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SPONTANEOUS PSEUDOPREGNANCY AND OBESITY IN THE RAT

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In the two preceding papers we discussed the normal hypothalamic integration of energy balance with the sex cycle in the rat, both as seen in the adult and during growth. In the course of this work we observed abnormal rhythms involving the same variables. Such abnormal cycles have been described before, but in different circumstances, and have been shown to consist of a succession of pseudopregnancies (Van der Lee & Boot, 1955; Richter, 1957). In this short paper we wish simply to record the factors which appeared to precipitate these cycles and to consider the possible mechanism.

METHODS

The animals and methods have been described in the preceding papers (Kennedy & Mitra, 1963*a*, *b*).

RESULTS

Abnormal cycles after food restriction. In earlier experiments 25 young rats between 60 and 110 days old were underfed by being given half their normal food ration for 10 days. The immediate effects have been described (Kennedy & Mitra, 1963*a*); after re-feeding the rats behaved quite normally until they were between 130 and 240 days old. The first sign of abnormality was a decrease in running activity and a break in oestrous cycle, with a little increase in food intake and gain in body weight. The length of the break in cycle varied from rat to rat, the extremes being 12 and 21 days. After a variable period of normal activity there was another interruption of the cycle, then a normal interval and so on for the rest of the rat's life (Fig. 1). The length of the inactive periods were fairly constant in any given rat; the normal intervals were more variable.

So far as changes in energy balance were concerned the subsequent history of the rats could be divided into two types. The first is illustrated in Fig. 1. Although in the recovery periods the rat tended to eat rather less than normal and to lose weight, it did not come back to its original level. Moreover, with each successive break in cycle the overeating became more

obvious, reaching as much as 40 g/day, or twice the normal intake and equivalent to hypothalamic hyperphagia. Sometimes the rat gained as much as 80 g in weight during a single break of cycle, only losing 30 or 40 g when the regular cycle returned. So the weight gain continued in a stepwise way until the rat became as obese as after hypothalamic operation, and the general level of activity fell progressively to virtually zero.

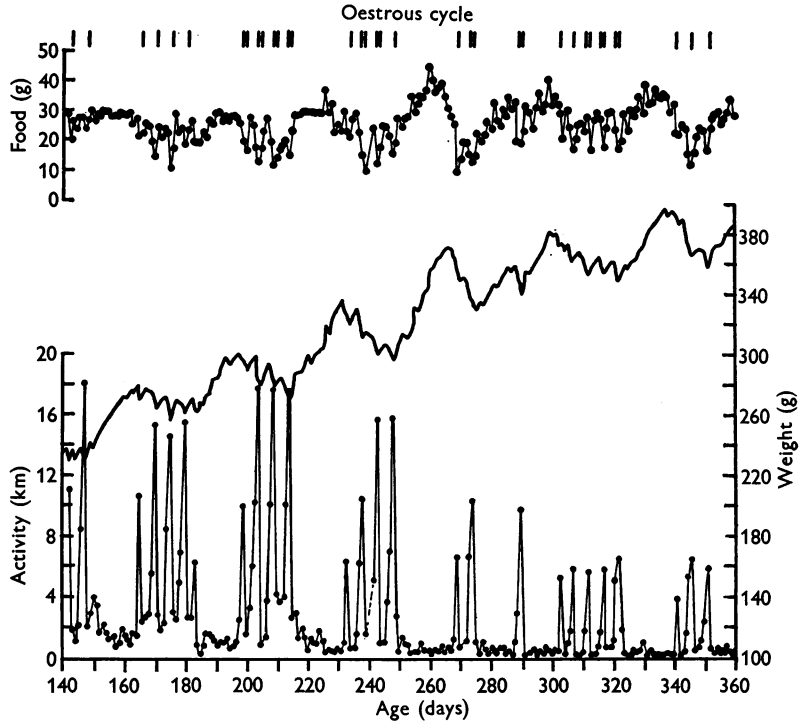

Fig. 1. Abnormal cycles of prolonged dioestrus and diminished activity associated with obesity. Female rats of this strain usually reach a maximal weight of 250 g.

The second type of progress is shown in Fig. 2. Here the weight gained during the abnormal cycle was lost during the recovery period, the fluctuations in food intake never built up to the hyperphagia level seen in the first type, and there was no more than the expected reduction in running activity with age during the periods of normal oestrous rhythm. In persistence and in all other ways the disturbance of oestrus was the same in this type of animal as in the first; it merely differed in the stability of long-term energy balance.

Spontaneous abnormal cycles. Another group of 25 rats were kept as controls to find whether abnormal cycles would appear without preliminary underfeeding. Since the underfed rats had been drawn from groups

growing at rapid, normal and slow rates (Kennedy & Mitra, 1963*b*), similar groups were included in the control experiment. The relative frequency of regular and irregular cycles in the two experiments is given in Table 1. There were slightly but not significantly more irregular cycles in the underfed group, but the incidence was only really high when underfeeding was combined with slow growth, when over half the rats had irregular cycles.

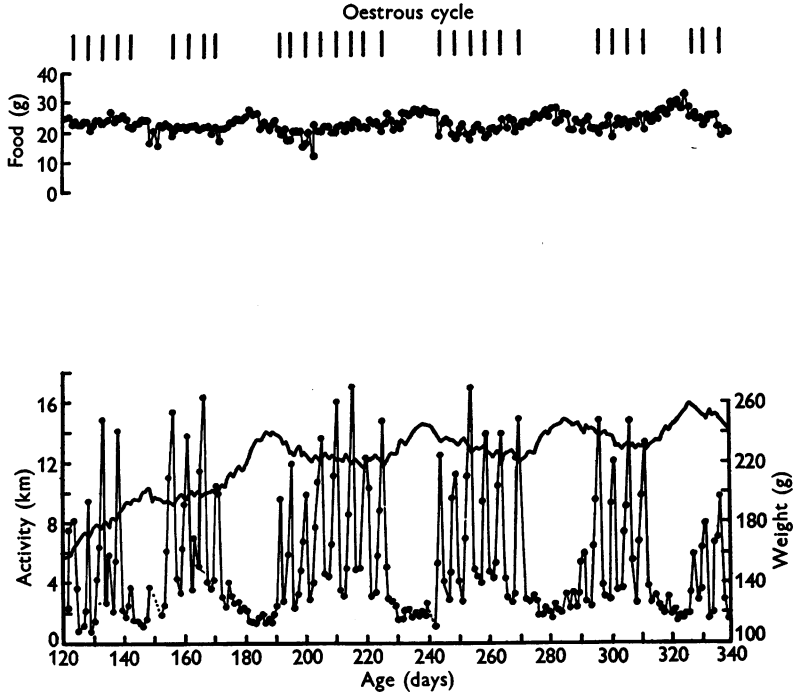

Fig. 2. Similar cycles of dioestrus and inactivity to Fig. 1, without obesity. Activity remained normal during the periods of oestrus.

Other factors affecting the cycle. Experiments had been conducted on rats in activity cages to determine the acute effects of D-amphetamine and of bovine growth hormone. Both had been found on occasion to interrupt the oestrous rhythm, so their chronic effect was studied, and is recorded in Table 1. Amphetamine did not affect the incidence of chronic irregularity, but in view of the high incidence of irregular cycles in small rats, the fall in incidence in growth hormone treated rats was suggestive.

Accordingly growth hormone (1 mg daily for 14 days) was given to 5 rats which had previously been fasting, and would have been expected to show a high rate of irregularity. None subsequently showed abnormal cycles.

TABLE 1. Relative frequency of normal oestrous rhythm and recurrent pseudopregnancy after a variety of treatments

In activity cages

Cause ...	Spontaneous		Underfeeding		Amphetamine		Growth hormone	
Rhythm ...	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Large	5	2	6	2	2	1	5	1
Normal	6	1	2	2	2	—	2	—
Small	7	4	5	8	1	2	2	1
Total	18	7	13	12	5	3	9	2

In ordinary cages

Cause ...	Spontaneous		Underfeeding	
Rhythm ...	Normal	Abnormal	Normal	Abnormal
Large	4	—	—	—
Normal	6	—	4	—
Small	20	—	5	1
Total	30	—	9	1

Abnormal cycles in ordinary cages. Van der Lee & Boot (1955) showed that mice kept in single cages showed a much lower incidence of abnormal cycles than those housed in groups. Assuming the same thing to be true of rats one might have expected little abnormality from the animals housed in separate activity cages, but this factor obviously needed investigation. Thirty rats were studied in individual 'mouse boxes' with opaque sides, $14 \times 6 \times 6$ in. ($35 \times 15 \times 15$ cm) in dimensions. They were otherwise fed and housed in the same way as those in the activity cages. A further 10 were subjected to a period of underfeeding as in the first experiment. As is shown in Table 1, only 1 rat of the 40 developed irregular cycles, but 6 of the 10 underfed rats showed persistent vaginal cornification on re-feeding, and this condition was permanent.

Endocrine investigations. The general characteristics of the abnormal cycles and the work of previous investigators made it almost certain that they represented pseudopregnancies. On the 5th day of abnormal cycles five rats were selected and a silk thread passed through a uterine horn under ether anaesthesia. The rats were killed and the uterus sectioned on the 5th or 6th subsequent day. Three of them showed typical deciduomata. The ovaries, pituitaries, adrenals and thyroids were also sectioned. The three latter were normal in appearance and the ovaries showed abundant corpora lutea.

DISCUSSION

Underfeeding, to which we first attributed the abnormal cycles in our rats, was at most a contributory cause. The same was true of retarded growth initiated while being suckled, although together these two factors had a significant effect, and it was interesting that this effect could be

counteracted by growth hormone treatment for as short a period as 14 days.

Leaving these minor causes aside, merely being in an activity cage was the major factor inducing abnormal cycles in our rats. We were well aware that a considerable environmental stimulus was necessary before rats would run successfully, and one has only to watch the rivalry between neighbours in these cages to realize that running is partly a social activity. One explanation of the abnormal cycles is that they were analogous to the crowding effects described in mice by Van der Lee & Boot (1955) and Mody & Christian (1962) and recently discussed by Parkes & Bruce (1961). This phenomenon seems to depend on smell and to be abolished by removal of the olfactory lobes. Similar abnormal cycles have been described in rats following quite different stimuli such as forced swimming (Richter, 1957), where the communal element is excluded from the initial stimulus. In Richter's experiments, however, the rats were subsequently housed in activity cages, and one cannot say how much this contributed to the abnormality. The same can be said of the observations of Richter, Jones & Biswanger (1959) on partial ablation of the thyroid with ^{131}I, which they found to be followed by pseudopregnancy cycles. It was noteworthy, however, that removal of the frontal lobes in these rats did not abolish the abnormal cycles.

A large variety of stimuli, the majority with no relation to reproductive function, can cause pseudopregnancy in the rodent, although few have been investigated to see whether they cause the recurrent form. From what is known of this phenomenon (Parkes & Bruce, 1961) it seems likely that it is initiated through the rhinencephalon, although the external stimulus is certainly not always olfactory. Indeed Maclean (1960) preferred limbic system to rhinencephalon simply because it avoided the implication of a restricted function, and he specified among its wider functions the control of the sex cycle and of energy balance.

The other persistent abnormality of the cycle which we observed, constant vaginal cornification, was probably also initiated by the limbic system. Its relation to underfeeding has been discussed in the preceding paper, and it is of especial interest therefore that even transient underfeeding during growth can so disorganize the limbic centres that the future release of luteinizing hormone is impaired. It has been shown by Barraclough & Gorski (1961) that injection of androgen during the early growth period in the rat causes a similar disorganization of future ovulation.

Still further evidence of the intimate association between the central regulation of the sex cycle and energy balance was the obesity which occurred in a number of the rats with irregular cycles. This has also been reported by Richter (1957) in his rats subjected to forced swimming. The

stepwise increase in severity of the obesity with successive pseudopregnancies is reminiscent of the common clinical condition in women which Sheldon (1949) called maternal obesity.

SUMMARY

1. A proportion of rats housed in activity cages developed abnormalities of oestrous rhythm, consisting essentially of recurrent pseudopregnancies interspersed with periods of normal rhythm.

2. The pseudopregnancies were accompanied by an increase in weight and food intake and a decreased running activity. Usually the weight returned to normal during the intervals of normal rhythm, but occasional rats developed severe obesity.

3. The frequency of abnormal cycles was increased by restricting food intake either during suckling or later in the growing period. The effect of such treatment could be annulled and abnormal cycles prevented by the subsequent injection of growth hormone.

4. Rats kept singly in normal cages did not develop pseudopregnancies, but in them transient undernutrition led to persistent vaginal cornification.

5. These abnormalities of the oestrous cycle were almost certainly hypothalamic in origin. No changes in size or histological appearance were found in the pituitary or adrenal glands to lend support to the idea that they were 'stress' phenomena.

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