EFFECT OF SALIVARY GLAND-ECTOMY ON THE RESPONSE OF THE TIBIAL EPIPHYSIS TO GROWTH HORMONE

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PLATE IV

In recent years, purification of the growth hormone from the anterior pituitary has made a remarkable progress and works with the use of purified preparation have been vigorously initiated. However, no clear-cut demonstration has yet been reported as to the significant as well as fundamental problem if the growth hormone, like other anterior pituitary hormones, do act through the agency of other kind of organ or organs. Meanwhile, we are afforded with remarkable informations concerning the incretory activity of the salivary gland from the well-known series of works of Ogata and his associates (1)(2)(3)(4)(5)(6)(7) including the work of chemical purification by Ito and Mizutani (5) who proposed the name Parotin for crystallized form of the hormone representing the active product of the salivary gland. On the biological properties of Parotin many reports have been published, according to which, growth and salivary gland hormones are similar in many respects. Besides, Akazaki (1) has shown that salivary gland-ectomy produces proliferative effect on the acidophils in the anterior hypophysis, and Tasaka (2) has reported that chronic treatment with Parotin brings about reduction of the cells in question. Such being the case, an assumption appears to be granted that the growth hormone may exert its influence primarily upon the salivary gland, whereby the activities of the hormone may be effectuated.

Present report concerns one of our attempts toward examination of this assumption as a working hypothesis.

MATERIALS AND METHODS

As experimental animals fifteen male rats 60-day-old were employed, and they were divided into the following 5 groups of 3 rats in each: the intact and untreated; the hypophysectomized and untreated; the hypophysectomized and growth hormone injected; the salivary gland-ectomized, hypophysectomized and growth hormone injected; the salivary gland-ectomized, hypophysectomized and Parotin injected. Hypophysectomy was performed exclusively at the 50th day of age. Salivary gland-ectomy was performed at the 35th day, both parotid and submaxillary glands being removed bilaterally. Intraperitoneal injection of growth hormone with daily dose of 0.5 mg. per individual and subcutaneous injection of Parotin with daily dose of 0.1 mg. per 100 gm. body weight were carried out for 7 days.

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beginning from the 53rd day to the 59th day. Untreated controls received saline injection in the same way. The growth hormone, which was kindly supplied through the courtesy of the Armour Laboratories (Lot # R 285128), had an activity of 100% of Armour Standard and its main contaminant was TSH 0.1 USPU/mg. After the 50th day of age animals were kept separately in individual cages at constant temperature of 28°C and maintained by means of pair feeding on a consistent diet of pressed barley boiled in fish soup. Besides, they were freely given of 1% saline as drink. The animals were fasted for 24 hours following the last injection and then brought to autopsy. They were decapitated, one or both tibias dissected out of soft tissues to be fixed in Zenker-formol for 3 days. They were then decalcified in 5% solution of formic acid in 10% formaline, sectioned and stained with hematoxylin-eosin, azan, van-Gieson or periodic acid-Schiff method.

RESULTS

The histological aspects of the proximal epiphyseal region of the tibia in each group of animals were as follows:

**THE INTACT AND UNTREATED GROUP** The cartilage cells were large and showed regular arrangement; long, delicate and parallel trabeculae consisting exclusively of lamellar bone were abundantly observed within the primary spongiosa (Fig. A). A number of osteoblasts being indicative of very active osteogenesis were seen on the surface of the trabeculae.

**THE HYPOPHYSECTOMIZED AND UNTREATED GROUP** As the result of reduction in size of the cells along with decrease in number of the cell layers in both the basophilic and vesicular zones, which indicated marked sign of retardation of proliferation, the decrease in the width of the epiphyseal cartilage became to be pronounced. Arrangement of the cartilage cells was rather irregular from the comparative point of view. Osteogenesis was also affected and the trabeculae adjacent to the epiphyseal cartilage were found to be scarce and coarse (Fig. B). Although there was no remarkable change in hemopoiesis in bone marrow, significant hyperemia was noticed.

**THE HYPOPHYSECTOMIZED AND GROWTH HORMONE INJECTED GROUP** The recovery in the non-calcified portion of the cartilage which was made up of enlarged cells was observed. And the width of the epiphyseal cartilage more or less increased as compared with that of the hypophysectomized and untreated group, though not so pronounced as in the intact and untreated group. Small amounts of newlyformed, delicate trabecular bone were seen and trabeculae were longer than those in the hypophysectomized and untreated group (Fig. C). Osteoblasts and osteoclasts were in active appearances and hyperemia in bone marrow was not remarkable.

**THE SALIVARY GLAND-ECTOMIZED, HYPOPHYSECTOMIZED AND GROWTH HORMONE INJECTED GROUP** The cartilage cells were smaller in size and decreased in number, and the epiphyseal cartilage was narrower. At different sites of the epiphyseal cartilage the characteristic picture of marked lacking of cell column was observed. The trabeculae adjacent to the cartilage were short and coarse (Fig. E).

**THE SALIVARY GLAND-ECTOMIZED, HYPOPHYSECTOMIZED AND PAROTIN INJECTED GROUP** The cartilage cells in both basophilic and
vesicular zones assumed rounded appearances and were large in number, indicating the active proliferation, and the width of the epiphyseal cartilage was much increased. Lack of cell columns in the epiphyseal cartilage was not the case in the samples of this group of animals. Although the trabeculae did not completely recovered their normal characters, histological aspects of cartilage region as a whole much simulated those in the intact and untreated group (Fig. F).

These results showed that the histopathological changes in the proximal epiphyseal region of the tibia following hypophysectomy tended to return towards normal upon growth hormone replacement and that the effect of the hormone was largely suppressed by removal of the salivary gland. And it was practically demonstrated that administration of the salivary gland hormone, Parotin, effectively favored the recovery of the histopathological changes in the absence of both the pituitary and the salivary gland.

**DISCUSSION**

As inferred in the introductory note, similarities have been disclosed between biological actions of the growth hormone and those of the salivary gland hormone. For example, promotion of gain of body weight, proliferation of cartilage cells in proximal epiphysis of the tibia, leucocytosis and proliferation of general mesenchymal tissues are all standing for the analogous effects of the two hormones. Moreover, lines of evidences suggest that the hormones do influence upon phosphorous, calcium, protein and carbohydrate metabolisms in similar ways.

As the histopathological changes resulting from salivary gland-ectomy, proliferation of the acidophils of the anterior pituitary and the epithelial cells in the thyroid gland, proliferation and swelling of Langerhans's islands in the pancreas, etc., have been reported by Akazaki. On the other hand, Tasaka has stated that degeneration of the parotid gland, roughening and numerical reduction of the anterior pituitary acidophils, degeneration and hypofunction of the thyroid gland result from chronic treatment with Parotin. These observations suggest that a certain hormone which stimulates the incretory activity of the salivary gland may in all possibility be produced from the acidophils of the anterior pituitary. In the current opinion of hormone production, it is conjectured that the cells in question are responsible for production of the growth hormone together with the prolactin hormone. Of the hormones concerned, the latter is known to stimulate the mammary gland development or production of progesterone from corpus luteum, so the former may be suspected to stimulate the incretory activity of the salivary gland.

Among various biological activities of the growth hormone, one of the most characteristic is that it stimulates the bony epiphysis and induces increase of the width in the epiphyseal cartilage of the hypophysectomized rat. In fact, some of the workers such as Greenspan et al. have employed the phenomenon for the assay of the hormone. In the present experiment, we also made use of the phenomenon to examine the proposed assumption that the growth hormone might act through the salivary gland and that the effect of the former would be rendered negative in the absence of the latter. This assumption was confirmed by our ex-
Experiment concerning the tibial epiphysis stimulating effect of the growth hormone. The fact already reported by Takizawa\textsuperscript{4}) that, in salivary gland-ectomized animals, degeneration of mesenchymal tissues especially epiphyseal cartilage is elicited, seems to support our assumption. Such inhibition will be elicited much more pronouncedly when the purified growth hormone is administered; we guess that the present result showing not so pronounced inhibition may be due to the stated contamination of the growth hormone by thyrotropic principle, since it has been demonstrated that apart from the growth hormone, certain other hormones, \textit{viz.}, thyroxine, cause a mean increment in the width of the cartilage plate of the tibia of the hypophysectomized rat (Marx \textit{et al.}\textsuperscript{6}), Becks \textit{et al.}\textsuperscript{2}) and Geschwind \textit{et al.}\textsuperscript{3}).

From the results dealt with in the foregoing accounts, it appears to be reasonably concluded that the tibial epiphysis stimulating effect of the growth hormone may be elicited through the possible agency of the salivary gland.

**SUMMARY**

Effect of salivary gland-ectomy on the proliferative response of proximal epiphyseal cartilage of the tibia of hypophysectomized rat to the growth hormone was investigated using young adult male rats of 60 days of age. Changes of the cells in the basophilic and vesicular zones, the decrease in the width of the cartilage plate and the reduction of osteogenesis in the tibia of the hypophysectomized animal showed a tendency of recovery to normal states following growth hormone replacement. Those morphological features in both hypophysectomized and salivary gland-ectomized animals did hardly regain their normalities as the result of growth hormone administration. Nevertheless, it was learned that the affected features in question, excepting the suffered osteogenesis, were in significant measure brought to recovery by injection of the preparation of salivary-gland hormone, Parotin.

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**REFERENCES**

EXPLANATION OF PLATE IV

Sagittal sections through the central portion of the proximal epiphyseal cartilage of tibia from 60-day old male rat. Van-Gieson stain. All ×55.

A, Intact untreated;  B, Control, hypophysectomized at the 50th day of age;  C, Hypophysectomized at the 50th day of age and subjected to growth hormone injection beginning from the 53rd day, 0.5 mg. daily for 7 days;  D, Same as C with exception of difference in daily dosage of growth hormone (1.0 mg.) (An example, of which mention is not made in the text, illustrated for the sake of reference);  E, Salivary gland-ectomized at the 35th day of age, hypophysectomized at the 50th day and subjected to injection of growth hormone in the same way as in C.  F, Same pretreatment as E, but injected of Parotin 0.1 mg./100 gm. body weight, daily for 7 days from 53rd day.