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BODY WEIGHT AND FOOD INTAKE AS INITIATING FACTORS FOR PUBERTY IN THE RAT

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In a preceding paper (Kennedy & Mitra, 1963) evidence was presented that the ventromedial nuclei of the hypothalamus lay at the junction of two regulatory areas, one running anteroposteriorly and concerned with the endocrine and behavioural aspects of oestrus, the other running laterally and concerned with energy balance and motivated behaviour. Both are likely to be involved in initiating puberty, but we could find no evidence that they worked together; but rather that they responded independently to some common stimulus connected with growth.

It is sometimes stated as an axiom that before an animal can breed it must first grow to maturity (Bullough, 1951), but little thought is given to how this synchronization is brought about, and whether maturity is to be measured on a chronological or ponderal scale. The purpose of the present paper is to find out more about this question by examining the effect of variation in growth rate upon energy exchange and the endocrine and behavioural aspects of puberty.

It is possible by chronic underfeeding to delay both growth and sexual development in rats (Ershoff, 1952). However, this by itself tells us little about the relation between puberty and body weight, because of the associated underfeeding. This confusion can be eliminated by the simple technique described by Kennedy (1957). Two litters born on the same day are thoroughly mixed and then returned to the mothers to be reared in groups containing widely different numbers. The sucklings in the small groups obtain enough milk to enable them to utilize their maximal growth potential from birth, but those in large groups are held back during the critical first week when under optimal conditions birth weight may be trebled. Although after weaning on to unrestricted food the retarded rats increase their weight each week by the amount appropriate to that age, the opportunity for rapid reproductive growth that they missed during the suckling period never recurs and they remain permanently small. In this way groups of rats with the same genetic background approach the

expected period of puberty normally nourished but differing widely in body weight. A preliminary study showed that vaginal opening was then more closely related to body weight than to chronological age (Kennedy, 1957).

METHODS

The animals and diet were as previously described (Kennedy & Mitra, 1963) as were the standardized conditions of temperature and lighting. Experiments were conducted during successive summers to avoid seasonal variations in the time of puberty.

Experiment 1. Pairs of litters born on the same day and each consisting of 9–10 pups were mixed, 3 sucklings returned to one mother and the remainder given to the other. When weaned at 21 days the average weight of the optimally fed young was over 40 g, while those reared in large litters weighed between 15 and 25 g. After weaning, both groups were fed *ad lib.* on M.R.C. Diet 41B. Only the females were used for further study. The effect of this procedure was studied on (*a*) somatic growth, (*b*) sexual development, (*c*) food intake and (*d*) sponaneous running activity.

Experiment 2. Twenty-five female rats reared normally in litters of 7–9 were weaned into individual small cages and restricted to a quantity of food which almost stopped growth. They were maintained at their weaning weight for 10 days, then given rather more food so that they increased by approximately 10 g, at which weight they were again held for 10 days, so gaining stepwise until they reached 75 g at 65 days, compared with 45 days for the retarded rats of experiment 1. They were then fed *ad lib.*

Experiment 3. A further 25 rats were treated in the manner of experiment 2, but returned to unrestricted feeding as soon as the vaginas of all of them had opened, when they were 65 g in weight and 55 days old.

Experiment 4. Another series of underfed rats was allowed to grow by 10 g steps to 145 g, pairs being killed before each change in weight to examine ovarian and skeletal maturation. In addition, at each weight over 65 g a pair of rats was injected with 10 I.U. of chorionic gonadotrophin (Pregnyl, Organon Ltd.) daily for 3 days and killed on the 4th day.

Somatic development. Apart from body weight, liver and kidney histology and epiphysial ossification in the skeleton was examined. The livers and kidneys were fixed and sectioned as previously described (Kennedy & Mitra, 1963). The skeletons were examined by first macerating eviscerated, skinned and formalin-fixed carcasses in KOH, as described by Williams (1941). After staining in alizarine the skeletons were visualized by clearing the muscles in glycerine and preserved in pure glycerine for examination. A typical cleared specimen is illustrated in Pl. 1.

Sexual development. The assessment of puberty was based on four criteria; (*a*) vaginal canalization, (*b*) the first oestrous smear, (*c*) the first time when the rat mated after being placed with a male for several hours on the evenings of pro-oestrus and oestrus and (*d*) the onset of pregnancy as judged by a break in the oestrous cycle followed by a successful gestation. All pregnant rats were allowed to rear their litters and the birth and weaning weights were noted. Food intake and locomotor activity were measured as previously described (Kennedy & Mitra, 1963).

RESULTS

Experiment 1

Somatic development. The growth in weight of these rats has been fully described elsewhere (Kennedy, 1957; Widdowson & Kennedy, 1962). The livers and kidneys of the slowly growing rats were retarded in development, but the signs of this were only visible histologically during the

suckling period and will not be described further. Abnormalities of skeletal development were visible throughout the period of sexual maturation, however, and will therefore be considered in greater detail.

The skeletons of normal sucklings confirmed the findings of Simpson, Asling & Evans (1950) that this was a period of very rapid development of new ossification centres, when one day in age caused a marked change in the appearance of the skeleton. The striking differences in appearance between large and small rats of the same age described by Dickerson & Widdowson (1960), which we confirmed, therefore require some sort of

TABLE 1. Apparent 'bone ages' of optimally grown (*O*) and retarded (*R*) rats when compared with 'normal' rats from litters of nine

Chronological age (days)	Group	Bone age (g)	Weight (g)	Weight of normal rat (g)
10	*O*	12	27	18
10	*R*	10	11	16
14	*O*	19	35	27
14	*R*	12	16	18
20	*O*	21	42	36
20	*R*	19	16	27

chronological scale for their quantitative assessment. By comparing them with 'normal' skeletons from litters of nine young, we obtained the 'bone ages' shown in Table 1 for large and small rats at 10, 14 and 20 days old. Neither the precocity of the large rats nor the retardation of the small was very striking when viewed in this way. There was little correspondence between the weights of the large or small animals and normals of the same bone age, but the picture at this stage was complicated by differences of nutrition as well as size.

The effect of variation of growth rate uncomplicated by undernutrition could be studied after weaning, but then few changes in ossification were taking place. Fortunately for our purpose, however, it was shown by Becks, Asling, Simpson, Evans & Li (1948) that there is a narrow bar of cartilage in the lateral epicondyle of the humerus in 35-day-old normal female rats, which fuses with the rest of the epiphysis between 35 and 40 days. This was found to be clearly visible in our rats through the dissecting microscope, and was chosen to examine the relation of bony development to puberty. Two large rats with open vaginas at 33 days had fused epicondyles, whereas small litter-mates killed on the same day had open cartilages. A further pair of large rats in which the vaginas opened at 35 days, together with their retarded litter-mates, confirmed the same finding. A group of small rats were killed at 45 days; those in which the vagina had opened showed fused epicondyles, those with closed vaginas open cartilages. It will be shown later that in rats reared in this way vaginal opening is closely related to ovarian maturation.

Sexual development. Because of the variability in weaning weight among retarded rats, they were subdivided into two groups, giving three groups in all, each consisting of at least twenty-five individuals. They are described as optimally grown (weaning weight over 40 g), retarded (weaned between 20 and 25 g) and very retarded (weaned at less than 15 g). Table 2 records the age and weight at which 3 of our 4 signs of sexual maturation were observed; mating was so nearly invariably followed by conception that the two are not separately recorded.

TABLE 2. The effect of growth rate and of feeding on the weight and age of puberty in rats. The groups are arranged in order of age at first oestrus

	Optimal growth	Retarded growth	Very retarded growth	Underfed (Expt. 3)	Underfed (Expt. 2)
Age (days: mean ± S.D.)					
Vaginal opening	38·1 ± 5	43·4 ± 4	49·0 ± 6	49·5 ± 12	46·7 ± 14
Oestrus	38·4 ± 5	46·6 ± 6	50·6 ± 6	61·3 ± 11	68·8 ± 14
Mating	41·1 ± 6	51·0 ± 10	56·7 ± 9	66·7 ± 9	76·3 ± 15
Weight (g: mean ± S.D.)					
Vaginal opening	91·3 ± 12	78·0 ± 9	83·2 ± 12	61·5 ± 17	62·7 ± 18
Oestrus	92·7 ± 11	89·2 ± 14	90·4 ± 14	93·2 ± 10	105·2 ± 9
Mating	102·4 ± 15	99·1 ± 21	102·0 ± 18	104·1 ± 19	124·2 ± 25

To consider age first. Whatever criterion was used, all three groups reached puberty at a different age ($P < 0{\cdot}01$). Moreover, within both retarded groups the various signs were seen at different times ($P < 0{\cdot}01$), although this was not so in the large rats. The significance of this difference was established both by analysis of variance of the actual ages, and by comparing by χ^2 the frequencies of the events in different days, taking day 1 for each rat as the day the vagina opened. In most optimally grown rats the vagina opened, oestrus occurred and successful mating took place on the same day, whereas most retarded rats did not show oestrus until a day or two after vaginal opening and even then did not mate until the second, third or even fourth cycle.

To look next at the weights; vaginal opening was more closely related to weight than to age, but there was still a significant difference in weight between groups ($P < 0{\cdot}01$). The difference was not apparent for the weights at first oestrus, and mating also occurred at virtually identical weights in the three groups. Although the large standard deviations indicate that other factors were probably concerned, weight was a much more important determinant of the onset of reproduction than age.

Food intake. Table 3 records the mean food intake of the groups of optimally grown and retarded rats expressed in relation to body weight. This was done by calculating the mean intake for each rat on the day it reached, say, 50 g, the day before and the day after and then taking an

over-all mean. It will be seen that the intake in absolute terms rose in virtually the same way in both groups. When it was expressed in terms of body weight, however, the intake fell by almost half between 50 and 150 g, but again in almost precisely the same manner in the two groups. Note particularly the identity of intake at 100 g, the approximate weight of puberty.

TABLE 3. The relation of food intake to body weight in rats growing at different rates

Body weight (g)	Optimal growth		Retarded growth	
	Mean intake (g/day)	Intake (g/100 g)	Mean intake (g/day)	Intake (g/100 g)
50	9·6	19·3	10·4	20·7
75	11·8	15·7	13·1	17·5
100	16·4	16·4	16·4	16·4
125	18·9	15·1	18·2	14·6
150	21·6	14·4	19·1	12·7

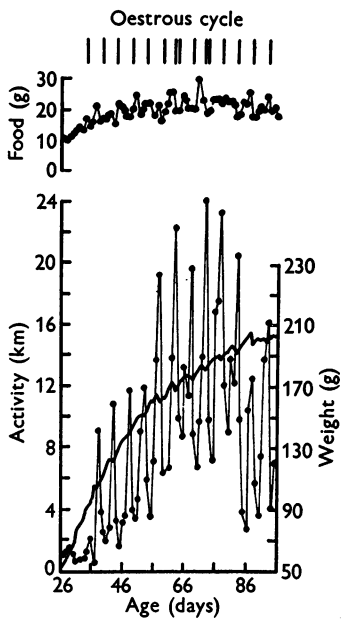
Activity. Although there was considerable variation, particularly in the retarded groups, and it was impossible to attach an arbitrary date to the onset of running in any given rat, nevertheless, there was an obvious delay in the development of the adult running pattern in the small rats. This is illustrated in Text-figs. 1, 2. Also included in the figures is a record of oestrous cycles; it can be seen that in the optimally grown rat activity built up quickly in the early cycles, whereas in the very retarded rat the sixth cycle was reached before activity began. This was of course one of the extreme cases, but it illustrates the same tendency as we found with mating for behavioural arousal to be delayed in these rats more than the endocrine aspects of oestrus.

Experiments 2 and 3

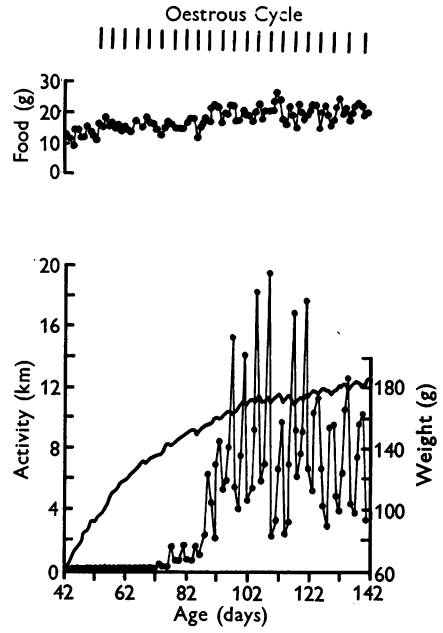
As will be seen from Table 2, the vaginas of underfed rats opened at about the same age as retarded rats, but at a much lower weight. In general, there was no oestrous cycle while underfeeding continued, although 4 rats of the 50 showed transient vaginal cornification within a day or two of vaginal opening.

When re-feeding began in experiment 2 the rats increased in weight very rapidly, from 75 g to an average of 105 g in 3 days. On the 3rd day their mean food intake was 16·7 g and therefore did not differ significantly from that of normally fed rats of the same weight, and 19 of the 25 rats were in oestrus. The remaining six showed oestrous changes within the following 3 days and the mean weight at first oestrus for the whole group was somewhat higher than the retarded rats. Because of the synchronous onset of cycles in so many of the rats it seemed possible that earlier refeeding might start cycles at a lower weight, so in experiment 3 it was begun at

65 g. Only 9 of the 25 rats began their cycles synchronously, after the same 3-day interval, and it appears that follicular development in them was sufficiently advanced for the additional stimulus of feeding to lead to virtually immediate ovulation; the latent period will be discussed later. The delay in onset of cycles among the remainder was rather greater than in Experiment 2, and the mean weight for the whole group was indistinguishable from that in the retarded groups. Both underfed groups were considerably older, however.



Text-fig. 1



Text-fig. 2

Text-fig. 1. Food intake, activity and body weight in relation to the onset of oestrous cycles in an optimally grown rat. Both oestrus and running activity began at about 36 days of age.

Text-fig. 2. The relation of activity to puberty in a very retarded rat; although oestrus began at about 52 days, activity was even further delayed.

Mating behaviour yielded somewhat similar results. In experiment 2 mating was delayed to an average weight substantially higher than in retarded rats, while in experiment 3 it occurred at the same weight as in the retarded animals. Only in experiment 2 was any significant delay in the development of full fertility noted; the majority of the rats did not then become pregnant on the first mating, but all did on the second. In all other groups the first matings were fertile in more than 90 % of cases. The length of gestation, litter size and birth weight of the young showed no significant difference from those of normal young adult females of the

colony, although the weaning weights of the young were a little lower than those of older mothers, as is usual with first litters in small rats. There were no differences in mortality in the young of optimally grown, retarded or underfed rats.

Experiment 4

Ovarian development. The ovaries of underfed rats of successive weights showed a steady increase in size, both of the organs as a whole and of the follicles. The pair killed at 65 g, just after vaginal opening, had a few small corpora lutea, but there was no further evidence of ovulation in the bigger rats. Most of the rats over 100 g in weight showed persistent vaginal cornification during life, and their ovaries contained follicles of all sizes, some cystic, but no corpora lutea; the interstitial tissue was normal. All the ovaries from rats injected with chorionic gonadotrophin had fresh crops of corpora lutea.

Skeletal development. The lateral epicondyle of the humerus was fused in all the rats of 75 g or over, the weight at which re-feeding led to immediate onset of cycles.

DISCUSSION

These experiments demonstrate that the onset of puberty in the rat is influenced both by body size and by food intake, and in conjunction with those reported in the previous paper give us some idea of the central nervous mechanisms involved. Information about the state of somatic development would appear almost essential to the hypothalamus in order to regulate not only puberty but other pituitary functions during growth. Such information might be conveyed by fairly direct chemical feed-backs, or it might be acquired indirectly from the alterations in energy balance which are at least in part due to the changing relation between the growing animal and its environment. In trebling its weight, say from 50 to 150 g, the rat only doubles its surface area. This altered relation between radiating surface and heat-producing mass requires a reduction of metabolic rate if the temperature gradient between the rat and its environment is to be maintained (Thompson, 1942; Brody, 1945). Its effect resembles that of a rise in environmental temperature, and hypothalamic centres which react to such environmental changes in the adult would be expected to respond similarly to effects of growth which simulate them. Marshall (1936, 1942) and others have stressed the importance of such exteroceptive factors as food intake, light and temperature in initiating sexual activity in seasonal breeders, and they can be shown to influence puberty in the rat (Luce-Clausen & Brown, 1939; Fiske, 1941; Ershoff, 1952). The stable relation between puberty and changes in energy balance when we varied growth rate suggested that food intake or its correlate metabolic rate may act as the normal signal to initiate puberty.

Donovan & Van der Werff ten Bosch (1959), Donovan (1960) and Harris (1961) have argued convincingly that before puberty the anterior pituitary and ovaries are functionally active and related by a feed-back in much the same way as in the adult, but the feed-back is so adjusted that ovarian activity is restricted to a low level. Moreover, these authors showed that lesions in the anterior hypothalamus led to sexual precocity, the opposite of what one might expect if puberty depended on the structural maturation of the C.N.S. One must conclude that growth somehow alters the feed-back between the ovaries and the hypothalamus. Harris (1961) suggested that the change might depend on a reduction in the sensitivity of the nervous centres to oestrogen, since Hohlweg & Dohrn (1931) showed that increasing doses of oestrogen were required as rats grew older to prevent histological changes in the pituitary following castration.

Could such a change in sensitivity be related to a change in metabolic rate? The action of thyroid hormone on ovarian function is probably a complex one, and effects on pituitary release on gonadotrophins, ovarian sensitivity, and peripheral inactivation both of gonadotrophin and of oestrogen have all been suggested (Jones & Ball, 1962). Nevertheless, there seems general agreement with Van Horn (1931) and Chu & You (1945) that thyroid-fed animals show inhibition of oestrous cycles, at least with large doses, and a high pituitary concentration of gonadotrophin. In this connexion it is significant that in the third and fourth week of life, when the metabolic rate of the rat rises to about twice the adult level (Brody, 1945) the concentration of gonadotrophin in the pituitary rises to a value many times that in the adult gland (Lauson, Golden & Sevringhaus, 1939). The close association we found between puberty and skeletal maturation also suggests a link with the thyroid, since Becks *et al.* (1946) showed that epiphysial development is under the control of the thyroid, whereas increase in size of the skeleton depends chiefly on growth hormone.

The possibility of more than one stimulus to the hypothalamus, or more than one site of action, was suggested by the way in which puberty, which had a monolithic character during optimal growth, broke up into a series of separate events when growth was retarded. Under these conditions vaginal opening bore little relation to the other signs of puberty, to age or to body weight. Although widely used as an index of ovarian development it is manifestly an unreliable one. The stress of food restriction or even of premature introduction into an activity cage (unpublished observation) sometimes caused the vagina to open very early, and this is in accord with the effects of other stresses described by Mandl & Zuckerman (1952).

When we retarded growth without chronic underfeeding the onset of cycles and of mating was dependent on weight, but the two events were separated in time. However, the beginning of ovarian activity was capable

of further sub-division, as we saw when growth was held back for a still longer period by restricted feeding. Although the follicles then grew normally they did not produce enough oestrogen to cornify the vaginal mucosa before the weight at which cycles began in normally fed rats. Even after that, cycles did not begin so long as food was short, although ovulation could readily be produced by preparations containing luteinizing hormone. Moreover, continued growth after the expected weight for puberty led to persistent vaginal cornification and the formation of cystic follicles. Now these latter changes can be induced in the adult rat by anterior hypothalamic lesions of the type said to interfere with luteinizing hormone release, and Flerkó (1954) showed that such lesions also prevented the gonadal atrophy normally induced by oestrogens. Conversely, transplants of ovarian tissue in the anterior hypothalamus depress the release of gonadotrophin (Flerkó & Szentágothai, 1957). This appears the most probable site for the feed-back of oestrogens on gonadotrophin release, as well as for any change in sensitivity of this feed-back which may take place during growth. As was discussed in the preceding paper (Kennedy & Mitra, 1963) the anterior hypothalamus is not essential for follicular growth, but it appears to exert an over-all regulation and in particular to control ovulation. It is therefore noteworthy that Van Dyke, Simpson, Lepkovsky, Koneff & Brobeck (1957) found that anterior hypothalamic lesions which induced constant vaginal cornification also doubled ^{131}I uptake by the thyroid, direct evidence of the intimate relation of this regulatory mechanism with thyroid function.

Follicular growth was sufficiently advanced as the underfed rats approached the expected weight for puberty for normal cycles to begin as soon as free access to food was allowed. The specificity of food as a stimulus was shown by the synchronous onset of cycles; a similar synchrony has been observed in response to exteroceptive factors in mice, with the same latent period of three days (Whitten, 1956).

Finally, as we have noted, mating behaviour was often absent in retarded rats even after normal cycles had begun. In general, when mating did take place it was fertile and so it can be inferred that ovulation was taking place. Perry & Rowlands (1962) point out that there is good reason to suppose that in many mammals ovulation and oestrus are not synchronized immediately at puberty. In our retarded rats this absence of appropriate motivation during the early cycles was accompanied by delay in running, which suggests an inadequate oestrogenic stimulus to the ventromedial hypothalamic centres. Under adverse environmental conditions, therefore, one can trace the operation at puberty of the same three central nervous mechanisms we discussed in the control of reproductive behaviour in the adult. All three appear to be sensitive to changes in

energy metabolism, although clearly they respond to other stimuli as well. The most direct relation to food intake is shown by the centres initiating ovulation.

SUMMARY

1. The growth rate of young rats was modified either by variation in the milk supply during suckling, followed by normal feeding, or by chronic underfeeding, and the effect of both methods on puberty was studied.

2. In optimally fed rats vaginal canalization, the first oestrus, mating and attainment of full fertility usually occurred on the same day, whereas in retarded rats these events became separated in time.

3. Retardation without chronic underfeeding led to delayed but normal oestrous cycles, which began at the same body weight as in optimally grown rats. Food intake and skeletal maturation also bore a constant relation to the onset of ovarian activity, independent of age.

4. Chronic underfeeding prevented normal cycles, but not follicular growth, which eventually led to the development of follicular cysts and persistent vaginal cornification. If groups of chronically underfed rats were re-fed when they reached the expected weight for normal puberty oestrus began synchronously after a delay of 3 days. Skeletal maturation and food intake were again appropriate to the weight.

5. In retarded rats the first few oestrous cycles frequently failed to provoke mating behaviour. Similarly, they were not accompanied by the running activity which the oestrous rat characteristically exhibits.

6. These observations were discussed in relation to what is known of the hypothalamic control of energy balance and ovarian function.

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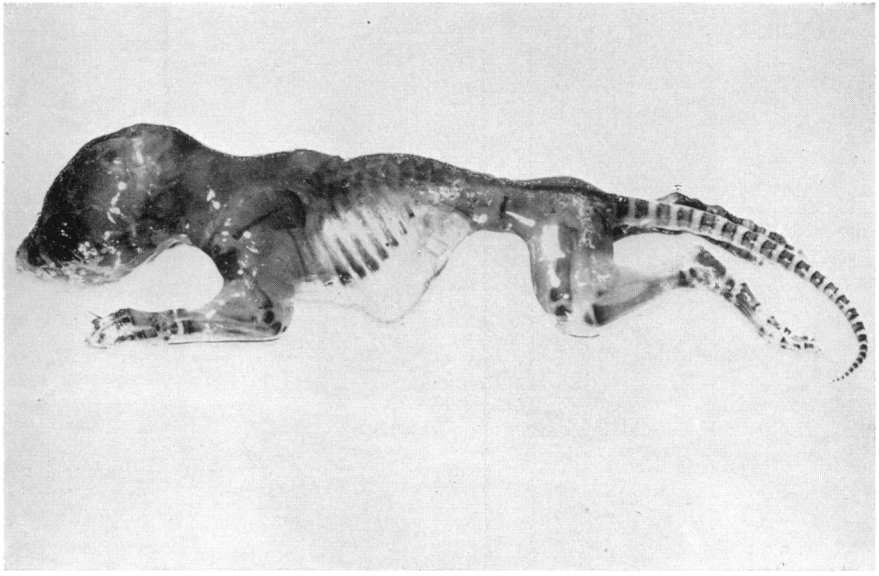
EXPLANATION OF PLATE

Skeleton of normal rat age 20 days to illustrate the alizarine staining method described in the text.



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