THE EFFECTS OF ACUTE INFECTION AND ACUTE STARVATION ON SKELETAL DEVELOPMENT

A Study of Young Rats

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Analysis of the data collected during the earlier years of the Oxford Child Health Survey showed that pre-school children who lived in poor homes grew, and matured skeletally, more slowly than did their co-evals living in prosperous circumstances. By relating stature to level of skeletal maturity rather than to age, it was shown that the poor children suffered a certain amount of stunting, and the conclusion was drawn that, should the environmental difference between the two groups remain unchanged throughout the growing period, the poor children would become the smaller adults (Acheson and Hewitt, 1954). Subsequently Hewitt, Westropp and Acheson (1955), using the same material, showed that the common intercurrent infections of childhood also interfered with increase in stature, causing children who suffered such an illness to grow about $\frac{1}{4}$ in. less in that year of their lives than did others of the same age and sex who remained healthy during the same year. In this analysis, however, the retardation noted in the skeletal maturation of the hand and knee did not reach the limits of statistical significance. Thus it seemed that the sick children were maturing without interruption, and unless they were capable of accelerating their growth they would close their epiphyses (*i.e.* stop growing) $\frac{1}{4}$ in. smaller than they would have been had they not been ill. There was no suggestion of such an acceleration during the year following the illness, and it was concluded that such seemingly trivial incidents as attacks of acute bronchitis or measles in early life might possibly cause minor degrees of permanent stunting of adult stature.

In the field of animal experiment an accelerated gain in weight in kittens following release from daily exposure to chloroform was demonstrated over fifty years ago by Schapiro (1905) and a similar dramatic weight gain described in rats, which were fed after prolonged inanition by Osborne and Mendel (1916). Subsequently many studies of growth following chronic malnutrition have been conducted (Jackson, 1936; Quimby, 1951; Fábry and Hrůza, 1956), but to our knowledge no investigation has been carried out upon the effect of acute illness or starvation, nor have daily measurements of length been made.

In order to pursue further the long-term effect of acute trauma on skeletal development, animal experiments were planned to examine the validity of the following hypothesis: "An acute adverse experience, whether infection or

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starvation, has a general retrading effect upon the skeletal development of the growing mammal. The retardation in linear growth is greater than that in skeletal maturation, and as a consequence, a minor degree of permanent stunting may occur." The animal selected for the purpose was the rat because it is easy to handle and it undergoes the major part of its skeletal maturation after it has been born. In the latter respect it is similar to the human, in striking contrast to other laboratory animals.

Difficulty was experienced in keeping an animal sick for more than 48 hr., and yet being reasonably certain that it would completely recover. Therefore, two separate experiments were performed : in the first a group of animals were infected intraperitoneally with a Type I pneumococcus and later treated with penicillin and in the second another group was subjected to starvation for several days, thereby giving an opportunity for the study of a slightly longer period of acute environmental stress.

MATERIAL AND METHOD

Experiment I.—Eight litters of albino rats of known age were used, each consisting of 8 animals. In one of these litters there were only 2 males, in three there were 3 males, in two there were 4 males and in two there were 5 males. In the three litters in which the sexes were divided evenly the rats were paired off by weight, so that there was a control for every animal to be infected. In the other five litters one control animal was used for two experimental ones, the matching again being done by weight, so that in all there were 29 male animals 12 of which were controls, and 35 females 15 of which were controls.

When the animals were 24 days old each had its weight, total length and tail length recorded while fully conscious (see Macintyre, Acheson and Oldham, 1958). The next day they were all radiographed under general anaesthesia, and the skeletal maturity status of each was assessed from the X-ray plates by the Oxford Method (Acheson, 1954, 1957 ; Macintyre, Acheson and Oldham, 1958). This method depends upon the recognition, on the radiogram, of shape changes in the parts of the maturing skeleton where osteogenesis is occurring. Such shape changes as are constant in all animals, regardless of sex, are described as maturity indicators, and the total number of indicators in any X-ray is known as the maturity score. Skeletal maturity scores for the rat ranged from a minimum of 22 at the age of 3 weeks to 100 at 16 weeks. Then the members of the experimental group (17 males and 20 females) were inoculated intraperitoneally with a Type I Pneumococcus. Each animal was inoculated with 0·1 ml. of a 1/10,000 saline dilution of a virulent strain which had just been cultured in blood broth for 4 hr. Eighteen hours later intramuscular injections of 5000 units of penicillin were given and repeated twice daily for 5 days.

Twenty animals (9 male and 11 female) failed to respond to penicillin in the first 24 hr., 4 died on the third day, 3 on the fourth day and one on the eleventh day. This animal, a male, after making a good initial response to the antibiotic, developed otitis media and despite strenuous treatment died aged 36 days. In the majority of these cases of premature death the control animal was killed. Eighteen animals, 9 from the experimental group and 9 controls, survived for the duration of the experiment.

Measurements of total and tail length, and of weight were recorded at the age of 26 days and daily thereafter to 40 days of age. They were repeated at the ages of 44, 54, 64, 94 and 114 days, when the animals were killed. X-rays were taken at the ages of 33, 39, 42, 55, 65, 95, and 114 days.

After death the animals were all dissected ; from the left side of the body the third metacarpal, radius, ulna, humerus, femur, tibia and third metatarsal were cleaned and the length measured with callipers accurate to 0·2 mm. The final value for each bone was the average of three independent readings (Acheson and Macintyre, 1958). The same bones from the right side, together with sections of the tail were decalcified and sectioned for histological examination.

Experiment II.—Five litters were used, each again consisted of 8 animals, but on this occasion the sexes were equally divided (4 males and 4 females). The animals were sexed, the males in each litter numbered 1–4, and the females 5–8 ; all animals were earmarked for

purposes of identification. Then one male and one female from each litter were selected to act as controls ; no account was taken of size when this selection was made.

In order to obtain a good baseline the same daily measurements as had been used in the first experiment were instituted at the age of 21 days, 5 days before the animals were starved ; these measurements were continued until the age of 35 days. They were then repeated at the age of 40 days, and on every tenth day thereafter until the age of 70 days, then again at 90 and 110 days when the animals were killed. X-rays were taken at the ages of 32, 42, 52, 63 and 110 days.

All food was removed from the cages of the experimental animals after they had been measured on the morning of the 27th day (26 days old) and they were left with a diet of water for 48 hr. On the 29th day these animals were allowed to feed normally for 24 hr. before having their food removed for a further 48 hr. (the 30th and 31st days). *Ad libitum* feeding was reintroduced on the 32nd day and continued until the end of the experiment. Two males died before the experiment started, 4 females failed to recover from the period of starvation, and one male died under anaesthetic aged 60 days.

After the remaining 33 animals had been killed, some bones from the left side were measured, and others from the right prepared for histological examination in the same manner as has been described for Experiment I.

Assessment of results.—In order to judge the immediate effect of the infection or starvation upon the experimental animals the following procedure was adopted. The difference d between the total length of each experimental animal and its control was calculated every time they were measured. If the value for the experimental animal was the smaller it was designated by a minus sign, and if larger by a plus. Then, since a difference of say 2 mm. between 25 day-old rats is a larger proportion of their total growth than is the same difference between two mature rats, the value d was expressed as a percentage of the mean length of all the control rats of the same age and sex. Finally the results for males and females were combined by estimating the weighted mean of the percentage values for each.

$$\text{Per cent deviation length} = \frac{\text{Length of experimental animal—Length of control}}{\text{Mean length of all controls of same age and sex}} \times 100$$

Per cent deviation of males and females combined

$$= \frac{(\text{Per cent male deviation} \times \text{No. of males}) + (\text{per cent female deviation} \times \text{No. of females})}{\text{No. of males} + \text{No. of females}}$$

The same procedure was then followed with respect to tail length, weight and skeletal maturity Finally, the difference was established between the lengths of the dissected third metacarpal and third metatarsal of each experimental animal and those of their controls.

RESULTS

The results of Experiment I are shown in Fig. 1. The horizontal line represents a mean difference of zero between the experimental animals and their controls ; all points below this line indicate that, on average, the experimental animals were smaller than their controls, and points above indicate the reverse. For the sake of simplicity tail length has been omitted from the graph because it follows the pattern of total length very closely. It will be seen that all the three facets of physical development studied are slowed in the sick animals, weight being the most profoundly affected and skeletal maturation the least. By the fifth post-infection day, however, the experimental group had started to gain weight on the controls ; this gain continued, albeit erratically to begin with, until the 15th post-infection day, at which time the rate of gain flattened out. Growth took longer to recover, and it was not until the seventh post-infection day that this process showed any sign of making good its losses ; in spite of the fact that clinically

the rats gave the appearance of having been cured by the third post-infection day. By the time the animals were killed the total length curve had almost returned to the level from which it had started, but the experimental animals were still 1·8 per cent lighter than their controls. However, these differences were not statistically significant, although the deviations throughout the greater part of the experiment had been highly significant.

The first gain for skeletal maturation is followed by a loss, but by the end of the experiment there is no difference between infected or uninfected animals in this respect either.

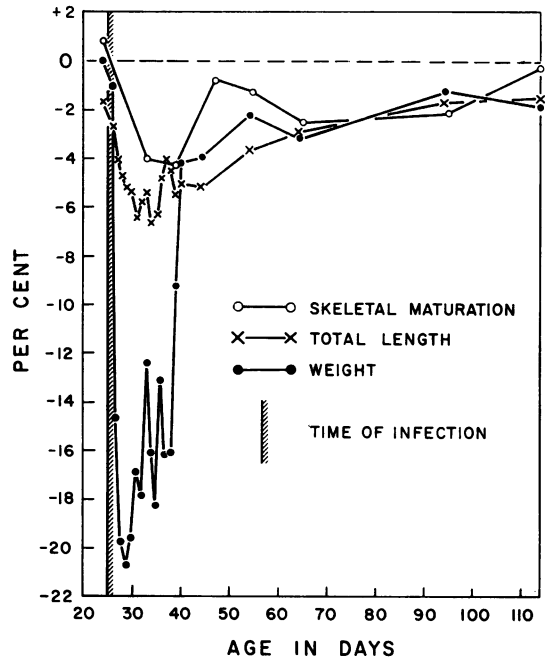

FIG. 1.—Differences between serial measurements of experimental and control animals in Experiment I, expressed as a percentage of the same measurements of all controls of the same age and sex.

Fig. 2 shows the results for the second experiment; it will be seen at a glance that the general pattern is the same, though the degree to which the animals were affected in the second experiment was much more profound. Between the first and second periods of starvation about 20 per cent of the weight loss is made up, while the growth process only just holds its own. Similarly after the second starvation period a dramatic weight gain (over 25 per cent of the total loss) is shown while the process of increasing in length is resumed, but at the same speed as that of the controls. This pattern is continued for 4 days after which the starved animals lose once more, although they are simultaneously gaining in weight. It is not until some 8–9 days after full feeding had been reinstituted that a definitive growth spurt began and the experimental animals started to catch up with their controls.

As in the first experiment skeletal maturation shows a loss to begin with and then, after a temporary gain, a second loss before returning to the same level as that from which it started.

Neither in linear growth, nor in weight increment, however, has the starved group succeeded in catching up with its controls, and the difference between the groups is highly significant (Table I).

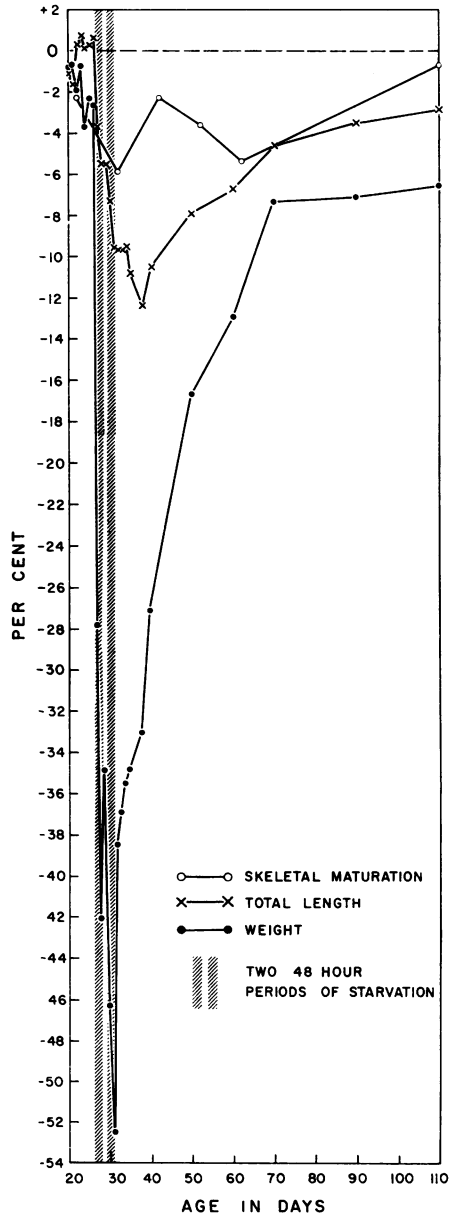

FIG. 2.—Differences between serial measurements made in Experiment II, expressed in the same way as was used in Experiment I (Fig. 1).

TABLE I.—*Mean Difference (and Standard Error of Mean) Between Experimental and Control Animals Aged* 110 *Days*, 79 *Days After Starving*

	Males only (12)		Females only (11)		All animals (23)	
	Mean difference	Standard error of mean difference	Mean difference	Standard error of mean difference	Mean difference	Standard error of mean difference
Length (mm.)	−1·78	±0·43	−0·41	±0·26	−1·13	±0·29
Weight (g.)	−37·8	±8·0	−2·7	±4·8	−21·0	±5·9
Skeletal maturity (points)*	−1·67	±1·42	+0·45	±1·06	−0·65	±0·91

See Acheson, 1957, Macintyre *et al.*, 1958.

It is well known that many of the growth cartilages in the rat never ossify, and that others maintain minor degrees of activity until senescence. Thus, although the animals have achieved full sexual maturity by the age of 110 days they continue to grow slowly and therefore retain the ability to make up lost ground much later than would the human and, had the experiment been prolonged, might have made good the leeway. The bones of the metacarpus and metatarsus, however, are more similar to the primate in that their growth cartilage plates are ossified shortly after puberty. Becks, Asling, Collins, Simpson and Evans (1948) showed that ossification of the growth cartilage plate in the third metacarpal was well advanced in the majority of animals they studied by the 110th day of life, and that all growth had ceased about 20 days previously. Our histological studies confirmed this report, and we found that the third metatarsal tended to reach maturity at much the same time as the metacarpal. A disadvantage of using these bones is that by the time our experiments started (25th day of life) they has already undergone about 80 per cent of their entire growth, whereas in total length, the animals had completed less than 50 per cent of their ultimate growth. Nevertheless, since these bones followed the human pattern closely, we made a special study of them. The differences between experimental and control animals are shown in Table II where it will be seen that while the difference between experimental and control groups has been made good in Experiment I, the results of Experiment II are again consistent with some genuine effect of stress.

TABLE II.—*Mean Difference in Millimetres Between Certain Bones of Animals Exposed to Infection or Starvation, and those of Litter-mate Controls of the Same Sex*

Experiment	Bone	Mean difference	*P*
I	Third metacarpal	−·0625	<·80
I	,, metatarsal	−·0333	<·90
II	,, metacarpal	−·0571	<·30
II	,, metatarsal	−·1111	<·20

Sex differences.—The survivors in the first experiment were too few to make a between-sex comparison. In the second experiment the males suffered a greater initial setback in every respect and showed more stunting at the end of the experiment than did the females. These sex differences are the same as those already

described in the rat by Jackson (1936). They are also similar to those postulated for the human in the Oxford Child Health Survey (Acheson and Hewitt, 1954; Hewitt, Westropp and Acheson, 1955; Acheson, Hewitt, Westropp and Macintyre, 1956), for not only is the retardation in the male greater, but its variability is also wider.

DISCUSSION

It is always dangerous to argue from one mammalian family to another. Nevertheless, observations on the rat have borne out the first part of our hypothesis based upon observations of the human, for there can be no doubt that the stress, whether it was an acute infection, or acute starvation, did have a general retarding effect on skeletal development. Furthermore, this effect was more profound on linear growth than it was on skeletal maturation.

We can add, with equal certainty, that the accelerated weight gain which earlier workers (Schapiro, 1905; Osborne and Mendel, 1916; Jackson, 1937) have observed to follow release from any retarding influence is accompanied by accelerated growth in length. Thus we confirm the work of Barnes, Sperling and McCay (1947) who based their conclusions on measurement of bones from X-rays of rats taken at intervals of some weeks. Jackson's (1936) suggestion that the early stages of weight gain are due to increased intestinal content may help to explain why growth in length lags behind weight gain for the first day or two. It does not explain why the experimental animals, having held their own for 4 days, lose ground a second time before making a definitive gain in length. This interesting observation cannot be explained on the present evidence and merits further investigation.

Taking into account our results and those reported in the literature, it would seem that the effect of stress on the final length of any bone depends upon three things, namely the amount of growth the bone has completed before the stress is introduced, the amount of growing time left after the stress has been removed, and the duration of the stress itself. The fact that the observed effect on the metacarpus and metatarsus was less than that on total length was probably due to the first of these considerations. In experiments where the stress is long drawn out, such as those of Barnes, Sperling and McCay (1947), the latter two factors must be related and supplementary, because a rat which has suffered malnutrition for a year or more is past its normal growing age when full feeding is reinstated.

Clarke and his colleagues (Clarke, Bassin and Smith, 1936; Clarke and Smith, 1938) concentrated their attention on bone length and composition and showed that, while mild dietary deprivation for a short period of time could result in over-compensation so that the experimental animals actually finished longer than their controls, prolongation of the stress invariably led to stunting, even though full diet was given for several mouths subsequently. Their work differed from ours in that the degree of under-feeding was so graded as to keep the body weight of the experimental animals constant, whereas we induced a profound loss of body weight in all the members of the experimental group. Thus, since our severe short-lived trauma wrought a similar effect to the milder, long-lasting stress used by the earlier workers, we feel justified in endorsing the conclusions of Clarke and his colleagues (loc. cit.) and of Jackson (1936) that the ultimate stunting effect is also dependent both upon the severity and duration of the stimulus which causes it.

More recently Fábry and Hrůza (1956) have exposed rats, over a period of 6 weeks, to intermittent starvation of a type similar to that used in the present study. Their animals were about 10 days older (to judge by reported weight) at the outset than ours, and weight alone was repeatedly assessed; yet it is of considerable interest that therapy with growth hormone during the recovery phase enabled the experimental group to grow at a greater rate than a control group which had also been starved, and thus, on the criterion of weight, to avoid being stunted. Quimby (1951), however, in a similar study, found that increase in skeletal length failed to make the same response as weight gain when an anterior pituitary extract was given during the recovery phase. The role of the anterior pituitary in interrupted growth is discussed more fully elsewhere (Acheson, and Macintyre, unpublished).

No clear grounds can be given for the different response of the two sexes, but it is of some interest that the rat also resembles the human in normal growth, for although in both the female tends to be smaller, her skeletal maturation proceeds more quickly than that of the male (Macintyre, Acheson and Oldham, 1958).

In conclusion, our findings have supported our hypothesis that an acute adverse experience such as septicaemia or starvation has a general retarding effect on the growing mammal, and that the retardation of linear growth is greater than that of skeletal maturation. There was, however, pronounced compensation during recovery so that a 48-hour illness, had no perceptible permanent effect upon the skeleton, but intermittent acute starvation lasting over 5 days did appear to cause minimal stunting of bones incapable of growth into old age. It is not unreasonable to suppose that some of the inter-current illnesses of childhood may also cause minor degrees of permanent stunting in the human. This could only be confirmed by making repeated careful measurements of a small group of children at monthly intervals for several years and correlating the results with detailed health records.

SUMMARY

Two experiments are described which were designed to test the hypothesis that an acute adversity such as an intercurrent infection or a period of total starvation so interferes with skeletal development as to induce minor degrees of permanent stunting.

Including controls a total of 104 3-week-old rats were used. It was found that both pneumococcal septicaemia, treated by penicillin, and also intermittent starvation lasting over 5 days, had a profound retarding effect upon increase in length, weight and skeletal maturity. The animals that suffered the infection which made them sick for about 48 hours had caught up with their controls by the age of 16 weeks, while those which had been starved showed residual stunting. This was true both of total length, which normally would have continued to increase several months after the experiment was complete and of bones of the metacarpus and metatarsus which had already ceased growing. The retardation experienced by the male was, in every respect, more severe than that experienced by the female.

It is concluded that a relatively short acute traumatic episode can cause permanent stunting of the skeleton in the rat and that the hypothesis is borne out for this animal. More research is necessary to ascertain whether similar stunting occurs in the human.

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