# Body growth, puberty and undernutrition in the male guinea-pig

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1. Male guinea-pigs were assigned to four different groups at birth. The heaviest group of animals was severely undernourished from 21 d of age (weight gain: 1·2 g/d *v.* 10 g/d for normally-fed animals).

2. At 35, 45, 55 and 65 d of age in normally-fed animals, and at 45 and 55 d of age in undernourished animals, blood testosterone levels were determined by radioimmunoassay, epididymidal tissue examined for the presence of spermatozoa and testes sectioned for rating of spermatogenesis using light microscopy.

3. Sexual maturity in terms of potential fertility (spermatozoa in the epididymis) was reached by all normally-fed animals between 45 and 55 d of age. High birth-weight animals had a higher incidence of the most advanced stages of spermatogenesis than low birth-weight ones at the various ages studied. In the undernourished animals spermatogenesis was clearly delayed.

4. Plasma testosterone concentrations were already in the adult range at 35 d and independent of age and birth-weight. The undernourished animals, however, had very low levels. Maintenance of spermatogenesis seemed compatible with low plasma levels of testosterone.

5. It is concluded that the timing of testicular development seems to be determined by the time the animals are born and appears to be unaffected by severe undernutrition from 21 d of age.

Investigations using various species have indicated that puberty may start earlier in individuals that are large than in those that are small during growth. Investigations have been made in the rat by Kennedy & Mitra (1963) and in man by Tanner (1962). In the female guinea-pig clear correlations have been established between body-weight at birth and the rate of post-natal growth in body-weight (Lister & McCance, 1965) and with the age at which the vagina first opened (Slob *et al.* 1973).

The present study was undertaken to examine the effects of body-weight at birth, and of undernutrition during growth, upon the time of onset of puberty in male guinea-pigs. Criteria for the onset of puberty needed to be established. Therefore, a mixed longitudinal-cross-sectional growth study was carried out, in the course of which animals were killed at regular intervals. Testes and epididymidal tissue were examined for sperm production. Blood was collected for testosterone determinations. Part of this study has been reported previously (Slob *et al.* 1975).

## EXPERIMENTAL METHODS

Albino guinea-pigs (30–45 d pregnant) were purchased from a commercial breeder (random-bred closed breeding colony; TNO, Zeist, The Netherlands). The breeding schedule was such that several litters were born on the same day. Thirty-eight females gave birth to a total of 128 live young (seventy-seven males and fifty-one females); thirteen (five males and eight females) were stillborn or died shortly after birth. Within 12 h of birth (day of birth designated day 0) the pups were sexed and weighed to the nearest g. Within 2 d of birth several litters were mixed and redistributed such that each new 'litter' contained three or four male and female pups; the weights of the pups were not taken into consideration. Each newly formed litter lived with a lactating dam in a plastic box (500 × 300 × 300 mm) which contained a layer of wood shavings.

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On the basis of body-weight at birth each male young was designated to be of low birth weight (range 58–89 g; LBW), to be of medium birth weight (range 92–102 g; MBW), to be of high birth weight (range 108–121 g; HBW), or of a very high birth weight (range 121–148 g UND). Within groups of LBW, MBW and HBW animals were randomly assigned to four sub-groups (five animals/sub-group) to be killed at 35, 45, 55 and 65 d of age.

On day 21 the male young of LBW, MBW and HBW were weaned and caged two or three animals to a cage, in plastic cages (330 × 200 × 160 mm) with wood shavings. Water and pelleted food (a complete diet with stable Vitamin C, Hope Farms, Woerden, Holland) were always available and were supplemented daily by endives (*Cichorium endivia*).

Group UND was subjected to inanition from the age of weaning (21 d) until autopsy at 45 or 55 d of age. These animals were caged individually (plastic cage 330 × 200 × 160 mm) and inanition was achieved by daily allowance of approximately 15 g pelleted food and supplements of endives; the quantity varying somewhat on the basis of the day-to-day changes in body-weight. Water was always available. Temperature in the animal-quarters was 21–23° and lighting was artificial only for 14 h/d.

The undernourished animals were weighed daily, the other animals every seventh day, between 09·00 and 10·00 hours. At autopsy (between 09·00 and 11·00 hours) each guinea-pig was anaesthetized with diethyl ether, weighed, and 6–10 ml blood was withdrawn from the ophthalmic venous plexus into a heparinized glass tube. Also the testes were removed, weighed to the nearest mg and prepared for histological examination. The epididymidal tissue was opened at the tail end and the contents examined under the microscope for the presence of spermatozoa.

Plasma samples were stored at −15° before being assayed individually for testosterone concentration using the radioimmunoassay described by Verjans *et al.* (1973). For determining adult levels of plasma testosterone eleven 4–6-month-old guinea-pigs were used.

Histological studies were performed on testicular tissues, which had been fixed in Bouin fluid and sectioned in paraffin at 10 μm; every tenth section was mounted and stained with haematoxylin and eosin. For each guinea-pig a rating of spermatogenic development was done according to a slightly modified version of the methods described by Johnson (1970) and Aafjes & Van der Vijver (1974).

Rating was done at a magnification of ×400 on at least 100 seminiferous tubules present in one to three cross-sections of a testis. Each of the tubules was categorized according to the descriptions listed in Table 1, and the results shown in Table 3. Furthermore, for each testis the spermatogenic score was calculated in the following manner: the number of tubules recorded at each score was multiplied by the score value, and the sum of the resulting values was then divided by the total number of tubules.

## RESULTS

### *Litter size at birth*

The four birth-weight groups were derived from litters with median numbers of 4·6 (mean 5·2) for LBW, 3·6 (mean 3·2) for MBW, 3·0 (mean 3·75) for HBW, and 3·5 (mean 3·7) for UND animals.

### *Normally-fed (LBW, MBW and HBW) animals*

*Body growth.* Curves of the weights (Table 2) at subsequent ages of animals autopsied showed that their pattern of growth was very similar to that found in earlier experiments (Slob *et al.* 1973). The weight velocity (weight gain; g/week) curves of the three groups showed differences in magnitude but not in the timing of changes in the rate of growth.

Age as well as weight at birth affected body growth (Table 2), as is indicated by the results

Table 1. *Criteria for rating of spermatogenic development in a tubule*

| Score | Descriptive category |
|---|---|
| 10 | More than five spermatozoa |
| 9 | All stages of spermatogenesis present but fewer than five spermatozoa |
| 8 | No spermatozoa but more than five spermatids present |
| 7 | No spermatozoa and fewer than five spermatids present |
| 6 | No spermatozoa, no spermatids, but more than five spermatocytes present |
| 5 | No spermatozoa, no spermatids, and fewer than five spermatocytes present |
| 4 | Only spermatogonia present |

of a two-way analysis of variance (age: $F$ 53·47, df 3/48, $P < 0{\cdot}005$; weight at birth: $F$ 5·62, df 2/48, $P < 0{\cdot}05$). There was no significant interaction ($F$ 0·58, df 6/48).

Subsequent analysis with the honest significance difference test (HSD, Kirk, 1968) revealed that LBW animals were significantly lighter than both MBW ($P = 0{\cdot}05$) and HBW animals ($P < 0{\cdot}01$) at all ages. The latter two groups were not significantly different.

Growth of an animal during the suckling period might have been influenced by the body size of the other animals in its group through competition for nipples. However, when body-weights at 21 d of age were examined, it appeared that eleven LBW animals that grew up in heterogeneous 'litter' groups (i.e. 'litter' groups that contained both LBW and HBW animals) had body-weights similar to those of six LBW animals that lived in homogeneous 'litter' groups (composed of LBW animals only). Also ten HBW animals from heterogeneous and five from homogeneous 'litter' groups had similar body-weights at 21 d of age. Thus, possible competition for food during lactation, between small and large members of a litter, does not appear to have affected growth.

*Testicular development.* Between 35 and 65 d weights of the testes nearly tripled. Analysis of variance (two-way) indicated that the differences in mean weights between the birth-weight groups were not statistically significant. ($F$ 3·02, df 2/48). The relative testis weights (per kg body-weight) increased with age ($F$ 55·48, df 3/48, $P < 0{\cdot}005$) but there were no differences between the birth-weight groups ($F$ 0·37, df 2/48). Spermatozoa were first found in the testes at 45 d (except in one animal which had spermatozoa at 35 d), and in the epididymidal tissue of all animals at 55 d but in none at 45 d. There were differences in the 'maturity' of the testes between the birth-weight groups, the HBW being more advanced at all three ages, as can be seen in Table 3 from the percentages of tubules that contained the various cell types. Analysis of variance of the spermatogenic scores showed an effect of age ($F$ 125·12, df 2/36, $P < 0{\cdot}005$) and weight at birth ($F$ 7·87, df 2/36, $P < 0{\cdot}005$). Subsequent analysis with HSD showed a difference ($P < 0{\cdot}01$) between HBW and the other two groups but none between the latter.

*Plasma testosterone.* Except for two unusually high values (see Table 2) testosterone levels ranged from 1·1 to 13·1 ng/ml in the developing guinea-pig and from 3·4 to 13·8 ng/ml in eleven adult animals. There was neither a significant age ($F$ 2·62, df 3/48) nor a birth weight effect ($F$ 0·14, df 2/48). Eleven samples, including six from UND animals were also assayed by gas–liquid chromatography using a method which has previously been used in our laboratory (Baum & Goldfoot, 1974). The results obtained by the two methods were highly correlated ($r$ 0·92) confirming that the radioimmunoassay procedure was measuring testosterone.

*Underfed (UND) animals*

*Body growth.* The food restriction regimen resulted in a very slow increase in body-weight (Table 2), so that animals gained on average 1·2 g/d between weaning at 21 d and post mortem at 45 or 55 d. The normally-fed animals gained approximately 10 g/d during the corresponding period. The severity of undernutrition is apparent from the sharp

Table 2. *Body-weight (g; BW), weight of two testes (mg; TT) and plasma testosterone (ng/ml; PT) in guinea-pigs grouped according to weight at birth and examined at different ages*

(Values are means with their standard errors for five animals/group)*

| Group | Range of body-wt at birth (g) | Age (d) 35 BW | 35 TT | 35 PT | 45 BW | 45 TT | 45 PT | 55 BW | 55 TT | 55 PT | 65 BW | 65 TT | 65 PT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Low birth wt | 59–89 | 381 | 856 | 6·9 | 488 | 1369 | 5·1 | 592 | 1993 | 5·0 | 627 | 2369 | 10·2† |
| | SE | 17·7 | 61·0 | 1·2 | 21·3 | 80·9 | 1·5 | 19·7 | 67·8 | 0·9 | 33·4 | 216·9 | 5·9 |
| Medium birth wt | 92–103 | 413 | 883 | 4·1 | 531 | 1566 | 5·0 | 627 | 2141 | 6·7 | 689 | 2606 | 7·9 |
| | SE | 26·0 | 108·9 | 0·9 | 34·2 | 154·0 | 0·9 | 15·1 | 124·5 | 1·8 | 22·5 | 191·0 | 1·6 |
| High birth wt | 108–121 | 480 | 1045 | 6·3 | 525 | 1576 | 3·6 | 629 | 2030 | 3·6 | 685 | 2844 | 14·5‡ |
| | SE | 19·5 | 93·5 | 1·3 | 17·7 | 103·3 | 1·1 | 45·3 | 183·8 | 0·6 | 15·2 | 102·0 | 8·1 |
| UND | 121–149 | | | | 330 | 839 | 1·1 | 362 | 884 | 0·3 | | | |
| | SE | | | | 22·4 | 84·3 | 0·4 | 4·7 | 115·5 | 0·1 | | | |

UND, severe undernutrition from 21 d of age. * Three animals in UND groups.
† 4·4 when one value of 33·4 is discarded. ‡ 6·5 when one value of 46·4 is discarded.

Table 3. *Spermatogenesis in guinea-pigs grouped according to weight at birth and examined at different ages*

(Values are for five animals/group.† Values in parenthesis are the spermatogenic development ratings described in Table 1)

| | | Mean percentage of tubules | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Spermatocytes | | Spermatids | | Spermatozoa | | Spermatogenic score‡ | |
| Age (d) | Group | Sperm-atogonia (4) | Few (5) | Many (6) | Few (7) | Many (8) | Few (9) | Many (10) | Mean | SEM |
| 35 | LBW | 0·9 | 5·2 | 58·4 | 17·3 | 18·2 | | | 6·5 | 0·13 |
| | MBW | 3·6 | 6·9 | 47·3 | 10·9 | 30·7 | 0·5 | | 6·6 | 0·35 |
| | HBW | 0·9 | 1·6 | 30·0 | 16·7 | 50·7 | | | 7·2 | 0·11 |
| 45 | LBW | | | 4·8 | 6·1 | 69·9 | 13·5 | 5·7 | 8·1 | 0·10 |
| | MBW | | 0·5 | 10·0 | 4·5 | 54·3 | 17·2 | 10·9 | 8·0 | 0·31 |
| | HBW | | | 1·1 | 3·9 | 57·1 | 18·4 | 19·7 | 8·5 | 0·18 |
| | UND | 2·3 | 6·1 | 14·5 | 8·4 | 33·4 | 17·3 | 17·9 | 7·9 | 0·19 |
| 55 | LBW | | | 0·2 | 0·2 | 25·3 | 22·1 | 52·2 | 9·3 | 0·15 |
| | MBW | 0·3 | 0·3 | 2·2 | 0·9 | 20·3 | 18·0 | 57·9 | 9·3 | 0·20 |
| | HBW | | | | 0·2 | 6·7 | 14·6 | 78·5 | 9·7 | 0·03 |
| | UND | 0·3 | 0·8 | 3·1 | 6·0 | 29·3 | 18·4 | 42·0 | 8·9 | 0·09 |

UND, severe undernutrition from 21 d of age.

† Three animals in UND groups.

‡ Number of tubules recorded at each score multiplied by the score value, and the sum of the resulting values then divided by the total number of tubules.

divergence in the growth of HBW and UND animals. Thus at 55 d the weight of UND animals was only 58% of that of HBW animals, whilst at 21 d of age the latter weighed less than the former (273 *v.* 319 g).

*Testicular development.* Testis growth was stunted so that at 55 d testes of UND animals were less than half the normal weight (Table 2). There was no overlap with any of the other groups at 45 and 55 d of age. Relative testis weights of UND animals were also lower than those of the other groups. Statistical analysis of values (analysis of variance and HSD) for relative testis weights at 45 and 55 d of age showed a significant effect of undernutrition ($F$ 4·35, df 3/28, $P < 0·025$; HSD: $P < 0·05$). Nevertheless spermatozoa appeared in testes (present at 45 d) and epididymides (between 45 and 55 d) in the same age-groups as in control groups. Detailed analysis of spermatogenesis showed that the transition from the prepubertal to the mature state of the tubules occurred more slowly. This is indicated by the fact that there were relatively more tubules with less advanced stages of spermatogenesis. Statistical analysis showed a significant difference only between UND and HBW animals ($F$ 5·04, df 3/28, $P < 0·025$; HSD: $P < 0·01$).

*Plasma testosterone.* The undernourished animals had very low plasma testosterone levels (at 45 d: 0·4, 1·1 and 1·7 ng/ml; at 55 d: 0·1, 0·3 and 0·5 ng/ml); there was no overlap with the other groups of the same age.

## DISCUSSION

Animals small at birth became small adults, while animals big at birth became big adults. In other words, the differences in body-weight at birth foreshadowed those in adulthood. Apparently the 'mechanisms' controlling adult body-weight in guinea-pigs, pigs and almost certainly man become 'set' some time before birth but in rats between the 3rd, 4th and

the 21st day after birth (see Widdowson & McCance, 1975). This view is supported by earlier work on guinea-pig (Lister & McCance, 1965; Slob *et al.* 1973) and rat (Slob *et al.* 1975).

Puberty in male subjects cannot be defined in terms of a single event like vaginal opening, menarche or first ovulation. In this work on the guinea-pig several aspects of testicular activity were evaluated.

The youngest group of normally-fed animals which had epididymal spermatozoa was aged 55 d. In the next younger group, examined at 45 d, the seminiferous tubules contained spermatozoa but the epididymis did not. In all but one of the 35-d-old animals spermatozoa were absent from the testis. In terms of possible fertility, therefore, somatic sexual maturity was reached between 45 and 55 d of age. This was true for all four groups of animals. Effects noted of the birth-weight differences and of the post-weaning undernutrition were of a quantitative nature. In each group HBW animals had a higher incidence of the most advanced stages of spermatogenesis. The timing of testicular development seems to have been set by the time the animals were born and appears to have been left undisturbed by undernutrition from the age of 21 d. Thus at 45 d the percentage of tubules containing spermatozoa was identical for the undernourished (which had the highest birth weights) and the HBW animals. The former had lower spermatogenic scores, because of a higher incidence of the least advanced types of tubules. Hormonal activity of the testes had 'matured' well before the first spermatozoa were found. At 35 d plasma testosterone levels were similar to those found in adult male guinea-pigs. It is of interest that Rigaudière *et al.* (1976) did not obtain adult levels of plasma testosterone until the age of approximately 50 d, i.e. 15 d later than in the present study. It appears that their guinea-pigs matured more slowly than those in the present study. This assumption is further supported by the fact that in the study of Rigaudière *et al.* (1976) spermatozoa were first found between d 55 and 60. Our finding of spermatozoa in seminiferous tubules at 45 d (10–15 d earlier than Rigaudière *et al.* 1976) accords with the work of Donovan *et al.* (1975). The finding that energy undernutrition in the developing guinea-pig results in a marked suppression of androgen secretion, whereas spermatogenesis seems only slightly affected corroborates results of studies on other species, such as the rat (see Talbert & Hamilton, 1955; Leathem, 1959; Widdowson *et al.* 1964; Howland, 1975) the bull (Davies *et al.* 1957; Baronos *et al.* 1969) and the pig (Dickerson *et al.* 1964). It is uncertain how undernutrition causes reduced androgen production. It has been reported for adult male rats that undernutrition caused decreased levels of luteinizing hormone (Howland, 1975) and diminished in vitro biotransformation in testicular tissue of pregnenolone and progesterone into androgens (Berliner & Ellis, 1965). The latter workers suggested that the diminished enzyme activity was due to a lack of gonadotropins. This unusual combination of low plasma testosterone levels and maintenance of spermatogenesis has also been experimentally obtained in hypophysectomized rats that were treated with pregnenolone (Vreeburg *et al.* 1964). These findings support the opinion that it is the concentration of testosterone in the testis that is important for spermatogenesis rather than the concentration in peripheral blood.

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