Studies on the hypophysectomized ferret

X. Growth and skeletal development

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[Plates 6-8]

The relation between the pituitary gland and body growth has been studied by means of hypophysectomy in a number of species of animals. Cushing (1911), from his results with hypophysectomy of the dog, concluded that the gland secreted a substance that controlled and maintained proportionate growth. The results of Smith (1927) and Collip (1934) and his co-workers on the rat show that there is little growth after removal of the pituitary, although the latter authors find that if the rat is hypophysectomized at an early age (body weight 25-35 g.) it continues to grow until a body weight of 60 g. is reached. The growth stasis following hypophysectomy seems to be characteristic of all mammals.

A detailed radiographical study of the effect of hypophysectomy on the development of bone in the rat has recently been made by Mortimer (1937). He finds that the degree of vascularity of the bone is greatly reduced, so that the resorption and deposition of calcium is lessened. Changes in the skeletal system are described as "an osteoporosis simplex with disorganization of the trabecular structure of bone and marrow aplasia". Special reference is made to the skull, since cranial growth, particularly antero-posterior growth, is greatly retarded by hypophysectomy.

Attempts have been made to ascertain which type of cell in the anterior lobe of the pituitary secretes the substance controlling growth. Evidence from clinical cases of pituitary tumours suggests that the eosinophil cells are concerned with this function of the pituitary. Further light has been thrown on this subject by Kemp's (1934) work on the hereditary dwarf mouse, the condition of which seems to be due to pituitary deficiency. Histological study of the pituitary of this dwarf shows a complete absence of eosinophil cells.
Further evidence of the role of the pituitary in normal body growth is shown by the increased skeletal effects that can be obtained by the administration of anterior pituitary extracts (Evans and Long 1921; Collip 1934; and Silberberg 1936). Evans and his co-workers (1933), however, were unable to obtain marked skeletal abnormalities by this means in the normal dog, although in the achondroplastic breeds (Dachs-
hunde) they were able to produce symmetrical giantism. In the hypophysectomized animal, growth and skeletal development can be maintained or restored by the use of extracts, as shown by Smith (1927), Collip (1934) and by Evans and his co-workers (1933, 1935). The degree of purity of the extracts seems to be of importance since both Evans and Collip have shown that with purified extracts continuous growth is not maintained. The former author was able, on the other hand, to produce indefinite growth by the use of crude pituitary extracts and to show that the rats resistant to the purified extracts responded to the crude preparations. The results of Mortimer (1937) suggest that although crude extracts produce massive growth of a poorly differentiated type, concentrated extracts produce growth which resembles more closely the normal structure of bone.

Material

Six young male and two young female ferrets of 500–600 g. were hypophysectomized by the parapharyngeal route described by Hill and Parkes (1932). Three of the males and the two females were born in the laboratory, the dates of birth being recorded. The remaining three males were purchased from dealers so that the exact age was not known. Control material consisted of five males and four females, the exact age being known in all except one male and one female.

The animals were weighed twice weekly while growing rapidly; subsequently weighings were made once weekly. The weights were recorded at a constant time relative to feeding. The sella turcica was examined histologically for pituitary fragments if there was any doubt as to the completeness of the operation. During the breeding season the condition of the external reproductive organs is an indication of the completeness of the operation.

In assembling the data on growth in the ferret, males and females have been grouped separately on account of the difference between the two sexes. Figs. 1 and 3 show the increase in weight with increasing age over intervals of 10 days. The arrow in each figure indicates the time at which the animals in each group were hypophysectomized. In these figures only animals that were born in the laboratory are included.
**Growth**

*Growth of the normal ferret.* The ferrets were first weighed at 1 month old, when they all weighed 170–190 g., there being little sex difference. It will be seen from figs. 1 and 3 that the growth rate in the two sexes is the same until the end of the second month, when the males begin to grow more rapidly than the females. In the females the adult body weight is reached in about 4 months' time, while the males continue to increase in weight for another month. The adult weight of the female ferret is about 1 kg., while the male is nearly double this weight. The largest male handled in this work attained a maximum weight of 2.6 kg. in the space of 6 months (fig. 2).

Growth of the ferret under laboratory conditions is not influenced by the date of birth; those born in August and September, when non-pregnant adults are entering anoestrus, grow throughout the autumn and winter at the same rate as ferrets born in June. It would appear, therefore, that the

![Graph showing growth of ferrets](image)
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The pituitary secretion responsible for the maintenance of growth is not subjected to seasonal variation such as is found in the case of the secretions regulating the gonads and the thyroid gland. In this respect the ferret

under laboratory conditions is different from British mammals in the wild state.

*The growth of the hypophysectomized ferret.* In the male ferrets of known age, hypophysectomy was performed at 51, 53 and 58 days respectively,

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**Fig. 2.** The body weight of a hypophysectomized male ferret, HYF 34 (○), compared with that of normal litter-mate control, HYF 30 (●).
and in the females at 67 and 72 days. Following the operation, the growth rate in the two sexes was greatly retarded. In the male, growth continued slowly until the age when growth in the normal ferret ceases. During this time, about 3 months, the maximum increment was approximately 300 g., as compared with 1200 g. for the normal animal. The male ferret, therefore, hypophysectomized at about 2 months old, is only half the normal weight at 4 months old (fig. 1).

Fig. 2 shows the growth rate of a male ferret hypophysectomized when 500 g., in comparison with that of a litter mate control. Both of these

![Graph showing growth rates](image)

Fig. 3. The effect of hypophysectomy on the growth rate of the female ferret. The mean body weight of two hypophysectomized ferrets (○) is compared with that of three normal female ferrets (□).

animals were fed on a similar diet and housed under identical conditions. They both lived for about 2½ years. The hypophysectomized one thus survived operation for over 2 years. He remained in good health and appeared normal except for his small size and failure to show enlargement of the testes during either of the two breeding seasons through which he lived. At autopsy, 16 November 1936, the reproductive organs were infantile, the weight of the os penis being only 70 mg. as compared with 420 mg. in the control litter mate. The thyroids and adrenals were very atrophic. No other pathological symptoms were noticed.

The rate of growth in the females following hypophysectomy was slower than in the males, but it is proportional in comparison with the normal growth rate. The maximum increase in weight following the operation was about 100 g., which is attained in three months (fig. 3).
Fig. 8

Fig. 9
These results, on the whole, are similar to those described for other species. In the males there is a great difference in weight between the normal and the hypophysectomized animal, while in the female, although the difference is not so pronounced, the curve obtained in the early stage following hypophysectomy is much flatter. The hypophysectomized ferret would thus be a good test animal for growth hormone.

**Skeletal development**

Study of the general skeleton, chiefly by X-ray photographs and prepared skeletons of the two ferrets whose body weights are plotted in fig. 2, shows that in the hypophysectomized ferret (HYF 34) there is a general stasis in the growth of bone so that the skeleton (fig. 5, Plate 6), although the animal is adult and 2½ years old, resembles that of an immature ferret 4–5 months old. The various processes and crests of the bones which develop with age are only very slightly developed. These are particularly well shown in the skull, in the spinous processes throughout the vertebral column, and in the scapulae. None of the smaller prominences and depressions characteristic of the surfaces of adult bones, associated with the development of muscles, appear; they retain the smooth rounded appearance of the bones of an immature skeleton.

Ossification is arrested in the hypophysectomized ferrets as shown by the open epiphyses of the long bones and the vertebrae (fig. 9, Plate 8), and in the degree of ossification of the pubic symphysis. This, however, has not led to any undue lengthening of the bones; it is seen from Table I that growth in length of bone is retarded, the length of skeleton of the hypophysectomized ferret being about 20% shorter than that of the litter-mate control. The ratio of the length of humerus to radius in these two ferrets seems to be disturbed by retardation of the growth of humerus in the hypophysectomized ferret and by the continued growth of the radius. This, however, is of doubtful significance. There is a marked decrease in the calcium content, which is particularly well seen at the ends of the long bones and in the cartilaginous parts of the ribs. There is some softening of the bones due to the low calcium content, which in turn has given rise to slight bending in some.

Skeletal differences between the skulls of normal and hypophysectomized ferrets are well marked (figs. 6, 7, Plate 7). In the young ferret of about 2 months of age all the cranial sutures are open and there is no development of the fronto-parietal and nuchal crests. At about 6 months of age, shortly after the animal has reached its maximum body weight, rapid
Table I. Comparison of Skeletal Dimensions of Normal and Hypophysectomized Ferret

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Normal</th>
<th>Hypophysectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of skeleton</td>
<td>57.4 cm</td>
<td>46.2 cm</td>
</tr>
<tr>
<td>Length of tail</td>
<td>16.2 cm</td>
<td>13.0 cm</td>
</tr>
<tr>
<td>Ratio humerus to radius</td>
<td>4.6 : 2.5</td>
<td>3.8 : 2.75</td>
</tr>
<tr>
<td>Skull:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall length</td>
<td>6.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Width across zygomatic arches</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Width between mastoid processes</td>
<td>3.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Width between supra-orbital processes</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Weight of skull</td>
<td>16.35 g</td>
<td>10.08 g</td>
</tr>
</tbody>
</table>

Calcification of bone occurs, as shown by the process of fusion which is taking place in the skull, and by the simultaneous development of the sagittal and nuchal crests. In the adult ferret, 1 year or more, the fusion of the sutures is complete. In ferret HYF 34, 2 years after hypophysectomy, most of the bones of the skull were distinct and separate. The parietals and the nasals have open sutures and the basilar-sphenoid junction remained open. Partial fusion, beginning posteriorly, of the frontal bones, has occurred. The fronto-parietal suture was open, and there was no formation of a sagittal ridge. The appearance of this and other skulls from ferrets hypophysectomized at about 2 months of age was similar to those of ferrets of approximately 4–5 months old. Complete fusion of the sutures and the full development of the sagittal and nuchal crests had been permanently inhibited.

The difference in size of the skulls of these two ferrets can be seen by the measurements given in Table I. The retardation in width of skull is somewhat greater than that of length. The difference in the extent of ossification of the skulls can be judged by their difference in weight, which amounts to over 30%.

We wish to thank Mr T. A. Webster for taking X-ray photographs of some of these ferrets during the course of this work, and also Dr Meyer, St Bartholomew’s Hospital, for taking the X-ray photographs shown on Plate 8.
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Summary

Six immature male, and two immature female ferrets were hypophysectomized at a body weight of 500–600 g. (7–10 weeks of age). Subsequent body growth in ferrets of both sexes was retarded; in the female the stasis was almost complete, but the males continued to grow slowly until the age when the growth in the normal ferret is complete.

Skeletal development was studied by means of X-ray photographs and prepared skeletons. It was found that skeletal growth had been arrested; the bones had a low calcium content and open epiphyses. Stasis in the development of the skull was most marked, this being shown by the absence of sagittal and nuchal crests, open sutures and the retention of all the characteristics of the skull of an immature animal.

References

Collip, J. B. 1934 *J. Mt Sinai Hosp.* 1, 28–71.
Cushing, H. 1911 *"The Pituitary Body and its Disorders."* Philadelphia.

Description of Plates 6–8

Plate 6

Fig. 4. Photograph of skeleton of normal adult male ferret (HYF 30), 2½ years old. × ⁴⁄₅.

Fig. 5. Photograph of skeleton of hypophysectomized male litter-mate ferret (HYF 34), 2½ years old. Hypophysectomy was performed at the age of 2 months. × ⁴⁄₅.

Plate 7

Fig. 6. Photograph of skull of normal ferret (HYF 30), showing the development of the sagittal and nuchal crests and the complete fusion of all sutures. × ⁵⁄₃.

Fig. 7. Photograph of skull of hypophysectomized ferret (HYF 34), showing the preservation of characters of the skull of an immature ferret. × ⁴⁄₃.

Plate 8

Fig. 8. X-ray photograph of posterior region of normal ferret (HYF 30). × ¼.

Fig. 9. X-ray photograph of posterior region of hypophysectomized ferret showing the open epiphyses of the caudal vertebrae. × ¼.