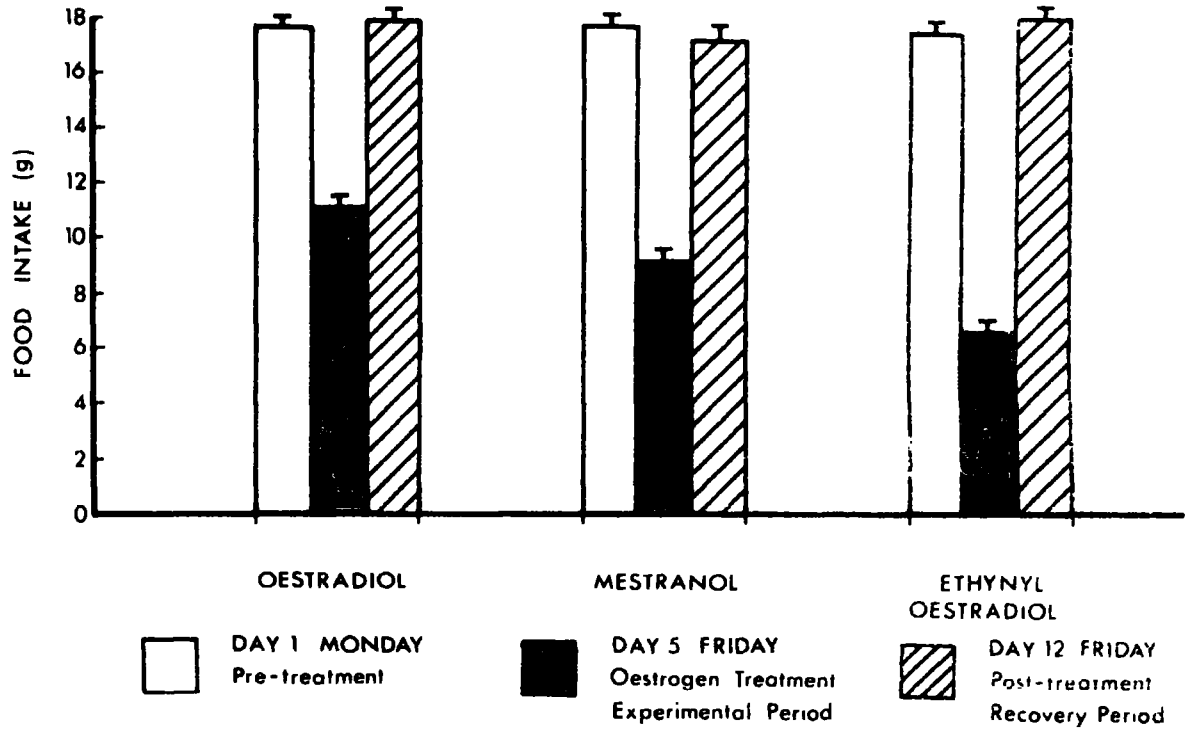


Figure 5.4

Experiment 12. Comparison of food intake of ovariectomized rats, injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, on separate days during the experiment. The values show the mean ± standard error. The days shown in the figure correspond to the time schedule of the experiment.

($F = 12.25$; with df. = 2, 34; $p < 0.001$). This was mainly due to the gradual increase in food intake of the mestranol treated rats which manifested itself in a significant days x treatment interaction ($F = 2.67$; with df. = 6, 102; $p < 0.05$). Post-hoc analysis using comparisons of weighted means revealed that 90% of the variance was attributable to the linear trend in food intake shown by rats treated with mestranol ($MS_{comp.} = 22.58$; $F = 14.38$; with df. = 1, 102; $p < 0.001$). The residual variance estimate was not significant ($MS_{resid.} = 0.51$; $F < 1$; with df. = 5, 102; $p =$ ns). However, by Day 12, food intake approached normal levels (Figure 5.4).

Water Intake

1. Experimental period:

A summary of the analysis of variance is presented in Table 5.4. Figure 5.5 shows that the depression of water consumption by oestrogen administration was similar to the depression in food intake, and the overall difference was significant ($F = 26.36$; with df. = 2, 34; $p < 0.01$). Individual comparisons, however, showed that there was no difference in water consumption between oestradiol and mestranol treated rats ($F = 4.08$; with df. = 1, 34; $0.06 > p > 0.05$). However, comparisons between these two treatment groups (between means on the fourth day of treatment), using the appropriate error term, revealed that there was a reliable difference in water intake ($F = 7.63$; with df. = 1, 102; $p < 0.01$) on that day. Rats treated with ethynyl oestradiol drank significantly less than mestranol treated rats ($F = 25.26$; with df. = 1, 34; $p < 0.001$). Figure 5.6 shows the difference between the pre-experimental, experimental and post-experimental levels in water intake.

2. Recovery period:

Figure 5.5 shows water intake on four days during the recovery period, and the analysis of variance is summarised in Table 5.5. There was no significant overall increase in water consumption over days, but it is

Table 5.4

Experiment 12. Effect of Ethynyl Oestrogens on Water Intake in the Rat: Experimental Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	4057.11			
Treatment	2	1441.14	720.57	26.36	< 0.01
Error	34	929.59	27.34		
Days	3	162.66	54.22	4.02	< 0.05
Error	51	687.74	13.49		
Days x Treatment	6	63.91	10.65	1.06	ns
Error	102	1026.63	10.07		
Within cell	198	4311.67			

Table 5.5

Experiment 12. Effect of Ethynyl Oestrogens on Water Intake in the Rat: Recovery Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	3555.94			
Treatment	2	566.36	283.18	7.15	< 0.01
Error	34	1345.78	39.58		
Days	3	68.76	22.92	1.99	ns
Error	51	585.94	11.49		
Days x Treatment	6	162.78	27.13	2.33	< 0.05
Error	102	1186.58	11.63		
Within cell	198	3916.20			

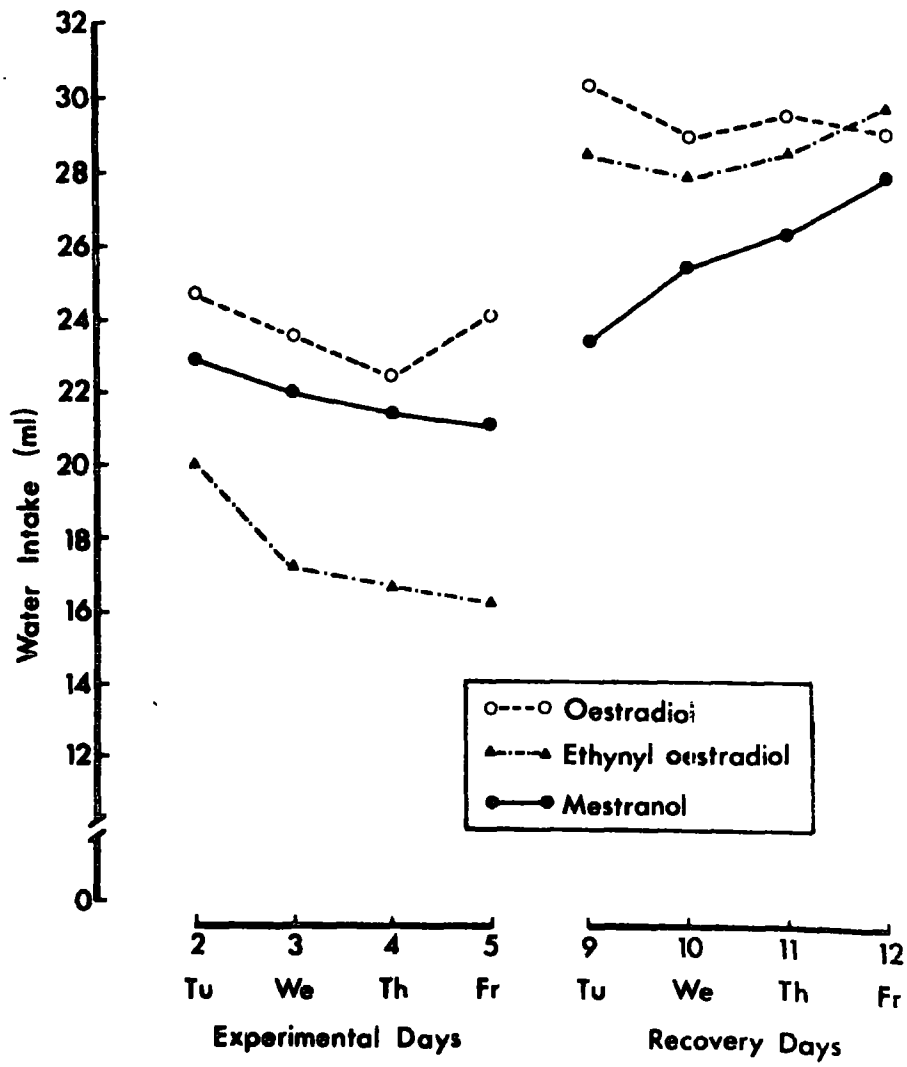


Figure 5.5

Experiment 12. Water intake (from Day 2 to Day 5) of ovariectomized rats injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, and after injections were stopped (Day 9 to Day 12). The days in the figure correspond to the time schedule of the experiment.

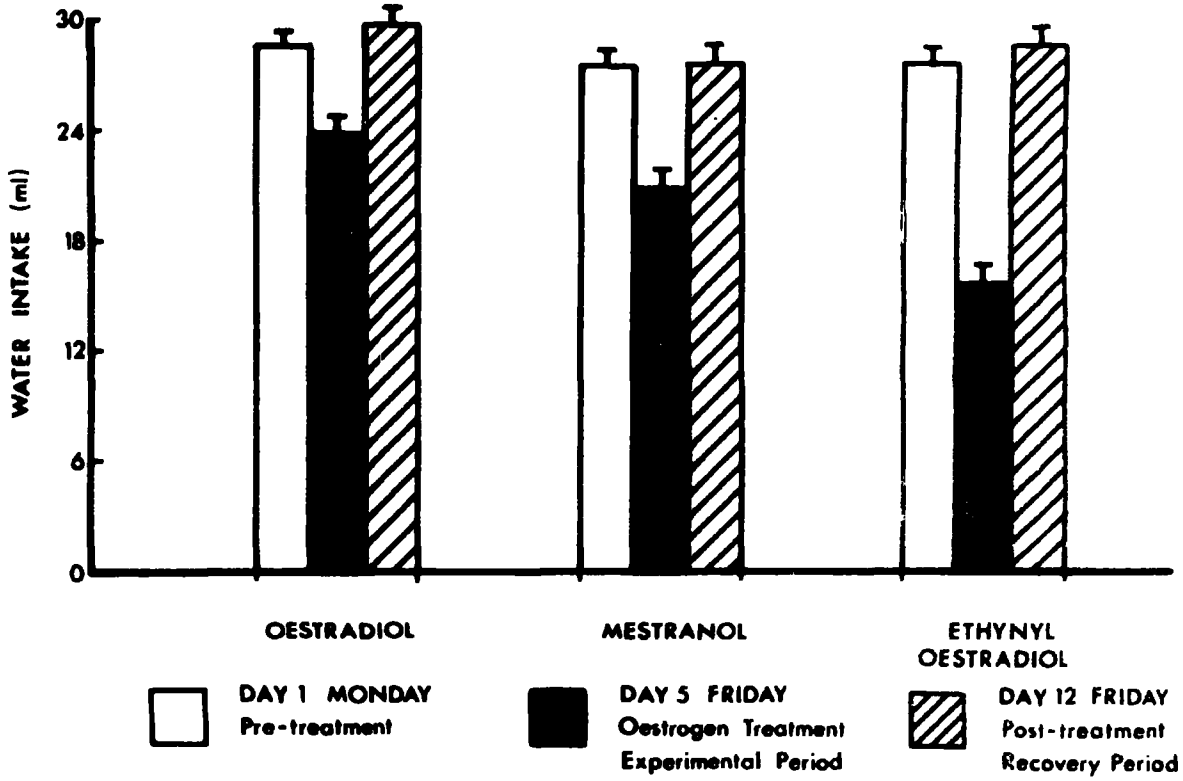


Figure 5.6

Experiment 12. Comparison of water intake of ovariectomized rats, injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, on separate days during the experiment. The values show the mean ± standard error. The days shown in the figure correspond to the time schedule of the experiment.

clear that mestranol treated rats showed a slower recovery in drinking than rats treated with either oestradiol or ethynyl oestradiol ($F = 2.33$; with df. = 6, 102; $p < 0.05$). Post-hoc analysis of this interaction attributed 81% of the variance to the linear trend in water intake shown by females treated with mestranol ($MS_{comp.} = 132.1$; $F = 10.84$; with df. = 1, 102; $p < 0.01$). The residual variance estimate was not significant ($MS_{resid.} = 6.14$; $F < 1$; with df. = 5, 102; $p =$ ns). Figure 5.6 shows that by Day 12 water intake was similar to normal or pre-experimental levels.

Body Weight

1. Experimental period:

The results were expressed as Per Cent Change in Body Weight and analysed with analysis of variance. Table 5.6 presents a summary of the analysis. Figure 5.7 shows that there was a significant change in weight with oestrogen treatment ($F = 20.23$; with df. = 2, 34; $p < 0.001$). Rats treated with mestranol and oestradiol did not show any difference in percentage weight change ($F = 0.67$; with df. = 1, 34; $p =$ ns), although by Day 5 a difference appeared ($F = 5.23$; with df. = 1, 102; $p < 0.05$). Rats treated with ethynyl oestradiol significantly lost more weight than mestranol treated rats ($F = 25.26$; with df. = 1, 34; $p < 0.001$). These rats also showed a greater weight loss over days than either oestradiol or mestranol treated rats ($F = 10.80$; with df. = 6, 102; $p < 0.001$). Body weight on pre-experimental, experimental and recovery days (expressed in grams) is shown in Figure 5.8.

2. Recovery period:

The prolonged recovery of body weight by rats treated with mestranol can be seen in Figure 5.7, and a summary of the analysis of variance in Table 5.7. Four days after the withdrawal of oestrogen treatment, rats which had been treated with either oestradiol or ethynyl oestradiol had

Table 5.6

Experiment 12. Effect of Ethynyl Oestrogens on Change in Body Weight in the Rat: Experimental Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	250.86			
Treatment	2	396.85	198.42	20.23	< 0.001
Error	34	333.55	9.81		
Days	3	504.97	168.32	203.45	< 0.001
Error	51	42.20	0.83		
Days x Treatment	6	52.05	8.68	10.80	< 0.001
Error	102	81.93	0.80		
Within cell	198	1411.55			

Table 5.7

Experiment 12. Effect of Ethynyl Oestrogens on Per Cent Change in Body Weight in the Rat: Recovery Condition. Summary of Analysis of Variance

Source	df	SS	MS	F-ratio	P
Subjects	17	414.31			
Treatment	2	257.74	128.87	4.86	< 0.05
Error	34	901.97	26.53		
Days	3	224.01	74.67	84.05	< 0.001
Error	51	45.31	0.89		
Days x Treatment	6	10.81	1.80	1.88	ns
Error	102	97.89	0.96		
Within cell	198				

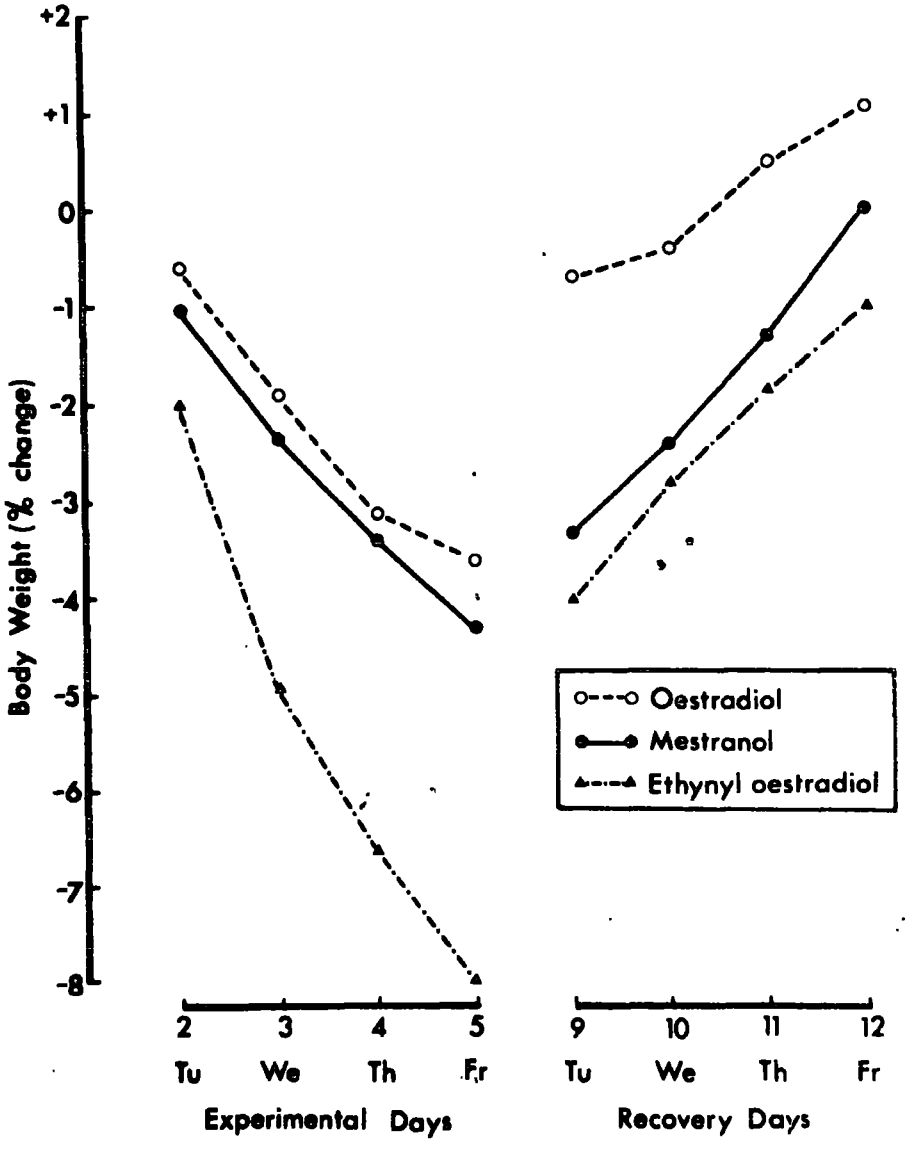


Figure 5.7

Experiment 12. Per cent change in body weight (from Day 2 to Day 5) of ovariectomized rats injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, and after injections were stopped (Day 9 to Day 12). The days in the figure correspond to the time schedule of the experiment.

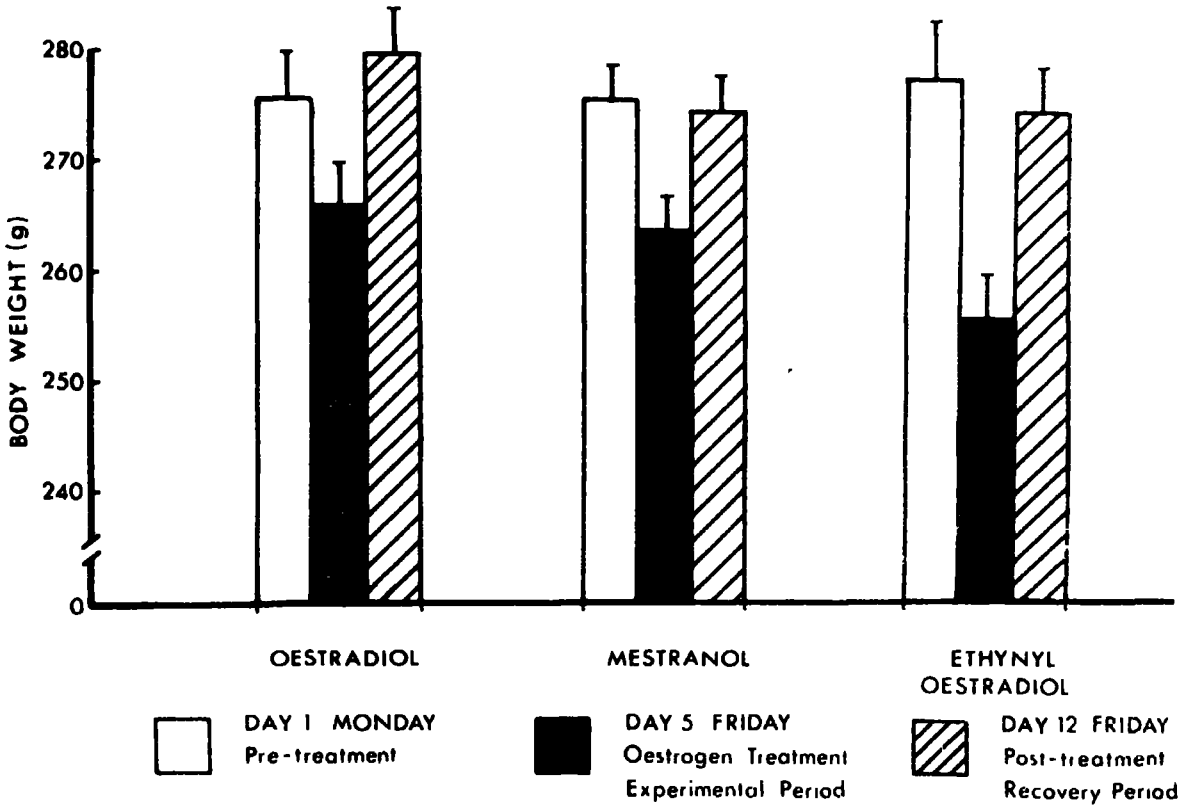


Figure 5.8

Experiment 12. Comparison of body weights of ovariectomized rats, injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol, on separate days during the experiment. The values show the mean (in g) ± standard error. The days shown in the figure correspond to the time schedule of the experiment.

recovered 3% and 4% of their body weight respectively, whereas mestranol treated rats had recovered only 1% of their body weight. The rate in weight gain was similar, and the increase in weight was significant after this period ($F = 84.05$; with $df. = 3, 51$; $p < 0.001$). Although an overall difference in oestrogen treatment was still evident during the recovery period ($F = 4.86$; with $df. = 2, 34$; $p < 0.05$), it was not great and the body weight of all the animals approximated normal levels by Day 12 (Figure 5.8).

5.3.3 Discussion

The anorexic effect of oestradiol-17β, and the related decrease in water intake and body weight are well documented (Tarttelin and Gorski, 1971; Drewett, 1973a,b; Redick, Nussbaum and Mook, 1973). The experiment reported here shows that this effect is also produced by the two synthetic oestrogens used in oral contraceptive formulations, ethynyl oestradiol and mestranol.

Mestranol and oestradiol-17β had similar effects, with one important difference: mestranol treated rats showed a slower recovery to "control" levels of food intake, water intake and body weight. Ethynyl oestradiol treatment produced the greatest depression of all three measures which were recorded during the experiment. Clearly, such effects must reflect differences in the pharmaco-kinetic properties of these oestrogens. An explanation which would account for the depression in food intake would necessarily explain the decrements in water consumption and body weight since changes in the latter were quite likely results of changes in the former. The rats ate less food and therefore drank less and also lost weight. Let us now look at one possible explanation which would account for the observed differences.

Although the metabolism of oestradiol to oestrone, leading to catecholoestrogen formation, is one step in oestradiol metabolism, it is

by no means unique. Competitive hydroxylation also occurs at the carbon-16 position, giving rise to oestriol formation, which is highly utero- and vaginotrophic (Fishman, Bradlow and Gallagher, 1960). On the other hand, hydroxylation at the carbon-2 position, and therefore catecholoestrogen formation, is the major metabolic process for ethynyl oestradiol. In the rat this process is in fact 50% higher than for oestradiol (Bolt et al., 1973). Because of the highly labile nature of the catechol-oestrogens, direct experimentation with these compounds is difficult. However, preliminary studies showed that catecholoestrogens significantly reduced food intake in rats (Garattini and Fishman, 1975; cited in Fishman, 1976). Therefore, one possibility is that the greater suppression of food intake by ethynyl oestradiol is in some way influenced by the increased metabolism of ethynyl oestradiol to catecholoestrogen. The mechanisms by which this could affect food intake have yet to be determined.

The differences in food intake observed in rats treated with mestranol are probably due to the action of its major metabolite, ethynyl oestradiol, since mestranol itself is of very low "oestrogenicity". Further, since mestranol is capable of binding to oestrogen receptors in a form with low oestrogenic activity (Hahn et al., 1971), the number of receptors available for the uptake of its active metabolite, ethynyl oestradiol, would thus be reduced. The "activation" of such receptors would depend, to a large extent, on (1) the high lipophilicity of mestranol, leading to its greater storage by body fat, brain and adrenal tissue, and consequently its slower release rate (Bolt and Remmer, 1972a,b) and (2) its rate of metabolism to ethynyl oestradiol by the liver.

Such an explanation would also account for the slower recovery to normal levels of feeding, drinking and body weight in mestranol treated rats, associated with the different pattern of excretion of mestranol from either ethynyl oestradiol or oestradiol-17β. Although these two oestrogens

have different excretory patterns, the similar recovery to normal levels of feeding, and, to a certain extent, drinking and body weight suggests that a factor or factors common to both oestradiol and ethynyl oestradiol may influence the mechanisms controlling feeding behaviour in the female rat treated with these steroids.

5.4 Experiment 13 : The effects of ethynyl oestrogens on sexual behaviour in the female rat

As yet, there has been no systematic investigation reporting the effects of ethynyl oestrogens on the sexual behaviour of the female rat. Watnick et al. (1964) make a cursory reference to the sexual receptivity of rats treated with ethynyl oestradiol and stated that this oestrogen increased receptivity in some of the females. No detail of measures of sexual receptivity was reported. Furthermore, this study used intact females, and the possibility of ovarian influences on the ensuing levels of performance of sexual behaviour could not be excluded.

If the absolute effects of the hormones are to be investigated, ovariectomized rats must be used in order to exclude interference or interactive effects of ovarian hormones with the hormones used in the study.

5.4.1 Method

Experimental animals

Twenty-four female Wistar rats, about 150 days of age, were used as the experimental animals. All the females were ovariectomized at least three weeks before the start of any experimental procedures, and were given sexual experience by allowing them ten mounts by active males on three separate occasions in a testing arena. The females were randomly divided into three groups, consisting of eight rats in each group. Twenty sexually experienced Wistar males (proven copulators) were used in the tests for sexual behaviour.

General procedure

The males had already been thoroughly habituated to testing arenas; nonetheless they were given two additional half-hour habituation periods in the testing arena. At the end of this period, all the males copulated within 2 minutes of being placed in the arena and presented with "teaser" females. All the females received three half-hour habituation periods on three separate days.

The testing arena was 90 cm in diameter, and had a circular blackened wall 30 cm high. Experimental testing was carried out during the dark period of the animals' light-dark cycle in a room lit by a 60 watt red light-bulb.

The female was placed with a sexually active male until the male performed 10 mounts with pelvic thrusting. The term "lordosis" was used to define pronounced or deep concave arching of the back by a female in response to mounting by a male. During testing, the number of lordosis responses by a female to ten mounts with pelvic thrusting by a male was recorded. In addition to this measure, a general measure of proceptive (or soliciting) behaviour (see Beach, 1976) was also recorded. "Hopping and darting" or hop-dart is a rapid staccato-like alternation between running and stopping, which is usually characterised as a major component of soliciting behaviour, and which is often exhibited by highly receptive females. Clawing at or nipping the male's face were also considered as proceptive behaviours and usually preceded hopping and darting (Hemmingsen, 1933; McClintock, 1974); but the most reliable were found to be the dart-hop movements. A measure of proceptivity was thus obtained by counting the number of inter-mount intervals during which some or all of these behaviours were observed to occur.

Experimental schedule

Each group of females received each hormone treatment once, and the

treatment order for each group was such that no two groups received identical treatments at the same time (3 x 3 Latin square design). There were three experimental (or testing) periods separated by ten days during which no treatment was given. This period minimised any possible interaction between the oestrogens.

Each experimental period consisted of four days during which the animals received subcutaneous injections of either oestradiol-17β at a dose of 5 μg/0.1 ml oil/rat/day, or 5.44 μg ethynyl oestradiol in 0.1 ml of oil per rat per day, or 5.69 μg mestranol in 0.1 ml of oil per rat per day. These injections were administered 96 hr, 72 hr, 48 hr, and 24 hours before experimental testing. On the fifth day all the rats were injected with 500 μg of progesterone in 0.1 ml arachis oil per rat, 6 hours before testing began. This commenced at 15.00 hrs, three hours after the start of the dark cycle, and usually lasted for about 2½ hours. It is obvious that testing could not be carried out instantaneously, so the running order of the animals was determined by a sequence of random numbers. A new sequence was generated for each experimental testing session. Moreover, during experimental testing, the observer was not aware of which group of rats received which treatment.

5.4.2 Results and Discussion

Because of evident "ceiling" and "base" effects inherent in data of this kind, a Friedman two-way analysis of variance was employed. The Wilcoxon matched-pairs signed-ranks test was used for post hoc comparisons between groups.

The overall difference in Lordosis behaviour was significant ($n = 24$; Chi-square = 37.02; with df. = 2; $p < 0.001$). The difference in the number of lordosis responses to ten mounts by a male, between the oestradiol and ethynyl oestradiol treated females and the mestranol treated females is evident from Figure 5.9. Females treated with ethynyl oestradiol, however,

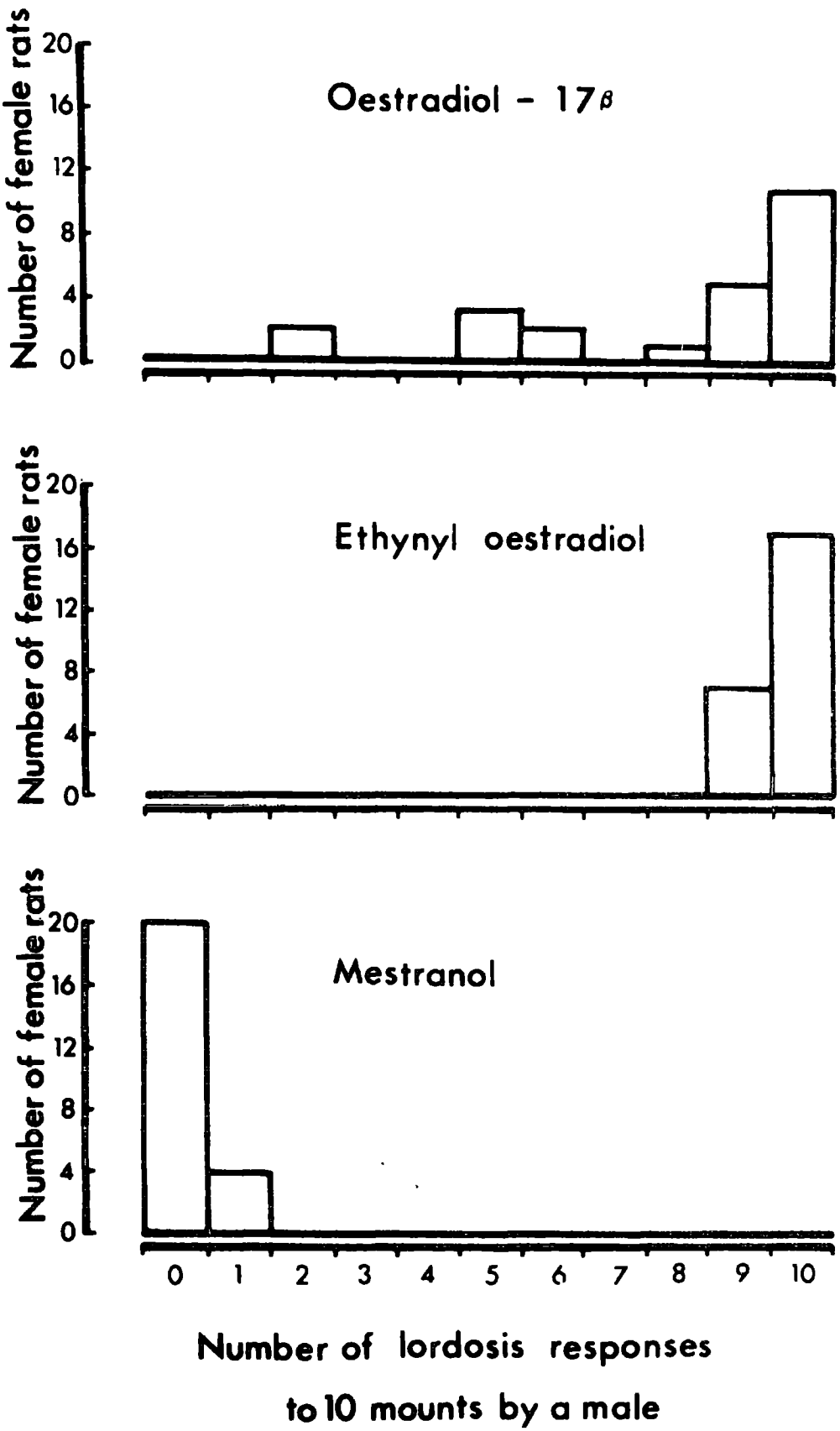


Figure 5.9

Experiment 13. The number of lordosis responses shown by ovariectomized rats, injected with equimolar doses of either oestradiol, or ethynyl oestradiol or mestranol for 4 days before, and 500 μg progesterone, 6 h before testing began.

showed a higher number of lordosis responses than females treated with oestradiol ($n = 24$; $Z = 2.551$; $p < 0.01$, two-tailed).

Proceptivity was also different between the groups ($n = 24$; Chi-square $= 36.02$; with df. $= 2$; $p < 0.001$); amd the rats treated with ethynyl oestradiol showed significantly more soliciting behaviour than females treated with oestradiol ($n = 24$; $Z = 2.229$; $p < 0.05$; two-tailed). Rats treated with mestranol showed no soliciting behaviour (Figure 5.10). However, these rats did not actively reject the males' attempts to mount. They all showed a noticeable stiffening of the tail, a characteristic also shown by sexually receptive females, but were remarkably passive during mounts with pelvic thrusting by the males (Figure 5.11). Some females sniffed or chewed the sawdust, and continued doing so during mounts. Others investigated the males' ano-genital region quite thoroughly, but did not show any soliciting behaviours. This passivity is uncharacteristic of dioestrous females, which show active rejection of the male (Pfaff and Lewis, 1974).

It is possible that the administration of exogenous progesterone may have inhibited the demethylation of mestranol, thereby rendering this oestrogen "hormonally inactive", as proposed by Jensen et al. (1966). This is unlikely for the following reasons. Firstly, Kappus, Bolt and Remmer (1973) suggest that this amount of progesterone is not sufficient to inhibit demethylation in vivo. Secondly, the progesterone administration was preceded by four days of oestrogen injections, which is a considerable period of time for the uptake of mestranol by neural tissue to take place; this generally occurs within a few hours after systemic injection (Kappus, Bolt and Remmer, 1972a,b). This possibility is, nonetheless, investigated in the next experiment.

5.5 Experiment 14 : A second experiment on the effects of ethynyl oestrogens on sexual behaviour in the female rat

Davidson, et al., (1968a) showed that daily injections of oestradiol-17β,

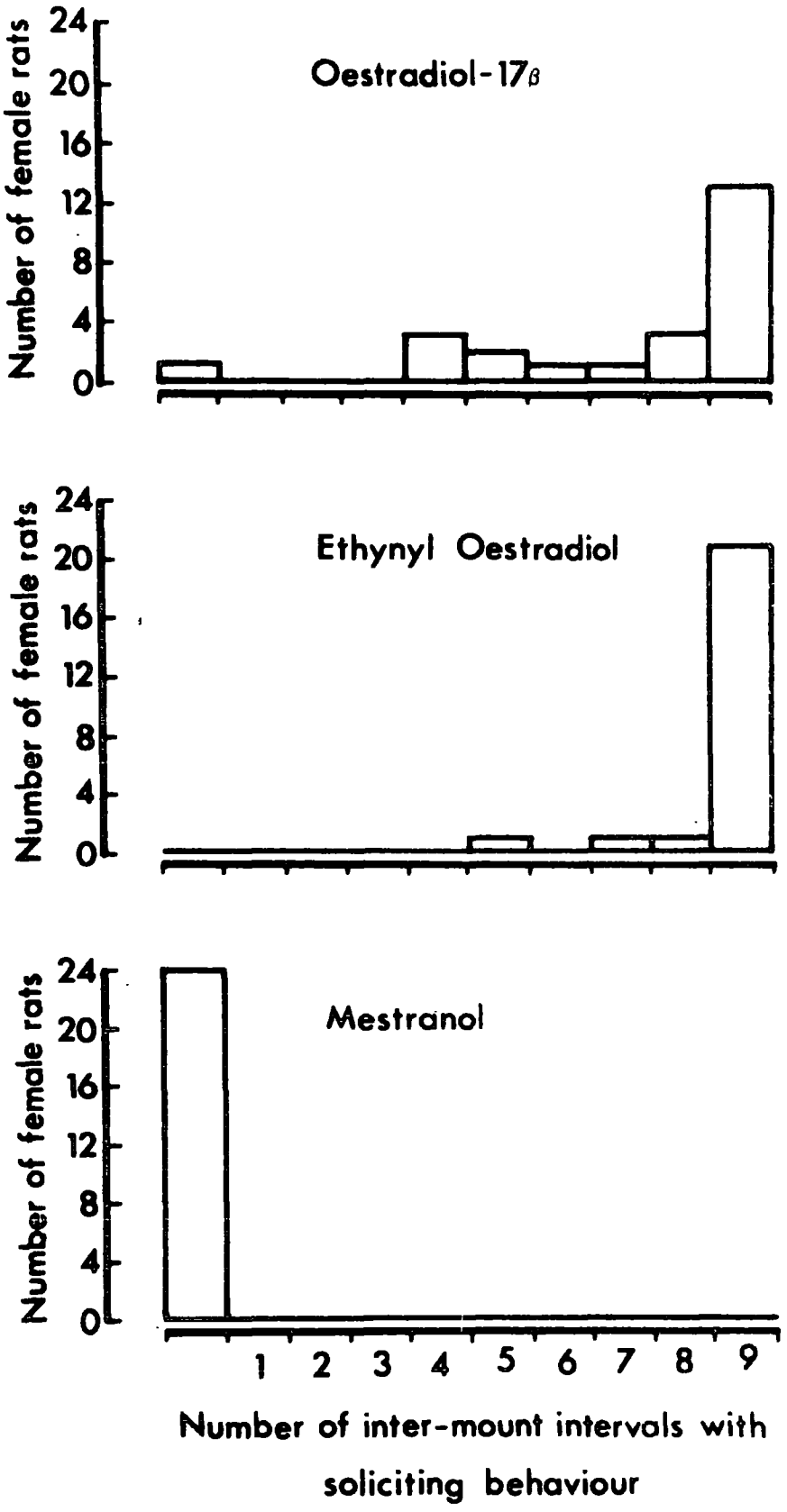
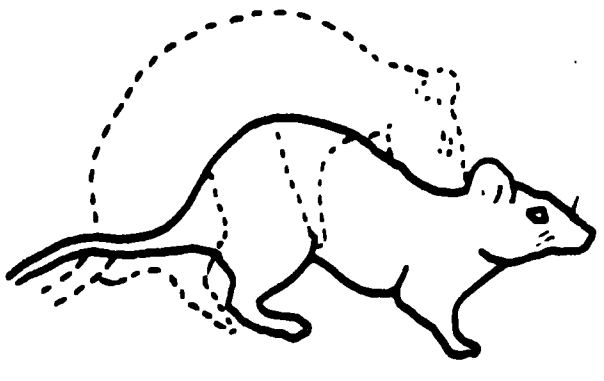
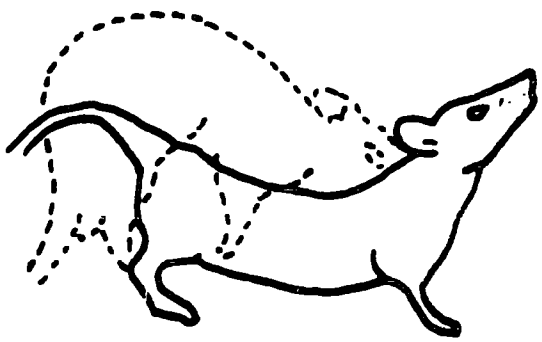


Figure 5.10

Experiment 13. The number of inter-mount intervals during which ovariectomized rats, injected with equimolar doses of either oestradiol or ethynyl oestradiol or mestranol (4 days before testing) and 500 μg progesterone (6 h before testing), showed soliciting behaviour.



(a)



(b)

Figure 5.11

Experiment 13. (a) Posture adopted by mestranol treated rats.

(b) Posture adopted by rats treated with either ethynyl oestradiol or oestradiol-17β.

using "physiological" doses induced high levels of sexual behaviour within five days of treatment. This response is not dependent on the production of progesterone by the adrenal glands, or any of the other adrenal steroids, since comparable levels of response to oestradiol were found in ovariectomized rats as well as ovariectomized-adrenalectomized rats (Davidson et al., 1968b; Pfaff, 1971). The following experiment investigated the effects of daily administration of equimolar doses of ethynyl oestrogens in the absence of exogenous progesterone. In addition, female rats were treated with oestradiol for comparative purposes.

5.5.1 Method

The animals were 42 female Wistar rats, 150 to 200 days of age. Eighteen of these rats were used in Experiment 13. The remaining 24 females were ovariectomized at least two weeks before the start of any experimental procedures.

All the rats were given sexual experience by allowing them ten mounts each by sexually active males, on three separate occasions, in a testing arena. Each rat was randomly allocated to one of three groups: one group received 5 µg of oestradiol in 0.1 ml arachis oil per rat per day. A second group received 5.44 µg of ethynyl oestradiol in 0.1 ml oil; and a third group received 5.69 µg of mestranol in 0.1 ml oil/rat/day. All injections were administered subcutaneously, at 09.00 h every morning. The difference in previous hormone treatment between these rats was compensated for by equating the previously treated rats over the three groups.

Experimental procedure

The room, testing arena and testing procedure were the same as those used in Experiment 13. In addition to measures of lordosis and proceptivity, a rejection measure was also obtained. This was done by counting the number of inter-mount intervals during which either kicking away the male, or fighting or "boxing" (as described by Pfaff and Lewis, 1974) were observed

to occur. These behaviours are also shown by dioestrous females in response to mounts or attempted mounts by a male.

The lordosis quotient was derived by applying the following formula:

$$\text{Lordosis quotient} = \frac{\text{Number of lordosis responses}}{\text{Number of mounts}} \times 100 \quad \ldots \text{Eq.(5.1)}$$

Similarly, for soliciting behaviour,

$$\text{Proceptivity quotient} = \frac{\text{Occurrence of soliciting behaviour}}{\text{Number of inter-mount intervals}} \times 100 \quad \ldots \text{Eq.(5.2)}$$

and, for rejection behaviour,

$$\text{Rejection quotient} = \frac{\text{Occurrence of rejection behaviour}}{\text{Number of inter-mount intervals}} \times 100 \quad \ldots \text{Eq. (5.3)}$$

The animals were tested according to a repeated counterbalanced sequence of numbers (a repetition of six possible permutations, i.e. 123, 132 etc. ...) and, except for one day (Day 6) all testing during the experiment was run "blind".

5.5.2 Results

The Lordosis, Proceptivity and Rejection quotients for each day were analysed using a Kruskal-Wallis one-way analysis of variance. Mann-Whitney U-tests were used in conducting post hoc analyses between groups.

Figure 5.12 shows lordosis quotients for all three oestrogen treated groups (expressed as medians per group per day). The scores on Day 1 were the same for all three groups, and were therefore excluded from the analysis. However, as Table 5.8 shows, there was an overall significant difference in the performance of lordosis between groups, from Day 3 to Day 9 inclusive. The difference in lordosis responses between ethynyl oestradiol and oestradiol treated females over mestranol treated females is obvious and large. (Two mestranol treated rats showed a very small number of lordosis responses on Day 8 and Day 9, which do not appear in Figure 5.12, since the results were expressed as medians.) Females treated with oestradiol and ethynyl oestradiol did not show a difference in lordosis behaviour up to and including Day 3; but ethynyl oestradiol treated females lordosed more

Table 5.8

Experiment 14. Kruskal-Wallis one-way analysis of variance: Lordosis responses (Scores for Day 1 were the same for all three groups, and were excluded from the analysis)

Day	Chi-Square
1	-
2	2.10
3	9.62 **
4	29.00 ***
5	31.55 ***
6	32.41 ***
7	31.78 ***
8	30.29 ***
9	31.50 ***

Two-tailed p: (**) < 0.01; (***) < 0.001

Table 5.9

Experiment 14. Mann-Whitney U-test summary table: Lordosis responses. Post hoc comparisons between oestradiol and ethynyl oestradiol groups. (Scores for Day 1 were the same for both groups, and were excluded from the analysis)

Day	U	Z
1	-	-
2	91.0	0.60
3	84.5	0.68
4	29.0	3.19 ***
5	25.0	3.38 ***
6	36.0	2.88 **
7	40.5	2.72 **
8	59.5	1.86
9	53.0	2.26 *

Two-tailed p: (*) < 0.05; (**) < 0.01; (***) < 0.001

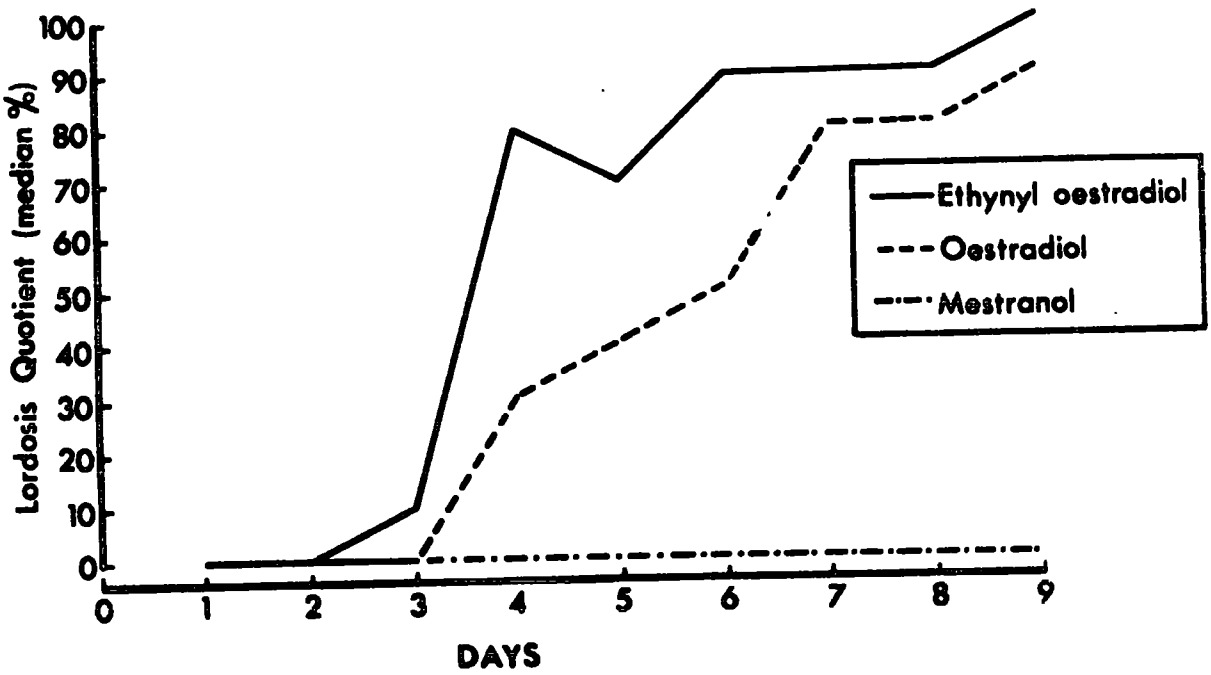


Figure 5.12

Experiment 14. Lordosis quotients of ovariectomized rats treated with equimolar doses of oestradiol, ethynyl oestradiol and mestranol daily for 9 days.

often in response to mounts by a male thereafter, with the exception of Day 8 ($Z = 1.86$; $p \leq 0.06$; Table 5.9). These results followed a similar trend to the one observed in Experiment 13.

Figure 5.13 shows the change in soliciting behaviour over days (expressed as median proceptivity quotient per group per day). There appeared a significant change after Day 3 of testing (Table 5.10). The mestranol treated females showed less soliciting behaviour than the two other oestrogen treated groups, and significantly less than the oestradiol group (Table 5.11). A difference in proceptivity between oestradiol and ethynyl oestradiol females did not appear until the fourth day of testing and lasted until the seventh day. Females from both groups showed a similar amount of soliciting behaviour on Day 8 and Day 9 (Table 5.11).

The rejection of a male by a female was frequent and similar for all three groups during the first two days of treatment (Figure 5.14). During the next seven days, however, there was an overall significant change in rejection behaviour (Table 5.12). Females treated with mestranol rejected the males' attempts to mount more often than oestradiol treated females; but females treated with ethynyl oestradiol rejected males on a fewer number of occasions than oestradiol treated females (Figure 5.14). This difference proved significant between Day 4 and Day 7 of hormone treatment (Table 5.13). It is interesting to note that females showed soliciting and lordosis behaviour to mounts by a male rat, while still showing some rejection of the male (for example, Quotients for Day 4 for females treated with ethynyl oestradiol).

The most frequently observed soliciting behaviours were the dart-hop movements by a female, and these proved to be the most reliable. Mestranol treated rats showed some passivity to attempted mounts by males, particularly during the last two days of treatment; but generally they were not as passive as the mestranol plus progesterone treated females in Experiment 13, frequently rejecting and fighting with the males.

Table 5.10

Experiment 14. Kruskal-Wallis one-way analysis of variance: Soliciting behaviour. (Scores on Days 1 and 2 were the same for all three groups, and were excluded from the analysis)

Day	Chi-square
1	-
2	-
3	5.19 ***
4	17.68 ***
5	24.92 ***
6	28.73 ***
7	31.99 ***
8	30.74 ***
9	35.66 ***

Two-tailed p: (***) < 0.001

Table 5.11

Experiment 14. Mann-Whitney U-test summary table: Soliciting behaviour. Post hoc comparisons between ethynyl oestradiol and oestradiol treated rats, and between oestradiol and mestranol treated rats. (Scores for Days 1 and 2 were the same for all three groups, and were excluded from the analysis)

Day	Ethynyl Oestradiol/Oestradiol		Oestradiol/Mestranol	
	U	Z	U	Z
1	-	-	-	-
2	-	-	-	-
3	90.5	0.433	77.0	1.797
4	63.0	1.663	56.0	2.689 **
5	50.5	2.212 *	35.0	3.496 ***
6	45.5	2.441 *	14.0	4.282 ***
7	51.0	2.369 *	0.0	4.764 ***
8	69.0	1.527	0.0	4.652 ***
9	77.0	1.797	0.0	4.736 ***

Two-tailed p: (*) < 0.05; (**) < 0.01; (***) < 0.001

Table 5.12

Experiment 14. Kruskal-Wallis one-way analysis of variance: Rejection behaviour. (Scores for Days 1 and 2 were the same for all three groups, and were excluded from the analysis)

Day	Chi-square
1	-
2	-
3	8.54 *
4	30.25 ***
5	31.94 ***
6	34.26 ***
7	30.19 ***
8	29.16 ***
9	33.02 ***

Two-tailed p: (*) < 0.05; (***) < 0.001

Table 5.13

Experiment 14. Mann-Whitney U-test summary table: Rejection behaviour. Post hoc comparisons between ethynyl oestradiol and oestradiol treated rats, and between oestradiol and mestranol treated rats. (Scores for Days 1 and 2 were the same for all three groups, and were excluded from the analysis

Day	Ethynyl Oestradiol/Oestradiol		Oestradiol/Mestranol	
	U	Z	U	Z
1	-	-	-	-
2	-	-	-	-
3	84.5	0.69	63.0	2.41 *
4	11.0	4.05 ***	41.0	2.98 **
5	8.0	4.20 ***	24.0	3.54 ***
6	16.5	3.80 ***	0.0	4.59 ***
7	36.0	2.99 **	8.5	4.16 ***
8	79.5	1.07	7.0	4.27 ***
9	69.5	1.83	0.0	4.61 ***

Two-tailed p: (*) < 0.05; (**) < 0.01; (***) < 0.001

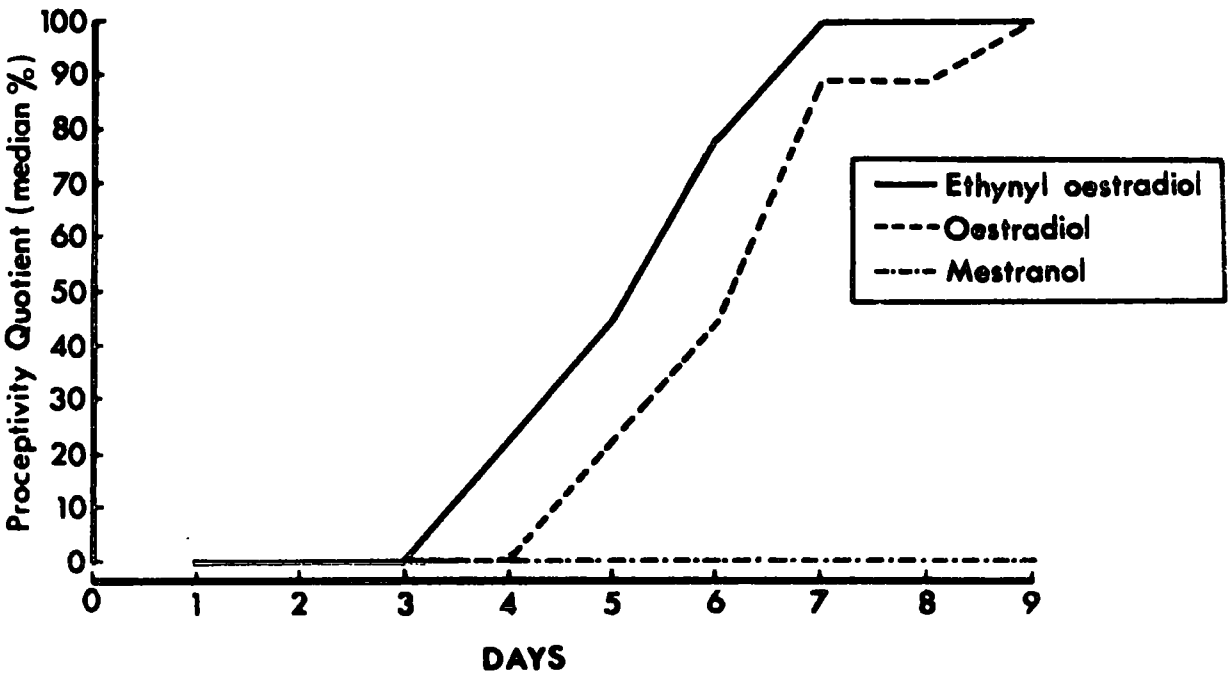


Figure 5.13

Experiment 14. Proceptivity quotients of ovariectomized rats treated with equimolar doses of oestradiol, ethynyl oestradiol and mestranol daily, for 9 days.

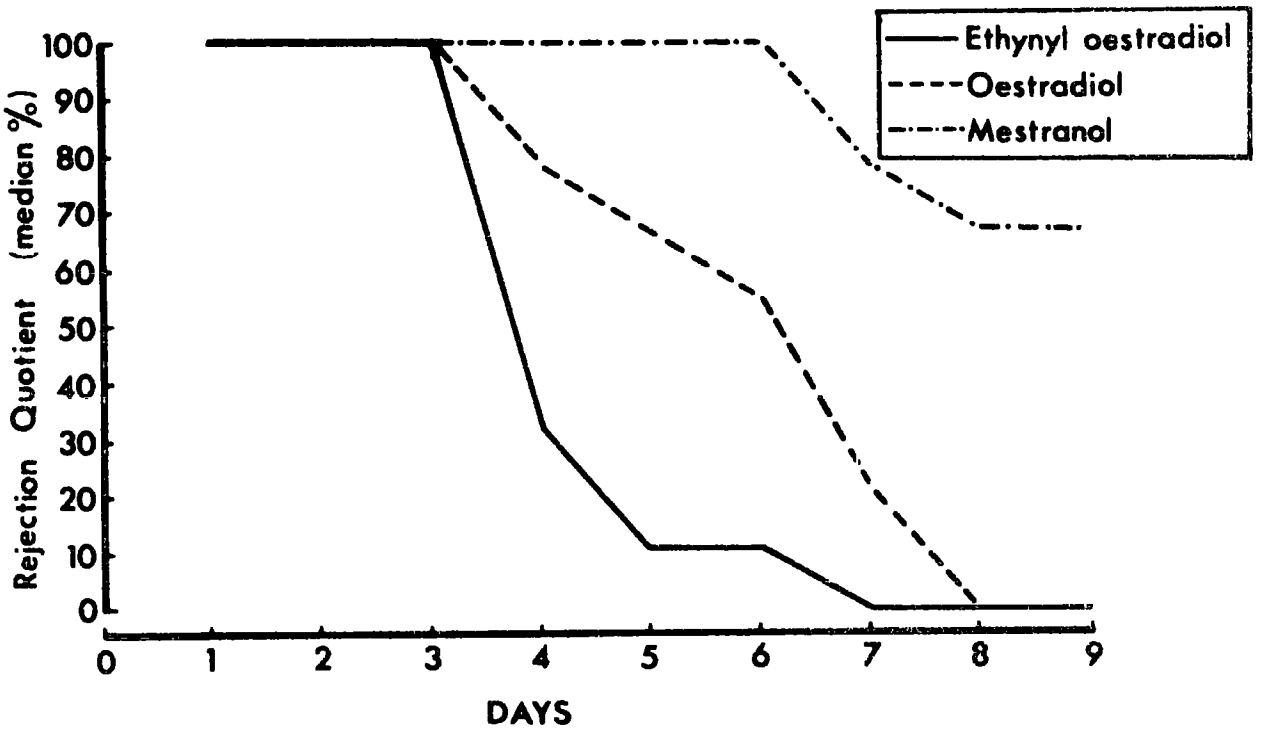


Figure 5.14

Experiment 14. Rejection quotients of ovariectomized rats treated with equimolar doses of oestradiol, ethynyl oestradiol and mestranol daily, for 9 days.

5.5.3 Discussion

Two main points have emerged from the present experiment. First, and perhaps the most obvious, the quality of female mating behaviour is affected by the administered oestrogen. Second, daily treatment with these oestrogens leads to the differential appearance of behavioural components of the mating sequence.

The most salient female sexual behaviours seen in response to administration of oestradiol-17β or ethynyl oestradiol were (1) lack of mate rejection, (2) lordosis behaviour, and (3) soliciting behaviour.

While female aggression was apparent during the initial days of hormone treatment, and characterized male-female interactions, females gradually showed lordosis to a male's mount (with the exception of mestranol treated rats). This type of lordosis behaviour seemed similar to the "forced" lordosis described by Zemlan and Adler (1977), the female sometimes moving away as the male dismounted. With continued hormone treatment, the elicited lordoses were more intense, with deeper arching of the back, and the posture was kept after the male dismounted. Komisaruk and Diakow (1973) have elegantly shown that the degree of concavity of the back during lordosis is more pronounced as the oestrous period progressed. Zemlan and Adler (1977) demonstrated similar effects with increasing, exogenously administered oestrogen doses.

In addition to lordosis behaviour, appetitive components of female copulatory behaviour were observed in this study. According to Hemmingsen (1933), soliciting behaviour consists of ear vibration, nipping the male's face and dart-hop movements. In the present study, high proceptivity quotients were associated with high lordosis levels. The spontaneous occurrence of dart-hop movements, ear vibration and lordosis is indicative of the very highest levels of receptivity at oestrus (Hemmingsen, 1933). Spontaneous lordosis was never observed in this experiment, and ear vibration was seen most frequently in females treated with ethynyl

oestradiol; but the amount of stimulation necessary to elicit lordosis behaviour was reduced with daily hormone treatment. Females which demonstrated intense lordosis showed the response when a male bumped into the back of a female, prior to mounting her.

Ethynyl oestradiol enhanced lordosis behaviour and reduced rejection behaviour in females to a greater extent than oestradiol. The transient difference in soliciting behaviour between the two female groups appeared more gradually and, by the final day, all the females treated with oestradiol or ethynyl oestradiol showed high and similar levels of soliciting behaviour. One point that is of interest is that during the development of sexual behaviour in females which were treated with either oestradiol or ethynyl oestradiol, females showed lordosis and soliciting behaviour as well as rejecting the males (for example, compare Figures 5.12, 5.13 and 5.14). These results suggest that although the females readily showed lordosis after an adequate mount by a male, they did not readily accept all his mounting attempts. The former is consistent with enhanced receptivity, but the latter is the converse. This was complicated even more by the fact that a proportion of the females displayed hopping and darting. Mestranol treated rats, however, did not exhibit any signs of receptivity. These various behaviours may, though not necessarily, have separate neuroendocrine control mechanisms involving different threshold responses and must necessarily reflect differences in the pharmaco-kinetic properties of oestradiol-17β and the ethynyl oestrogens. Luttge and Whalen (1972) suggest that oestradiol uptake by the brain "... is a comparatively irreversible process. This is not meant to imply that once a molecule of oestradiol had been 'bound' it will remain bound forever". Associated with the uptake and storage qualities of mestranol are its release or "unbinding" from tissue (and brain oestrogen receptors?) and metabolism to ethynyl oestradiol. These properties have been discussed earlier in this chapter. The

observation that mestranol suppressed food intake, but failed to stimulate sexual behaviour may be attributable, in part, to the different response thresholds of these two behavioural systems to oestrogen; for example, Drewett (1973b) demonstrated that doses of oestradiol which reliably depressed food intake were considerably lower than those which stimulated either running activity (Young and Fish, 1945; Finger, 1969) or sexual behaviour (Davidson et al., 1968a; Zemlan and Adler, 1977) in the female rat.

These experiments provide a first demonstration of certain behavioural changes caused by ethynyl oestrogens, in the female rat. As they are used in oral contraceptives, however, these oestrogens are combined with progestogens. The ensuing experiments, therefore, investigate the behavioural effects of combinations of this sort.

5.6 Experiment 15 : Behavioural effects of oestrogen-progestogen combinations in the female rat

Clinical investigations on mental, somatic and behavioural changes of women taking contraceptive steroids have assumed that the progestogens in the pill are responsible, in part, for the observed changes (e.g. Grant and Pryse-Davies, 1968) through their direct effects on central nervous system function, and have ignored possible interactive effects with oestrogens, or effects mediated solely by the oestrogen component with no progestogenic involvement. It is worth mentioning again that investigations into the behavioural effects of these steroids using animal models are still lacking. These would be of particular relevance because progestogens have varying levels of oestrogenicity, particularly those derivatives of oestrane, as measured by the Allen-Doisy (cornification of the vaginal mucosa) and other tests. Other progestogens, usually derivatives of androstane, and some derivatives of pregnane, have anabolic effects, and yet others have none of these properties (Brotherton, 1976, gives an excellent treatment of

the subject).

It would be of interest to see whether the oestrogen and progestogen components interact in such a way that the behavioural effects shown by the ethynyl oestrogens, and particularly ethynyl oestradiol, are suppressed or enhanced by progestogenic action on the central nervous system, an analogy with the interactions of oestradiol and progesterone. Oestradiol suppresses food intake and body weight (Drewett, 1973a,b; Wade 1975), whereas progesterone alone has a slight anabolic effect (Wade, 1975). However, when the two hormones are administered together over a period of days, there is no depression of food intake and body weight and no induction of sexual behaviour.

In the following experiment, the possible interactive effects between an ethynyl oestrogen and two progestogens on feeding, drinking, sexual behaviour and body weight were investigated. Ethynyl oestradiol was chosen as the oestrogen component because of its potent behavioural effects (as described in this chapter). The two progestogens used were Norethisterone acetate, which is an oestrane derivative and has a considerable degree of oestrogenic activity; and Medroxyprogesterone acetate a pregnane derivative with no oestrogenic activity but with some anabolic characteristics (Brotherton, 1976). These two progestogens are widely used in combination-type oral contraceptives.

5.6.1 Method

Experimental animals

The animals were twenty-four female Wistar rats about 210 days of age, and weighing 284 $\pm$ 14 g. The animals were previously used in Experiment 14, and had therefore received the same sexual experience. Seven days before the start of any hormone injections, the rats were housed singly in plastic cages, which had been previously modified for the measurement of food intake as described in Chapter 2.

Hormone injections

The steroid combinations used were based on two commercially available oral contraceptive formulations - "Anovlar-21" and "Nogest". Anovlar-21 contains 50 µg of ethynyl oestradiol plus 4 mg of norethisterone acetate. Nogest contains 50 µg ethynyl oestradiol plus 5 mg Medroxyprogesterone acetate. The above quantities, reduced ten-fold, were used in preparing the hormone solutions. Consequently, one group of rats received 5 µg ethynyl oestradiol in 0.1 ml arachis oil/day plus an oil blank solution of 0.1 ml. A second group received 5 µg ethynyl oestradiol in 0.1 ml arachis oil plus 400 µg norethisterone acetate in 0.1 ml oil/day; and a third group received 5 µg ethynyl oestradiol in 0.1 ml oil plus 500 µg medroxyprogesterone acetate in 0.1 ml oil/day.

Experimental schedule

Each rat was randomly allocated to one of three groups: one group received ethynyl oestradiol, another group, ethynyl oestradiol plus norethisterone acetate, and a third group, ethynyl oestradiol plus medroxyprogesterone acetate. Body weight, food and water intake were measured to the nearest 1.0 g, 0.1 g, and 0.1 ml respectively, on six consecutive days prior to steroid treatment; and then on the first four consecutive days of hormone injections. The rats were injected for five days, and on the fifth day they were given sexual behaviour tests. The room, testing arena and testing procedure have already been described in the "General procedures" section of Experiment 13. Thus lordosis quotients were obtained for the three groups of rats using formula 5.1 described in Experiment 14. The testing of the groups was run "blind", and proceeded using a generated sequence of random numbers.

5.6.2 Results

Baseline body weight, food and water intake were determined by averaging data for the three days prior to hormone treatment, when the

three measures had stabilised. The data for each group were then expressed as per cent change from baseline. The results are self-evident.

Food intake

All three groups showed an initial large decrease of about 50% in food intake with hormone treatment (Figure 5.15) during the first day. The mean standard error for all three groups was $\pm$ 5.5%. Taking this into account, it is obvious from Figure 5.15 that there is no interaction between the different hormones, and no significant difference between treatments.

Water intake

The per cent change in water intake followed a similar trend to the one observed for food intake, and is presented in Figure 5.16. Again, a large initial decrease of about 50% in water intake is evident after one day of hormone treatment. The mean standard error was $\pm$ 6.9%; and it is also clear from Figure 5.16 that there is no difference and no interaction between the various hormones.

Body weight

The change in body weight is presented in Figure 5.17. The animals showed a decrease in body weight of about 9%, after four days of hormone treatment. The mean standard error for each group was $\pm$ 0.8%, and the lack of interaction between the groups is obvious from Figure 5.17.

Sexual behaviour

Figure 5.18 shows the mean lordosis quotient ($\pm$ standard error) for the three groups, and it is evident from this that there was no difference between the groups.

5.6.3 Discussion

These results show that ethynyl oestradiol does not interact with either Norethisterone acetate or Medroxyprogesterone acetate; and that the observed effects were solely due to ethynyl oestradiol. Three

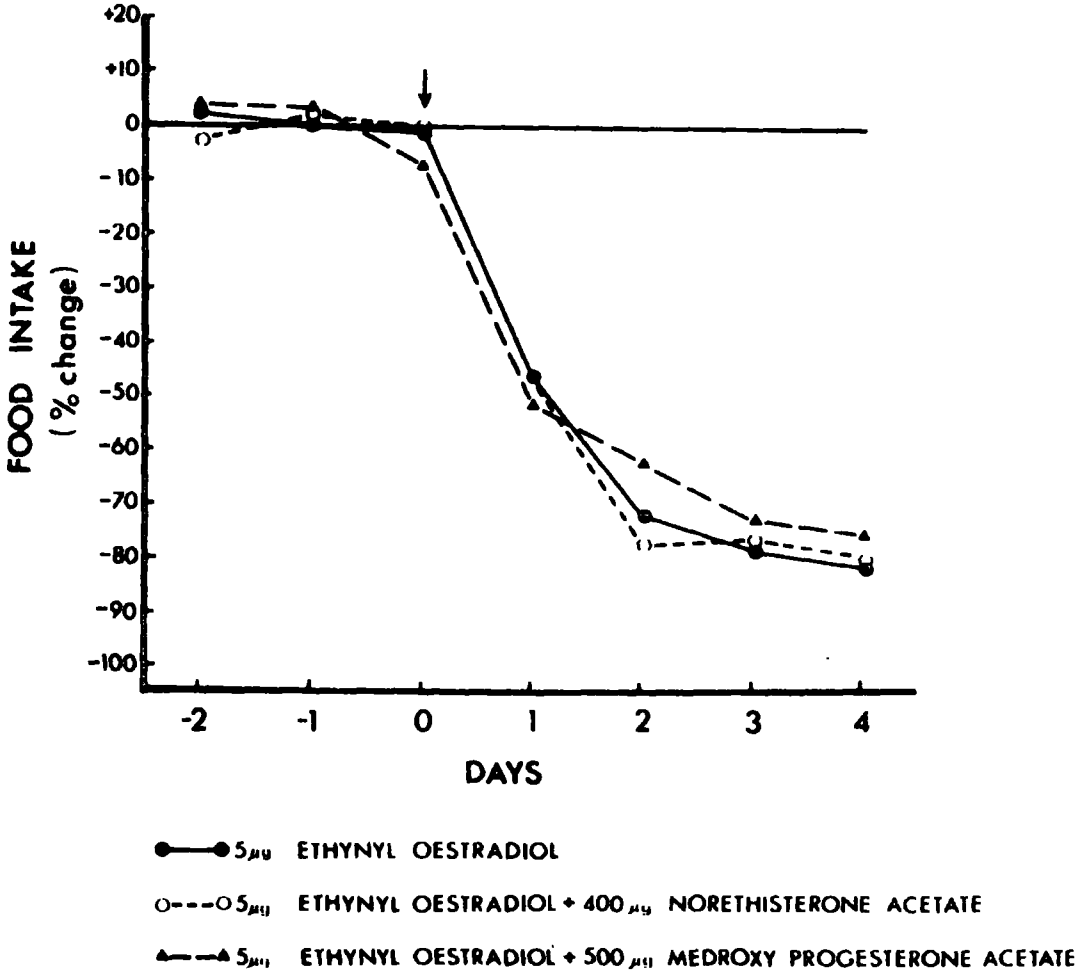


Figure 5.15

Experiment 15. Food intake (% change) of ovariectomized rats injected with either ethynyl oestradiol alone or in combination with either norethisterone acetate or medroxyprogesterone acetate, for 4 days. The start of the injections is indicated by the arrow (Day 0), after a 3-day baseline.

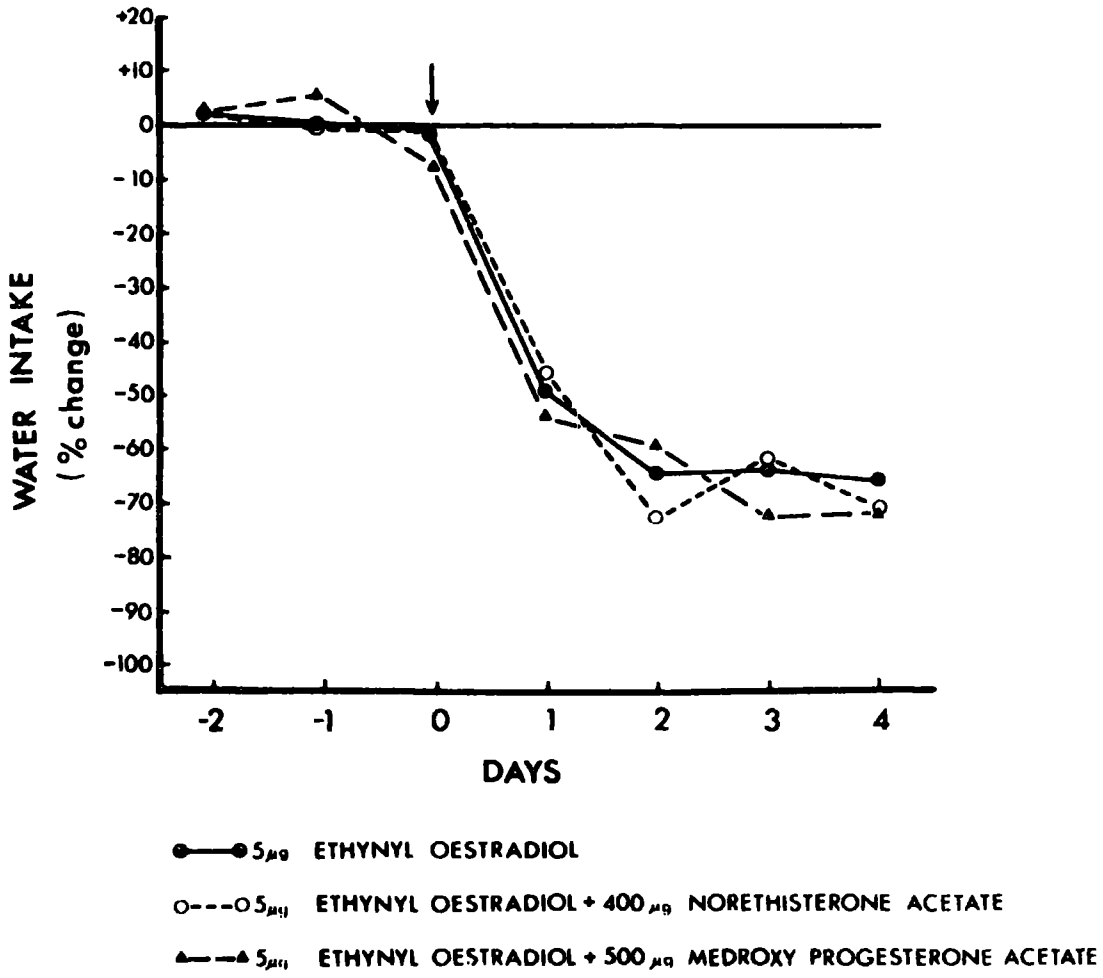


Figure 5.16

Experiment 15. Water intake (% change) of ovariectomized rats injected with either ethynyl oestradiol alone, or in combination with either norethisterone acetate or medroxyprogesterone acetate, for 4 days. The start of the injections is indicated by the arrow (Day 0), after a 3 day baseline.

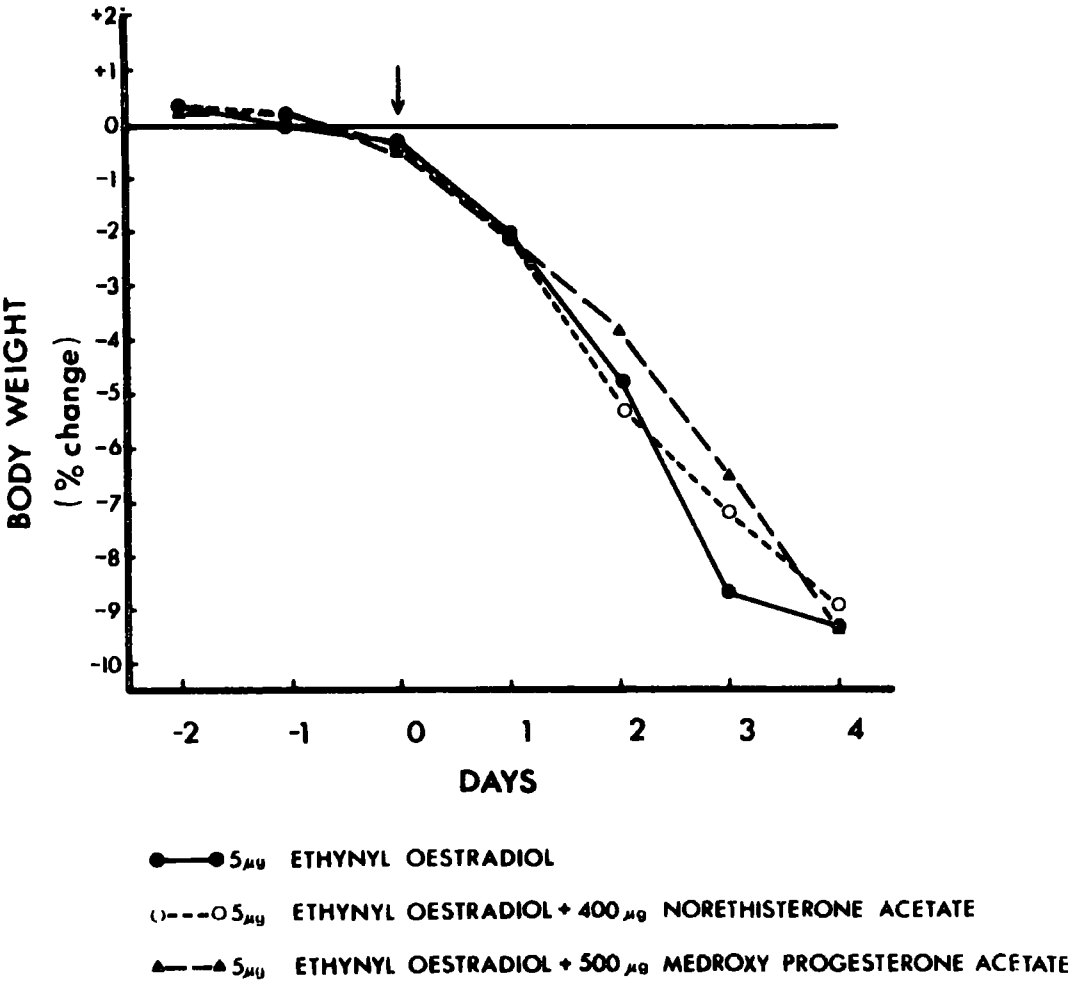


Figure 5.17

Experiment 15. Body weight (% change) of ovariectomized rats injected with either ethynyl oestradiol alone, or in combination with either norethisterone acetate or medroxyprogesterone acetate, for 4 days. The start of the injections is indicated by the arrow (Day 0), after a 3-day baseline.

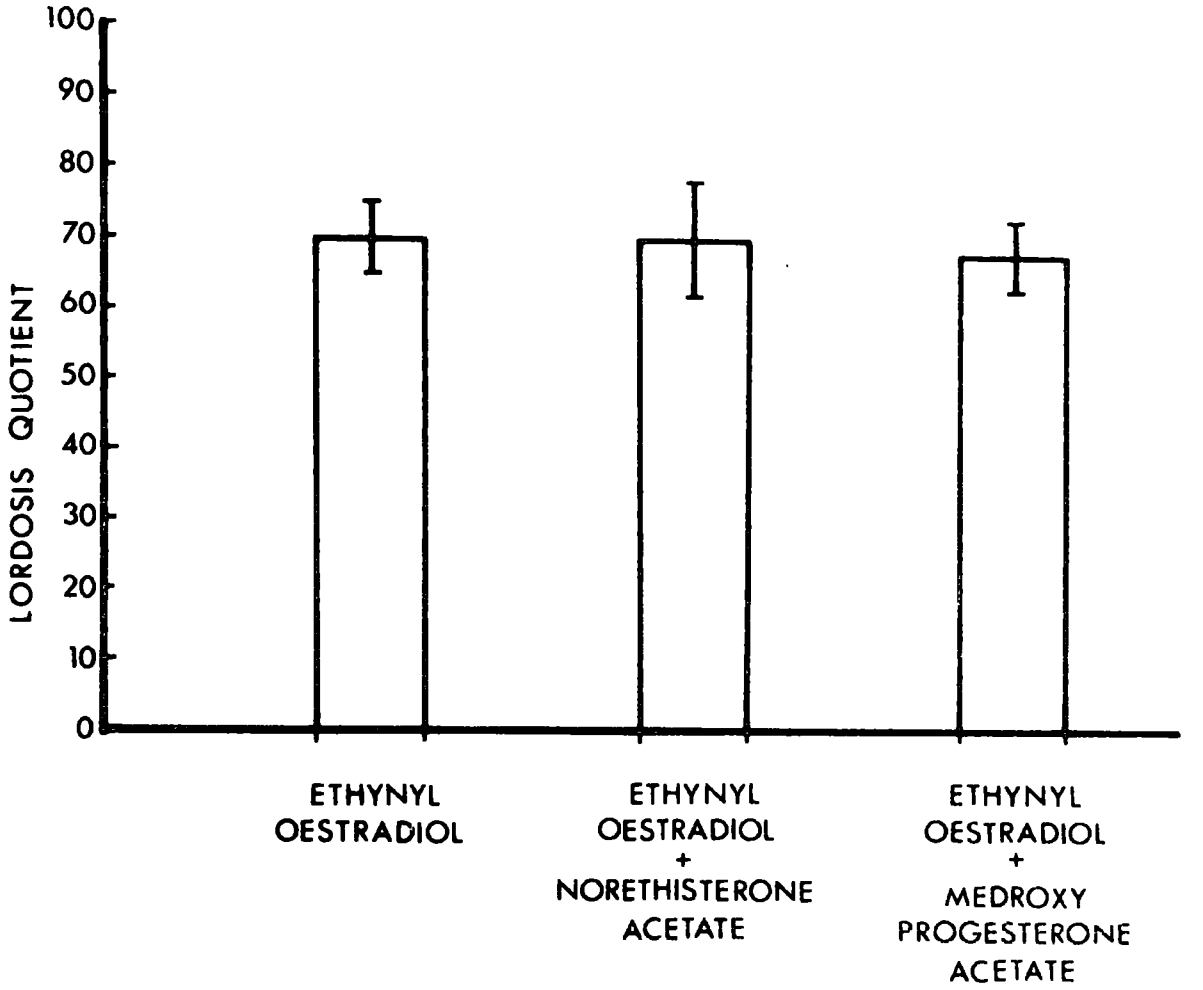


Figure 5.18

Experiment 15. Lordosis quotients (%) of ovariectomized rats injected with either ethynyl oestradiol alone, or in combination with either norethisterone acetate or medroxyprogesterone acetate, for 5 days.

explanations present themselves. First, neither of the progestogens crossed the blood-brain barrier. Secondly, the progestogens crossed this barrier, but were not taken up by any (progesterone?) receptors. Thirdly, the progestogens were bound by receptors, in the brain, in an inactive form.

The oestrogenic properties of norethisterone acetate and the strong progestogenic properties of the pregnane derivative, medroxyprogesterone acetate, do not seem to mediate behavioural changes, suggesting that these two progestogens do not influence central nervous mechanisms controlling feeding and sexual behaviour in the female rat.

5.7 Experiment 16. A second experiment investigating the behavioural effects of oestrogen-progestogen combinations in the female rat

It is possible that, in intact rats, ovarian secretions may interact with the administered steroids by attenuating the effects of these hormones. The next experiment, therefore, investigates the effects of ethynyl oestradiol administered in combination with norethisterone acetate on feeding, drinking, body weight and sexual behaviour in cycling female rats.

5.7.1 Method

Experimental animals

The animals were eight female Wistar rats, about 120 days of age, and weighing 261 $\pm$ 20 g. Two weeks before the start of hormone injections, the rats were housed singly in plastic cages, modified for the measurement of food and water intake as described in Chapter 2. Food and water intake, and body weight, were measured daily.

Experimental schedule

After one week the rats were tested daily for lordosis behaviour, at 14.00 h, by simultaneous flank and anogenital palpation manually (as described by Zucker, 1967; this method does not give false positive results). Females showing lordosis were considered to be in behavioural oestrus. Hormone injections commenced on the morning following the start

of the next behavioural oestrus. The rats were injected with 5.0 μg ethynyl oestradiol in 0.1 ml arachis oil/day plus 400 μg of norethisterone acetate in 0.1 ml oil for five days. Food, water intake and body weight were measured over the first four days; and the rats were given sexual behaviour tests on the fifth day of hormone injections. The method of testing has been described previously in Experiment 13.

5.7.2 Results

The data from all the rats were aligned together by matching behavioural oestrus for each rat, and expressed as per cent change from baseline.

The results for food and water intake and body weight are presented in Figures 5.19 and 5.20. On the fourth day of hormone injections, the rats ate less food than on the day of behavioural oestrus. A matched-pairs t-test between the mean values for Day 0 and Day 4 showed that this difference was significant ($t = 3.1775$; with $df. = 7$; $p < 0.02$). The mean of the difference of the change was about 31%. On the other hand, water intake on Day 4 was not significantly different from intake on Day 0 ($t = 0.7149$; with $df. = 7$; p = ns). The slight levelling off in food and water intake during the first two days after behavioural oestrus may have been possibly due to the ovarian secretion of progesterone.

The per cent change in body weight, however, showed a significant difference between Day 0 and Day 4 ($t = 3.2813$; with $df. = 7$; $p < 0.02$). The mean of the difference of the change was 4.4%. As for sexual behaviour, on the fifth day after behavioural oestrus the mean lordosis quotient was 92.5%. This value suggests that the ethynyl oestradiol-norethisterone acetate combination maintained sexual receptivity in the female rat after the period of behavioural oestrus.

5.7.3 Discussion

These results are consistent with the findings of Experiment 15, in that ethynyl oestradiol in combination with norethisterone acetate reduced food

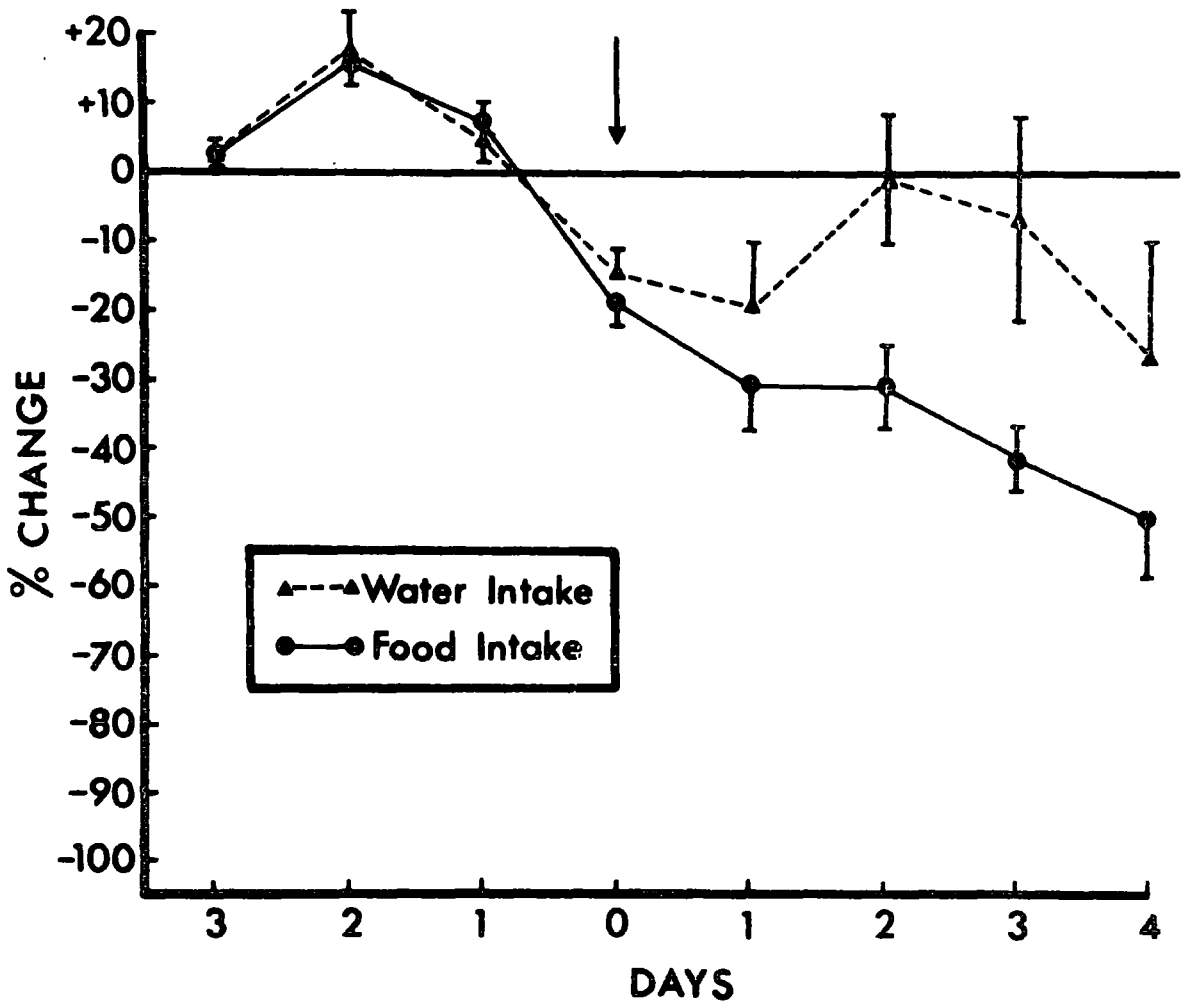


Figure 5.19

Experiment 16. Food and water intake (% change) of cycling female rats injected with ethynyl oestradiol plus norethisterone acetate for 4 days. Injections began after a 4-day baseline. Arrow (Day 0) indicates the morning following behavioural oestrus tests, and the start of injections.

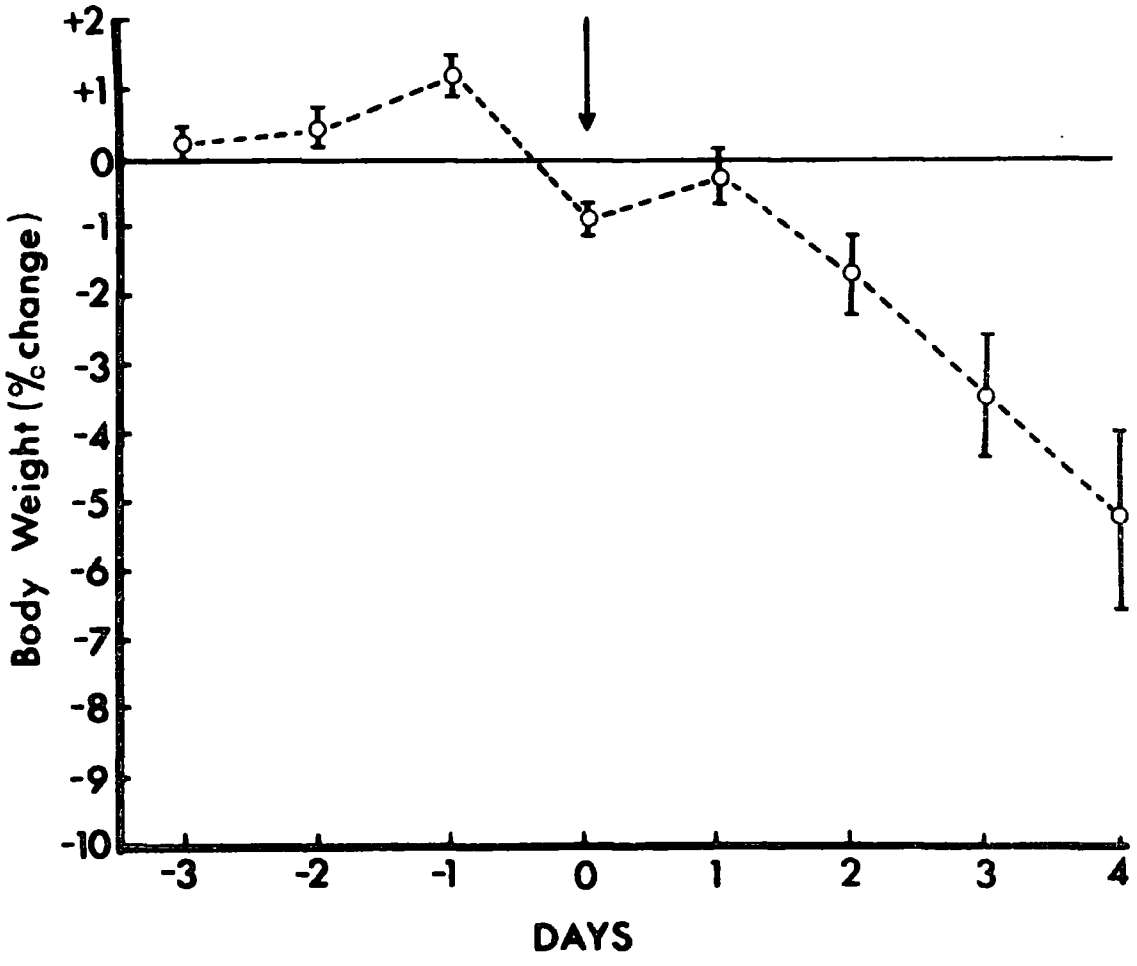


Figure 5.20

Experiment 16. Body weight (% change) of cycling female rats injected with ethynyl oestradiol plus norethisterone acetate for 4 days. Injections began after a 4-day baseline. Arrow (Day 0) indicates the morning following behavioural oestrus tests, and the start of injections.

intake and body weight significantly. The decrease in water intake was similar to the decrease observed at oestrus; but it was significantly different from intake during the rest of the cycle. The secretion of ovarian progesterone may have been responsible for attenuating the decrements in feeding, drinking and body weight on the first two days after oestrus. Thereafter, food intake, body weight, and to a lesser extent water intake, showed a decrease, which may have been due to a decreasing interference by ovarian progesterone.

Injections of oestradiol benzoate on any day of the oestrous cycle, except on the second day after the display of spontaneous oestrus, prolong the duration of receptivity in the female rat (Södersten and Hansen, 1977), and this is probably due to the synergistic action of oestradiol benzoate with progesterone secretion by the ovary. It is likely, then, that in this study ethynyl oestradiol maintained sexual receptivity in female rats by its synergistic action with the (subsequent?) ovarian secretion of progesterone. Further studies are needed, in this respect, on the effect of various hormones used in oral contraceptive formulations administered during various stages of the oestrus cycle, similar in method to the investigation by Södersten and Hansen (1977). It is hoped that these experiments will provide the necessary stimulus for doing so, since it is of major importance that the behavioural effects of these synthetic steroids be evaluated in a wide range of animals in view of their widespread use as anti-fertility agents by women.

CHAPTER 6

BEHAVIOURAL EFFECTS OF CONTRACEPTIVE STEROIDS IN THE HUMAN FEMALE

6.1 Sexual Behaviour in the Human Female

6.1.1 Changes during the menstrual cycle

Although it is often claimed that sexual responsiveness in human females is independent of hormonal control, there is no substantial body of evidence which would allow direct comparisons of endocrine effects in women with those known to exist in females of other species. Feminine sexual characteristics have not been conceptualized in terms of attractivity, proceptivity and receptivity as they have been for the rat, other rodents and primates; and the methods currently being used for studying human sexual behaviour make it very difficult to distinguish between proceptivity and receptivity.

Udry and Morris (1970) have reported rhythmical changes in sexual interactions in humans during the menstrual cycle, although contradictory reports continue to appear (Udry, Morris and Waller, 1973), and maximum levels are as commonly found early in the follicular phase as at midcycle (McCance, Luff and Widdowson, 1937; Hart, 1960; James, 1971; Spitz, Gold and Adams, 1975). It has been reported that female sexual activity declines during the luteal phase (as in monkeys) of the menstrual cycle, and that this decline can be prevented by taking contraceptive steroids (Udry, et al., 1973).

Spitz, Gold and Adams (1975) present three findings which may have direct relevance to the general understanding of human female sexual behaviour: (1) the post-menstrual peak and menstrual trough in intercourse during the cycle of menstruation, (2) the correlation of sexual arousal with type of sexual encounter, and not with periods of the menstrual cycle, and (3) the effect of oral contraceptives, which led to a greater

number of intercourse-days and a smaller number of intercourse-sessions per intercourse-day among women taking oral contraceptives compared to women using a diaphragm or women whose partner used a male prophylactic. These general findings are in agreement with Ford and Beach's (1951) proposals regarding social and cultural influences on human sexual behaviour. Ford and Beach, and more recently Beach (1976), contend that throughout the higher primates, and particularly in man, sexual behaviour has become progressively less influenced by hormones and more influenced by learning, social and cultural conditioning.

The consequences of inhibiting or removing endocrine secretions, or treatment with steroids, have usually been assessed and categorized in terms of loss or gain of "libido", although some studies have used other criteria such as the occurrence of orgasm or "coital satisfaction". These studies do not indicate, however, whether loss of libido involves changes in receptivity, proceptivity or both. Furthermore, changes in libido may occur secondary to those in attractivity, as in monkeys (Baum, Everitt, Herbert and Keverne, 1977), or to mental or somatic changes.

It is generally agreed that ovariectomy does not diminish the human female's libido consistently (Kinsey, Pomeroy, Martin and Gebhard, 1953), and the same conclusions have been reached in studies of menopausal women (Money, 1961). Insofar as comparisons are justified, this correlates with findings in ovariectomized monkeys (Baum et al., 1977). Adrenalectomy, however, has been found to diminish libido in the human female (Waxenburg, Drellich and Sutherland, 1959), and testosterone administration to intact women stimulated sexual interest in many cases (Salmon and Geist, 1943). This suggests that there may be a role for androgens in the human female's sexuality comparable with that experimentally determined for the rhesus monkey, but not the rat, although exact correspondence awaits further clinical investigation.

6.1.2 Ovarian control of human pheromones?

Several authors have presented evidence for the possible existence of human pheromones (Comfort, 1971; Schneider, 1971; Michael, Bonsall and Warner, 1974). Michael et al. (1974) suggest, for example, that vaginal secretions may communicate some information related to the stage of the menstrual cycle. The proportions of the components of vaginal secretions vary across the cycle (Michael et al., 1974; Preti and Huggins, 1975), and the odours of secretions from the menstrual or luteal phases of the cycle (Doty, Ford Preti and Huggins, 1975).

In the female macaque, pheromonal production is facilitated by oestrogen and suppressed by progesterone. The latter hormone also inhibits both female sexual activity and the attractiveness of the female to the male (Michael, Herbert and Welegalla, 1967). Further, vaginal secretions collected from oestrogen treated monkeys increased the sexual attractiveness when applied to the sexual skin of untreated, ovariectomized females (Michael, Keverne and Bonsall, 1971).

It has been demonstrated that many mammals can discriminate between the odours of conspecifics (see Miller-Schwarze, 1974, for a review). More recently, Wallace (1977) showed that both men and women could discriminate between human odours on the basis of olfactory cues from the hand. The accuracy of female observers was improved when the two stimulus individuals were genetically unrelated, and when they were on different diets.

Pietras and Moulton (1974) showed that the performance of four-day cycling female rats in an odour detection task fluctuated with the phases of the oestrous cycle. This cyclicity occurs for α-ionone, eugenol, cyclopentanone and Exaltolide, is significant for every compound but the last, and is not odour specific. Ovariectomy eliminated the cyclicity in performance, but elevated performance scores above those of controls. Exaltolide is a synthetic compound which can be detected by adult women, but

not by men or pre-pubertal girls. The ability to perceive this odour reaches a maximum during the follicular phase and a minimum during the luteal phase of the menstrual cycle, when progesterone levels are high. Similar hormone-dependent responses in human males have not yet been demonstrated.

It is interesting to note, then, that Michael (1969) suggests that oral contraceptives may adversely affect the sexual behaviour of men via changes in odour and behaviour of their female partners, caused by these steroids. Male monkeys found females less "attractive" after the females had been treated with oral contraceptive preparations. Such effects could be mediated by the progestogenic component of oral contraceptives; but the evidence for this is still lacking.

6.1.3 Oral contraceptives and sexual behaviour

The most common form in which steroids are given to women is the contraceptive pill, which usually consists of a variable mixture of an oestrogen and a progestogen. A number of investigations on the effect contraceptive steroids have on libido present equivocal and conflicting results. Table 6.1 is a brief survey of some recent controlled studies on the mental and somatic effects of oral contraceptives. Most of these studies indicate that some women develop mental and somatic symptoms during the first months of oral contraceptive use. These reactions seem to disappear after the initial months, however. In some cases, "loss of libido" has been reported, and this has been a major factor in women discontinuing their use of the pill (for example, the studies by Grounds, Davies and Mowbray, 1970; Marcotte, Kane, Obrist and Lipton, 1970). The study by Grounds et al. reports libido loss in eight out of a sample of ten patients during the first month; but only in two out of ten "double-blind" controls. Marcotte et al. report a loss of the capacity for orgasm in the three subjects (out of four) who completed the study. Another

study reporting a decrease in libido is the one by Grant and Pryse-Davies (1968). However, in spite of thorough questionnaires and psychiatric assessment, there is no evidence for a primary decrease in libido in the other controlled studies (Nilsson and Sölvell, 1967; Herzberg, Draper, Johnson and Nicol, 1971; Cullberg, 1972). The way in which such data are collected may have a crucial effect on the apparent change in behaviour. Furthermore, if direct effects on behaviour do occur, they may be attributed as much to social or cognitive factors as to endocrine factors. However, women taking the pill excrete less androgen metabolites (Bullbrook, Hayward, Herian, Swain, Tong and Wang, 1973) and have no mid-cycle peak in either testosterone or oestrogen, which may provide an endocrine explanation for some cases of decreased libido.

Data from a study by Morris and Udry (1971) investigating the effects of hormones on coital frequency, agrees with such an idea. Morris and Udry found no difference in intercourse rates between a group of women randomly selected to use oral contraceptives and another group randomly selected to use placebos. This finding deserves some attention because hormonal factors and cognitive factors associated with different contraceptive methods were not confounded. All subjects were instructed to continue using their usual (non-pill) means of contraception. In this respect, the Herzberg et al. (1971) results should be assessed in connection with the fact that oral contraceptive users as a group seem to differ from non-users in several important respects, for example, smoking habits, social class and sexual activity (Kay, Smith and Richards, 1969; Appelgren, 1972, cited in Cullberg, 1972). This would suggest that there may exist differences in mental attitudes between women using intra-uterine device and those using oral contraceptives, making the former group less suited as a control group.

There are other complications. Clinical depression, of which

diminished libido is one symptom, sometimes occurs in women taking contraceptive steroids (see Table 6.1); the converse (euphoria) may also occur, although this has not been demonstrated to date. For example, in the study by Grounds et al. (1970), nine out of ten patients showed an increase in depression during the first month of oral contraceptive use, and this was not related to questionnaire-related neuroticism. The way in which such data are collected may have a crucial effect on the apparent change in behaviour, and may raise interpretative problems. Such a problem can be found in the investigation by Silbergeld, Brast and Noble (1971). They studied mental and somatic parameters in eight female volunteers during a period of four months. The women were given "Enovid" (5 mg Norethynodrel + 0.075 mg mestranol) during two months and placebo treatment during another two months in a double-blind, cross-over design. Thus Enovid on the one hand was found to have a very significant tranquilizing effect as measured by one method (questionnaire) and on the other hand the self-rated "Anxiety" and "Hostility outwards" were found to increase.

6.1.4 Possible oestrogenic and progestogenic effects on central nervous system functioning

All of the studies listed in Table 6.1, as well as in the text, attribute some degree of effect to the progestogenic component of the pill, the oestrogenic component or to both. Some of these investigations failed to differentiate between first, and the most obvious from Table 6.1, the different preparations themselves. Secondly, the parent derivatives of the progestogens: some progestogens are derivatives of Gonane (for Norgestrel only), others of Oestrane (and have some oestrogenic properties), from Pregnane, and yet others of Androstane (and possess some anabolic effects). Thirdly, the type of oestrogen used in the different oral contraceptive preparations (whether ethynyl oestradiol or mestranol). Fourthly, and with the exception of one study (Cullberg, 1972), the oestrogen-

TABLE 6.1

Controlled studies on mental and somatic symptoms of oral contraceptives. Positive number of subjects (in brackets) indicates control group size; negative number indicates number of subjects not completing the study

AUTHOR	DURATION	EXPERIMENTAL DESIGN	PLACEBO	NUMBER OF SUBJECTS	CONTRACEPTIVES USED	MENTAL, SOMATIC AND SEXUAL CHANGES	PSYCHIATRIC ASSESSMENT
Nilsson, L. and Sölvell, L. (1967)	4 cycles	Double-blind Cross-over	None	159	Volidan (EE) Lyndiol mite (EE) Anovlar mite (EE) Ovulen (EE)	Volidan caused high spotting and breakthrough bleeding. Ovulen less than Volidan but sig. more than Lyndiol and Anovlar; also more nausea than other preparations. Anovlar produced sig. weight increase, higher than Ovulen or Lyndiol. No changes in Libido in any group. During first month a sig. increase in tiredness and/or depression.	No assessment
Nilsson et al. (1967)	2 years	No control group	None	344 (-63)	Anovlar (EE)	Relief of premenstrual tension and dysmenorrhoea. Sig. increase in weight. Change in Libido: 2.3% reported increase; 21.3% reported decrease; 28.7% reported no change. Increased sense of security led to an improvement of sexual adaptability in 45.3% of women. Women with a previous history of psychiatric symptoms reported these to a greater extent.	Postal Questionnaire (including personality characteristics)
Grounds et al. (1970)	2 cycles	Double-blind	Yes	10 (+10)	Ovulen (M)	9 out of 10 patients showed increase of depression during first month; in controls 2/10. 8 out of 10 patients reported sexual disturbances.	Psychiatric evaluation and inventories and rating scales
Herzberg, B. and A. Coppen (1970)	11 months	Two groups: o.c. and control. Drop-out of 32% in control group not discussed	None	152 (+40)	Gynovlar (EE) Anovlar (EE) Volidan (EE) Ortho-Novin (M) Lyndiol (M) Ovulen (M)	Difficult to interpret: both increase and decrease in premenstrual depression and irritability. Relief of Dysmenorrhoea in majority of patients. No report on Libido changes and no report on different preparations.	Postal Questionnaire and investory
Marcotte et al. (1970)	3 cycles	Double-blind	Yes	4 (-1)	Ortho-Novum (M)	Mild depression, lethargy, altered sleep patterns and decrease in Libido in all three women, including capacity for orgasm. Decreased levels of excretion of catecholamine metabolites.	Psychiatric evaluation and inventories

continued .

TABLE 6.1 (continued)

Controlled studies on mental and somatic symptoms of oral contraceptives. Positive number of subjects (in brackets) indicates control group size; negative number indicates number of subjects not completing the study

AUTHORS	DURATION	EXPERIMENTAL DESIGN	PLACEBO	NUMBER OF SUBJECTS	CONTRACEPTIVES USED	MENTAL, SOMATIC AND SEXUAL CHANGES	PSYCHIATRIC ASSESSMENT
Goldzieher et al. (1971)	5 cycles	Double-blind, Cross-over	Yes	398	Oracon (EE; seq.) Ovulen-21 (M) Norinyl-1 (M) Chlormadinone acetate	Significant increase in nausea and vomiting, headache and nervousness in high oestrogen preparation (Oracon). Sig. increase in nervousness on 1st cycle in both Ovulen and Oracon groups. No report on Libido changes. No report on different preparations.	Simple non-psychiatric assessment
Herzberg et al. (1971)	1 year	4 groups. Control group consisted of IUD* women	None	218 (+54)	Gynovlar (EE) Lyndiol 2.5 (M) Norinyl-1 (M)	No clear difference between groups (all group totals were pooled). No difference in Libido in o.c. groups; but a significant rise in Libido in IUD group.	Psychiatric evaluation and inventories and rating scales
Silbergeld et al. (1971)	4 cycles	Double-blind, Cross-over	Yes	8	Enovid (M)	Difficult to interpret: "Very significant" tranquilizing effect; but self-rated anxiety increased. Self-rated feelings of irritability and aggression decreased. Dysmenorrhoea relief. Physical side effects of nausea and vomiting, drowsiness and increased water retention.	Psychiatric evaluation and inventories and rating scales
Cullberg, J. (1972)	2 months	Double-blind, Randomized	Yes	322	Ethynyl oestradiol (0.05 mg) + 1.0 mg Norgestrel + 0.5 mg Norgestrel** + 0.06 mg Norgestrel	Relief of Dysmenorrhoea with progestogen dominated preparation in a dose-related response. Impairment in premenstrual tension relief with oestrogen dominated medication. Women without premenstrual irritability reacted adversely to the progestogen dominated treatment. Absence of direct negative effects on Libido.	Psychiatric evaluation and inventories and rating scales

* Intra-uterine device
** Ovral

Abbreviations: EE = Ethynyl oestradiol as the oestrogen base.
M = Mestranol as the oestrogen base.
seq.= Sequential

type/progestogen-derivative ratio in the formulations used.

Consequently, in studies where pooled data from women on different oral contraceptives are used (e.g. Herzberg and Coppen, 1970; Herzberg et al., 1971) a reported "no change in Libido" may have been due to a cancelling out of effects. Other undetected changes in mental or somatic parameters can be attributed to such pooling of oral contraceptives and the ensuing data. Grounds et al. (1970) and Marcotte et al. (1970) report a decrease in libido in women taking either "Ovulen" or "Ortho-Novum". Both of these preparations contain mestranol as the oestrogen component. In this respect it is interesting to note that mestranol was found not to facilitate sexual behaviour in the ovariectomized female rat (Chapter 5). Although oestrogens are not thought to have a pronounced effect on human female sexual behaviour (Kinsey et al., 1953), mestranol may be actively competing for receptor sites which are, in some way, involved in the control of sexual behaviour; or by interfering with synaptic transmission. It must also be remembered, however, that the studies by Grounds et al. and Marcotte et al. were conducted over a very short period of time, using a small number of women. On the other hand, these studies were very thorough.

Of particular interest here are studies which try to differentiate between progestogen and oestrogen dominated pills. There is, however, no clearcut evidence as to which preparations show more adverse mental effects. Whereas several studies report adverse mental reactions to formulations with higher progestational content (e.g. Grant and Pryse-Davies, 1968; Petersen, 1969), other authors report the opposite (e.g. Goldzieher, Moses, Averkin, Scheel and Taber, 1971; Kutner and Brown, 1972; Cullberg, 1972). Grant and Pryse-Davies (1968) report a "dramatic rise" in monoamine oxidase (MAO) activity of the endometrium in the late secretory phase of the menstrual cycle. They also find a rise in monoamine oxidase activity if the patients received a preparation containing a strong progestogenic compound in

combination with a weak oestrogen. It is hypothesised that progestogen stimulation causes a general change in monoamine oxidase activity, and that this "... may be a factor in the occurrence of depression and loss of libido in susceptible women". This presupposes that monoamine oxidase increase also occurs in the central nervous system. As yet, the direct effects of synthetic progestogens on central nervous system functioning have not been demonstrated. Mental symptoms associated with oral contraceptive use are generally of the dysphoric-irritable type with no diurnal rhythm, and consequently not typical of the endogenous depressive syndrome.

In healthy women, plasma MAO activity is lowest at the time of ovulation, when oestradiol production is greatest; but activity increases during the luteal phase when progesterone is secreted (Briggs and Briggs, 1972). Contrary to the findings of Grant and Pryse-Davies (1968), Briggs and Briggs found that plasma MAO activity was significantly reduced in women taking oral contraceptive preparations (these authors, however, do not mention which type of oral contraceptives were used), but not in women receiving depot injections of medroxyprogesterone acetate. Grant and Pryse-Davies measured endometrial enzymic activity, whereas Briggs and Briggs monitored plasma enzymic activity. These findings argue against using plasma MAO activity as an individual measure of brain MAO activity.

However, there is evidence from work with female rats that brain MAO levels may be influenced by oestradiol. The area with highest MAO activity in female rats is the medial hypothalamus (Zolovick, Pearse, Boehlke and Eleftheriou, 1966), which is also known to contain large numbers of oestrophilic cells (Pfaff and Keiner, 1973). The activity of brain MAO varies considerably throughout the oestrous cycle. It is lowest during dioestrus and progressively increases to a maximum at oestrus (Zolovick et al., 1966). If brain MAO is influenced by natural and synthetic sex

hormones in the same way as the plasma enzyme, then this would offer a method of investigating differences in brain function between women receiving different oral contraceptive formulations.

Cullberg's (1972) and Kutner and Brown's (1972) results give some evidence that the progestogens might have sedative properties similar to those of natural progesterone. Such effects were first reported by Selye (1942) who found that progesterone was one of the most effective compounds among the sedative steroid hormones. The marked decline in progesterone levels before menstruation and after parturition could therefore be implicated in mental disturbances at these times. Ladisich (1977), however, could not establish a clear relationship between progesterone levels in women and progestogen (Medroxyprogesterone) treatment. Ladisich suggests that progesterone affects serotonin metabolism in the brain; and he based this suggestion on his findings using ovariectomized female rats. Such extrapolations, if at all justified, must be treated with caution. It is possible that <u>exogenous progestogen</u> treatment may affect <u>endogenous progesterone</u> uptake or metabolism, and that such disturbances could well be related to affective changes. This is yet to be shown.

6.2 Food Intake in the Human Female

6.2.1 Changes during the menstrual cycle

Ovarian control of sexual behaviour in women has received a good deal of attention, but the studies have not been very methodical and the results equivocal and difficult to interpret. However, the possibility of ovarian control over food intake has scarcely been considered. The decrease in food intake at oestrus has been thoroughly investigated in the rat (Drewett, 1973a), and to a certain extent in other rodents; but there are few objective studies with primates.

Gilbert and Gillman (1956) found a correlation between food intake and circulating oestrogen levels in their study of appetite patterns during

the menstrual cycle of the baboon. They found an increase in appetite from two to five days before menstruation (during the luteal phase), and a decrease during the follicular phase (between Day 7 and Day 11 of the cycle). Krohn and Zuckerman (1937), observing food intake during three menstrual cycles of the pig-tailed macaque, and Czaja (1975) in the rhesus monkey, found identical feeding patterns. Gilbert and Gillman also showed that progesterone injections increased food intake in an intact baboon when given during the follicular depression of its menstrual cycle.

There are obvious difficulties with conducting such studies on women. Data would be particularly hard to collect as the diet and activity of humans cannot be easily controlled. Monitoring feeding patterns would present an additional problem. However, some dieticians have reported on their own food intake during the menstrual cycle. Yudkin (1951) reports a study by five dietetic students who measured their own food consumption during one menstrual cycle. Yudkin comments that "there was no obvious relationship between food consumption and menstruation". Chappel (1955) and Taggart (1962) did not find any evidence for cycle influence on food intake, water intake, water balance or body weight. They comment on the fact that changes due to the menstrual cycle may have been masked by variability from other sources, which was considerable. There are, however, clinical reports which show that appetite <u>increases</u> pre-menstrually (for example: Thorn, Nelson and Thorn, 1938; Critchley, 1962; Dalton, 1964). In this respect it is interesting to note a study by Crean (1963) who reported increases in gastric emptying rates during the luteal phase (presumably relative to the follicular phase in the cycle).

6.2.2 <u>Oral contraceptives and food intake</u>

Studies on the effects of oral contraceptive preparations on food intake and meal patterns are totally lacking. However, there are sparse reports on changes in weight (see Table 6.1). Nilsson and Sölvell (1967) and

Nilsson et al. (1967) report weight gain in women taking Anovlar (a combination of ethynyl oestradiol and norethisterone acetate); but it is not known whether such an increase is due to an increase in food intake or water retention. In Chapter 5 it was shown that this hormonal combination reduced food intake and body weight in both the ovariectomized and intact rat. Obviously, comparisons between effects observed in rats and in humans should not be made too readily. The pattern of weight gain was not reported in human studies. It is possible that the weight gain was caused by an increase in food intake by women as a reaction to an initial weight loss. From studies presented in Table 6.1 and others, the general impression is that weight change is highly variable: some women put on weight while others lose weight. It is quite likely that this variability is due to the lack of control in these studies. Strict control over a more constant diet in addition to a more uniform environment would be necessary to show such changes rigorously. It would be of particular interest to see whether the initial nausea and vomiting which occur in women taking certain oral contraceptives (those with a high oestrogen content) are in any way related to changes in food intake.

6.2.3 Oestrogens and feeding disorders

The question of whether food intake in women is free from oestrogenic influences is of more than academic interest. Certain eating disorders show, firstly, a remarkable sex difference in their incidence, and secondly, a frequent association with changes in or disorders of gonadal function. A clinical syndrome associated with disturbances in food intake is anorexia nervosa, which occurs ten times as frequently in women as in men. Anorexia nervosa is becoming more common (Garrow, 1976), but the reason for this is not known.

Both ovarian (Drewett, 1973a) and neuroendocrine (Russell, 1975) involvement in the manifestation of anorexia nervosa have been proposed;

but conclusive evidence is still lacking. These possibilities stem from the intriguing fact that most, if not all, of the symptoms shown by anorexic patients can be reproduced in some species by endogenous or exogenous oestrogens. The drop in food intake and body weight at oestrus (Drewett, 1973a), and similar phenomena shown by ovariectomized rats treated with oestradiol (Wade, 1975) are well documented. Further, ethynyl oestrogens, and particularly ethynyl oestradiol, have been shown to reduce food intake and body weight considerably in the female rat (Chapter 5). Vomiting, which is a common symptom in women with anorexia nervosa, is impossible in the rat (Code and Schlegel, 1968). However, nausea is a common side effect of oestrogen administration in women (Astwood, 1965), and in women taking combined oral contraceptive formulations (see Table 6.1).

6.2.4 A possible association between oral contraceptives and anorexia nervosa

It has already been mentioned that the incidence of anorexia nervosa is on the increase. Oral contraceptive use has also increased dramatically over the last decade. It would not be unreasonable to look for a possible association between the two. Studies on the behavioural effects and metabolism of oestradiol and the ethynyl oestrogens, both in rats and in humans, may shed some new light on the possible involvement of contraceptive steroids with certain feeding disorders.

Fishman, Boyar and Hellman (1975) investigated the metabolism of oestradiol in girls with anorexia nervosa, and compared it with obese subjects and normal weight, age and sex-matched controls. It was noted that the malnourished females converted oestradiol to the catecholoestrogen, 2-hydroxyoestrone, to a far greater extent than either obese patients or the normal weight controls. Increasing body weight was accompanied by a sharp decrease in catecholoestrogen formation and an increase in oestriol production. The reciprocal relationship found in women with anorexia nervosa

and obese women provides strong evidence that the alterations in oestradiol metabolism are not specific to these disorders, but are related to changes in body weight, body composition and/or nutrition. It is possible that the excessive 2-hydroxyoestrone formation was the cause rather than the result of the hypophagia in these women. In this respect, Garattini and Fishman (1975; cited in Fishman, 1976) evaluated the effect of this oestrogen on food intake in rats. They report that in initial trials 2-hydroxyoestrone significantly reduced food intake.

The demonstrated role of body weight in influencing the direction of oestradiol metabolism may play a significant role in such body weight and body composition related endocrine-controlled events as the onset of puberty, anovulation and breast or endometrial carcinoma. Anorexia nervosa begins most frequently in adolescence, and anorexic patients behave as if they had an abnormally low body weight "set point" (Russell, 1975; Russel, Campbell and Slade, 1975), but equally they might experience a threshold weight below which they need to be. Drewett (1973a) suggests that anorexia nervosa may represent a hypersensitivity reaction to oestrogens during puberty. Adolescents taking oral contraceptives may, likewise, show a reaction to the oestrogen component in the pill. This could give rise to changes in oestradiol metabolism leading to decreased food intake and consequently, lower body weight. This new low level in body weight could then influence subsequent oestrogen metabolism; that is, a positive feedback system. An analogous situation occurs in obese women: the increase in the amount of adipose tissue results in further raised levels of oestriol (Wolff, 1976). This oestrogen is highly uterotrophic, and further it is not converted to catecholoestrogen (Fishman et al., 1960).

Ethynyl oestradiol is used extensively as the oestrogen component in combined-type contraceptive steroids. In this thesis, evidence was presented (Chapter 5) that both ethynyl oestradiol and mestranol reduced

food intake significantly, and more so than oestradiol, in the female rat. It was argued that the greater metabolism of ethynyl oestradiol to catecholoestrogen may have caused, partly, the severe decrease in food intake shown by rats treated with this oestrogen. Clearly, much confirmatory work needs to be done; but the role of catecholoestrogens in the control of food intake warrants attention.

6.2.5 General considerations

As with sexual behaviour, the choice of food and the meal patterns associated with food intake in humans seem to have become more influenced by social and cultural conditioning, to the extent that hormonal effects are masked. It is possible, however, that changes in the former lead to changes in the latter, with serious consequences. For example, changes in eating habits influenced by current social and cultural trends, and which give rise to a decrease in body weight, could cause hormonal changes in some women. These changes could be a prelude to the appearance of certain clinical disorders, such as anorexia nervosa. The additional introduction of potent, exogenous oestrogens in the form of contraceptive steroids may be a further stimulus in triggering metabolic changes, particularly in the immediate years following, puberty. Prospective studies employing randomized clinical studies, and other screening procedures over not less than four years are needed and are ethically justified.

6.3 Concluding Comments

In this chapter, an attempt is made to relate work on the control of feeding and sexual behaviour in the rat to clinical observations of behavioural changes in women, with particular reference to the effects of hormones used in oral contraceptives on sexual behaviour and food intake. It is not easy to demonstrate any relationships that may exist with satisfactory rigour, largely because of the lack of adequate experimental studies on mental, somatic and sexual changes in women taking contraceptive

steroids. It is of some concern that so many women the world over are taking oral contraceptives, which have not as yet been tested for behavioural effects, although much has been done with regard to their pharmacological properties. It is hoped that experiments presented in this thesis will provide the necessary stimulus and outline the trend for such work.

APPENDIX A

This Appendix presents tables of means for Experiments 5(a), 5(b), 8 and 10. A note regarding the Analyses of Variance presented in this thesis is included at the end of the Appendix.

Table A1 (i)

Experiment 5(a). Reassessing sexual motivation in the female rat. Daily mean runway times (in seconds) of sexually receptive females running to castrate males, over trials

Day \ Trial	1	2	3	4	5	6
1	44.85	137.64	180.00	180.00	-	-
2	51.22	129.35	111.99	143.72	-	-
3	51.34	77.92	111.33	92.49	-	-
4	18.20	70.09	76.26	52.93	-	-
5	30.26	93.28	120.38	86.29	-	-
6	10.97	24.33	50.63	21.22	-	-
7	7.10	33.80	32.02	36.82	-	-
8	12.36	15.60	21.59	10.46	-	-
9	6.23	14.47	35.78	30.05	23.64	10.60
10	4.73	9.27	11.00	18.55	34.46	32.50

Table A1 (ii)

Experiment 5(a). Reassessing sexual motivation in the female rat. Daily mean runway times (in seconds) of sexually receptive females running to sexually active males, over trials

Day \ Trial	1	2	3	4	5	6
1	35.14	121.18	116.31	118.62	-	-
2	40.76	112.45	89.50	121.54	-	-
3	15.31	40.91	72.01	67.58	-	-
4	8.77	32.24	25.93	35.97	-	-
5	6.44	37.88	21.43	21.31	-	-
6	5.63	13.69	26.86	22.90	-	-
7	3.83	9.78	7.77	9.98	-	-
8	4.89	8.45	7.80	14.66	-	-
9	3.76	7.21	4.44	8.16	8.18	5.80
10	2.84	2.81	5.65	7.00	9.81	7.18

Table A2

Experiment 5(b). Sexual motivation in the oestrous or anoestrous rat. Mean runway times (in seconds) of oestrous and anoestrous females running to sexually active or castrate males, over trials

	Sexually Active Males		Castrate Males	
Trials	Oestrous Females	Anoestrous Females	Oestrous Females	Anoestrous Females
1	4.38	5.59	6.43	8.86
2	6.07	6.31	24.49	10.80
3	8.56	10.24	46.75	11.98
4	8.93	22.88	18.43	39.15
5	6.78	11.07	20.17	37.67
6	24.56	18.74	24.43	24.86
7	9.94	28.26	29.64	70.60
8	7.38	44.75	27.79	77.07

Table A3

Experiment 8. The sexual attractiveness of male rats: An endocrinological dissection. Daily mean runway times (in seconds) over trials

(i) Females running to oil treated castrates

Day \ Trial	1	2	3	4	5	6	7	8
1	68.68	118.80	107.66	145.49	172.70	175.11	143.66	130.66
2	35.34	56.44	61.71	97.84	103.06	76.58	62.56	88.67
3	16.86	22.02	69.11	49.71	76.03	60.08	91.26	98.72

(ii) Females running to castrate males treated with dihydrotestosterone (DHT)

Day \ Trial	1	2	3	4	5	6	7	8
1	48.89	16.32	61.51	74.81	106.67	125.68	103.14	95.37
2	13.56	40.11	76.86	68.58	58.57	64.97	69.59	41.16
3	6.63	4.91	14.42	17.88	41.00	27.59	27.45	16.23

(iii) Females running to castrate males treated with DHT in combination with oestradiol benzoate (DHT-OB)

Day \ Trial	1	2	3	4	5	6	7	8
1	52.28	22.74	47.64	20.24	25.80	24.87	46.02	23.55
2	12.04	7.71	5.53	11.35	12.75	13.70	13.49	18.27
3	6.83	5.83	9.83	4.14	16.51	11.33	9.10	22.55

Table A4

Experiment 10. A second study on the sexual attractiveness of male rats: Olfactory and behavioural components. Daily mean runway times (in seconds) over trials

(i) Females running to oil treated castrates

Day \ Trial	1	2	3	4	5	6	7	8
1	27.68	63.03	117.51	145.82	117.22	150.41	125.52	89.42
2	25.35	67.53	45.75	80.10	80.91	34.23	96.69	102.76
3	31.70	42.62	54.74	116.92	87.95	69.04	57.47	71.65

(ii) Females running to castrate males treated with dihydrotestosterone (DHT)

Day \ Trial	1	2	3	4	5	6	7	8
1	24.58	30.41	63.54	36.53	91.69	56.03	73.95	99.44
2	11.16	26.50	25.74	74.08	46.10	25.46	61.53	32.24
3	7.80	16.95	15.03	19.76	23.32	31.42	17.52	19.42

(iii) Females running to castrate males treated with DHT in combination with oestradiol benzoate (DHT-OB)

Day \ Trial	1	2	3	4	5	6	7	8
1	27.92	20.43	17.93	15.86	38.46	39.00	33.86	33.91
2	9.69	10.34	14.00	13.76	12.26	8.82	11.17	8.40
3	4.61	6.29	6.70	6.78	6.39	7.53	4.93	7.72

Analysis of Variance

Analyses of variance were performed using a standard, tested computer programme - "BWANOVA". The output (means, sums of squares, ratios, etc. ...) from this programme was given accurate to 4 decimal places. However, the values presented in summary tables, and tables of means, were corrected to 2 places of decimal.

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