THE EFFECTS OF CASTRATION AND ANDROGEN REPLACEMENT THERAPY ON THE ABILITY OF THE MALE TO INFLUENCE THE OESTROUS CYCLE OF THE UNDERFED RAT

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It is established that stimuli associated with the presence of a sexually mature male can influence the oestrous cycle of the underfed rat (Cooper & Haynes, 1967). This effect is still present when physical contact between the sexes is prevented, the lengthened and irregular cycles characteristic of undernutrition becoming significantly shorter (McNeilly, Cooper & Crighton, 1970; Purvis, Cooper & Haynes, 1971). A similar response to the proximity of the male has been demonstrated in the mouse, the lengthened and irregular oestrous cycles usually observed when mice are grouped not being found in the presence of a male (Whitten, 1957). Castration of the male prevented the acceleration of oestrous behaviour, but the stimulus value of the male for the female returned following androgen therapy (Bronson & Whitten, 1968). The object of the work described here was to determine whether the reduction in oestrous cycle duration induced in underfed rats by the proximity of a male was also androgen-dependent.

All the rats used were weaned at 21 days of age and housed in groups of ten to twelve in large communal cages until selection. The female rats were allocated to the experiments when they were approximately 5 months of age and weighed 150 to 200 g. The male rats were selected at approximately 6 months of age and weighed 250 to 300 g. Castration of a number of males was carried out when they were 28 days old. During the experimental period, all animals were housed individually in metal battery cages. Each cage was divided into two equal parts by means of a wire barrier as described by McNeilly *et al.* (1970) in order to minimize direct physical contact between animals.

Two experiments were carried out. In Exp. 1, forty female rats were allocated at random to the following treatment groups: ten animals were allowed unrestricted access to food. The remaining thirty animals were subjected to a restricted dietary regimen consisting of 65% by weight of the mean daily food consumption of the unrestricted group. Ten of these animals were fed this restricted diet only, ten were underfed in proximity to intact males and ten were underfed in proximity to castrate males. For Exp. 2, twenty female rats were allocated at random to two treatment groups. Ten animals were fed a restricted diet as described in Exp. 1 in proximity to an intact male and the

remaining ten animals were underfed in proximity to a castrate male which had been given a subcutaneous injection of testosterone propionate (30 µg/100 g body weight) each day for a period of 14 days preceding introduction to the cages in which the females were housed.

From the time of the first vaginal smear, all females were allowed to complete two oestrous cycles on an unrestricted feed intake and the underfed females completed either two or three (Exp. 1) or three (Exp. 2) oestrous cycles when subjected to dietary restriction. Thus, changes in dietary regimen were made

TABLE 1
DURATIONS OF OESTROUS CYCLE IN RATS (EXPERIMENT 1)

Sequential treatments	Duration of oestrous cycle (days)			
	Exposed to males		Controls	
			Underfed throughout	Unrestricted throughout
Unrestricted feeding for two oestrous cycles	4.2 ± 0.40 4.6 ± 0.18	3.6 ± 0.24 4.1 ± 0.20	4.6 ± 0.18 4.0 ± 0.24	4.1 ± 0.24 4.8 ± 0.20
Underfeeding for two oestrous cycles	5.3 ± 0.29 5.0 ± 0.34	5.0 ± 0.41 5.3 ± 0.29	4.9 ± 0.35 4.6 ± 0.31	4.0 ± 0.21 4.6 ± 0.26
Underfeeding + male for one oestrous cycle	Intact males 3.7 ± 0.29	Castrate males 5.6 ± 0.41	5.8 ± 0.43	4.3 ± 0.24 4.5 ± 0.34

TABLE 2
DURATIONS OF OESTROUS CYCLE IN RATS (EXPERIMENT 2)

Sequential treatments	Duration of oestrous cycle (days)	
Unrestricted feeding for two oestrous cycles	4.0 ± 0.15 4.2 ± 0.26	4.5 ± 0.22 4.4 ± 0.43
Underfeeding for three oestrous cycles	4.9 ± 0.46 5.0 ± 0.71 5.0 ± 0.56	5.9 ± 0.71 7.2 ± 0.75 6.9 ± 0.51
Underfeeding + male for one oestrous cycle	Intact males 3.8 ± 0.28	Castrate males after androgen therapy 4.3 ± 0.33

at different stages in the cycle in different individuals. The males were placed in proximity to the females following this period of four or five oestrous cycles for the duration of one cycle, introduction being made on the day of metoestrus in each case, following which the experiment was terminated. The males were introduced into the battery cages overnight for a total of 15 hr (from 18.00 to 09.00 hours) in each 24 hr, the pattern previously established by Cooper & Haynes (1967) and McNeilly *et al.* (1970). During the intervening period, all males were housed in communal cages and selected at random for introduction to the cages of the females each evening since previous work showed that females

appeared to become less sensitive to the presence of a particular male with time (McNeilly *et al.*, 1970). Oestrous cycles were monitored by the examination of daily vaginal smears.

The results of the two experiments are summarized in Tables 1 and 2. Statistical evaluations were by analysis of variance. Underfeeding for two oestrous cycles caused an overall significant increase in cycle length ($P < 0.05$). The proximity of intact males for one oestrous cycle significantly shortened this cycle length to a value more closely associated with that found in unrestricted animals ($P < 0.01$). This response to the male was not observed when underfed females were in proximity to castrate males, but was obtained with castrate males following the period of androgen replacement therapy ($P < 0.01$).

The experiments described confirm the observation of Cooper & Haynes (1967) and McNeilly *et al.* (1970) that a marked shortening of the duration of the oestrous cycle occurs when an underfed rat is placed in proximity to a male. The ability of the male to induce the acceleration of oestrus is dependent upon the presence of testes, the response not being apparent after castration. The finding that androgen replacement therapy is able to restore the stimulus value of the male for the female is similar to that in experiments carried out on mice (Bronson & Whitten, 1968) and indicates that whatever mechanism is responsible for the normal expression of male sexual stimuli, it is probably to a large extent dependent upon testicular steroid secretion. It is not possible to state with certainty the exact nature of the stimulus which can be transmitted from one animal to another under conditions where physical contact was not completely excluded but it seems likely, on the basis of comparable experiments carried out with mice, that this is largely pheromonal.

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