

SUMMARY

Amputation of supernumerary hind limbs was performed in 62 tadpoles (*Rana ridibunda* Pall). Regeneration of the principal and supernumerary limbs takes place with recurrence of the topographic-morphological relationships formed during the embryonic stage of development. Atypical formation of distal portions of the newly formed limbs takes place with appearance of external differentiation. Low temperature promotes a more stable recurrence of morphological signs of duplicate limbs by the regenerates.

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A TRIAL OF GROWTH HORMONE PREPARATIONS IN RATS WHOSE THYROID FUNCTION HAS BEEN BLOCKED WITH METHYLTHIOURACIL

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The discovery of Evans and Long of the growth hormone in 1921 prompted numerous attempts to isolate this substance in the pure form, which was achieved only in 1945 by the production of a highly purified preparation in the form of an individual protein [6]. However, the search for new, simpler and more convenient methods of biological testing of this hormone remains a problem of great importance; the most widely used tests, requiring preliminary hypophysectomy, are too complicated to be used as standard methods. Ia. M. Kabak and E. B. Pavlova [3] proposed the use of rats treated with methylthiouracil as test animals for growth hormone preparations, showing that blocking the thyroid gland function (as also by thyroidectomy) leads to cessation of formation of the growth hormone in the anterior lobe of the pituitary gland: the animals stop gaining weight, and in the anterior lobe of the pituitary the acidophil cells [2] which are generally held to produce the growth factor disappear; then, as a result of administration of growth hormone, growth is restored, while in the pituitary the histological changes do not take place.

* In Russian.

** See English translation.

This present work was undertaken as a development of these investigations with the following aims: 1) to confirm, by using one of the most specific indices of growth — the width of the cartilaginous growth plate of the proximal epiphysis of the tibia — that in rats receiving methylthiouracil the gain in weight which occurs as a result of the growth hormone is in fact due to true growth; 2) to investigate this test in rats with blocked thyroid gland function in greater detail, in particular to discover any relation between the dose of the pituitary preparation and the effect produced.

Replacement of hypophysectomy by blockage of thyroid function, in our opinion, not only simplifies the test procedure but has other advantages over hypophysectomy: absence of surgical trauma; exclusion not only of the growth factor of the pituitary but also of thyroid gland function which is especially important in the case of an inadequately purified preparation, since synergism between the growth hormone and the thyreotropic hormone (in the presence of a functioning thyroid gland) may result in an incorrect assessment of the activity of the growth hormone preparation [7].

Some of the findings presented here formed the subject of a lecture given to the All-Union Society of Endocrinologists [1].

EXPERIMENTAL METHOD

In this experiment rats of both sexes were used, the majority weighing 40-60 g. The diet of the animals consisted of porridge, to which was added fish oil, milk and finely chopped meat. Methylthiouracil (MTU) was mixed with this diet in a dose of 20-30 mg per rat (depending on the weight of the animal) per day. The amount of food given was roughly that consumed in the course of 24 hrs. In addition the animals received carrots or greens. The rats were kept on this diet for 35-62 days; every 5-7 days they were weighed and, after they had ceased to gain in weight, in addition to the continued administration of MTU they were given subcutaneous injections twice daily containing a preparation of pituitary growth hormone (PGH) prepared in accordance with the description by Ia. M. Kabak and E. B. Pavlova [3]. Besides the body weight the index of growth was the width of the epiphyseal cartilage of the proximal end of the tibia [6].

The animals were killed 24 hrs after the last injection, the tibia removed and treated by a method described by Greenspan et al. [5]: the tibia was cleared of soft tissues, divided in the median sagittal plane, and the halves of the bone either stained directly or first fixed in 10% neutral formalin. The staining procedure was as follows: the halves of the bone were immersed for 30 min in water, transferred into acetone for a minimum of 1 hr, and again washed in water for 30 min. Next they were placed for 1-2.5 min in a freshly prepared 2% solution of silver nitrate, washed in water and, without being taken out of the water, exposed to bright light until the calcified part had acquired a dark brown color. Afterwards the bone was immersed for 25-30 sec in a 10% solution of sodium thiosulfate and then rinsed in running water for 30 min. The width of the epiphyseal cartilage was measured under the low power of the microscope by means of an ocular micrometer. We took the average figures of 5-6 estimations of the transverse section of the cartilage.

EXPERIMENTAL RESULTS

We divided 23 male rats which received MTU for 62 days into 3 groups: one group acted as a control, the second was injected daily with 15 mg PGH per rat for 5 days, the third — 15 mg PGH per rat for 13 days. The results of the experiment are shown in Table 1.

As can be seen from Table 1, the rats receiving PGH injections for 5 days gained 12.5% of their original weight, and the mean width of their epiphyseal cartilage was 211% of that of the controls. Rats receiving PGH for 13 days gained 19.4% of their initial weight, and the mean width of their epiphyseal cartilage was 312% of that of the controls. The weight of the control rats receiving MTU was practically unchanged during this period.

We carried out a further trial of various doses of PGH. It was important to establish if the reaction of the epiphyseal cartilage of the tibia in these rats receiving MTU to PGH was quantitative in character. Table 2 answers this question in the affirmative, although variations are seen, connected with their individual characteristics. Altogether 5 experiments were performed on 108 rats of both sexes and receiving MTU for different periods of time.

TABLE 1

Changes in the Body Weight and the Width of the Epiphyseal Cartilage of the Tibia in Rats Receiving MTU After Injection of PGH (mean values)

Effect of	No. of animals	Body weight in g		Change in body weight		Width of epiphyseal cartilage	
		before injection of prep.	before death	in g	in %	in μ	in % of control
MTU (control)	7	85.8	86.2	+0.4	0.4	113	100
MTU + 15 mg PGH for 5 days	8	77.8	87.6	+9.8	12.5	239	211
MTU + 15 mg PGH for 13 days	8	92.5	110.5	+18.0	19.4	353	312

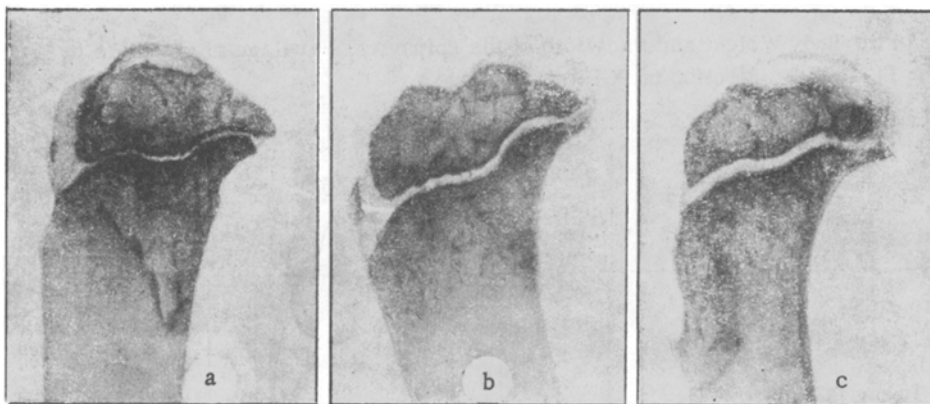
TABLE 2

Changes in the Width of the Tibial Cartilage of Experimental Rats After Injection of Different Doses of PGH (mean values)

Expt. no.	No. of animals in group	Dose of PGH (in mg)	Period of action of MTU in days	Width of tibial cartilage	
				in μ	in % of control
1	6	—	55	84	100
	5	0.5		116	138
	5	1.0		116	138
	5	2.0		129	153
	5	15.0		148	176
2	6	—	57	105	100
	6	2.0		189	180
	6	5.0		185	176
	7	15.0		213	208
3	5	—	46	110	100
	5	5.0		185	168
	5	10.0		191	174
	4	15.0		217	200
4	5	—	41	144	100
	5	5.0		202	140
	5	60.0		217	151
5	5	—	35	157	100
	6	7.5		234	149
	6	15.0		234	149
	6	30.0		239	150*

Before administration of the preparation was started the animals were divided into groups so that each group was made up as far as possible from males and females; one of these groups acted as a control in each experiment and received no other preparation than MTU; the rats of the other groups received two daily injections of the following doses of PGH: 0.5, 1, 2, 5, 7.5, 10, 15, 30 and 60 mg. Injections were give for a period of 4 days.

On the 5th day the rats were killed (in view of the short period of administration of the preparation these rats did not show any increase in weight).



Longitudinal sections of the proximal ends of the tibia of rats fed with MTU, stained with silver nitrate. a) Control (no PGH); b) PGH in a dose of 5 mg; c) PGH in a dose of 15 mg.

As may be seen in Table 2, a dose of 15 mg of PGH caused the most reaction in the cartilaginous plate, which increased in width roughly twice as much as that of the control (experiments 1, 2, 3). The least dose used — 0.5 mg — was also found to be active, giving a 38% increase in the width of the cartilage (experiment 1).

After feeding on MTU for 35 days (experiment 5) the width of the cartilaginous plate in the control rats was 157μ , and in this group the reaction of the cartilage was the same to different doses of PGH. With a more prolonged action of MTU — 41 days — (experiment 4), 46 days (experiment 3), 55 days (experiment 1), 57 days (experiment 2) — the width of the cartilage in the control animals varied from 144 to 84μ , while the reaction of the cartilage to PGH in all these experiments was quantitative in character, admittedly shown only very weakly in experiment 4, but well marked in experiment 3 (see figure), i. e., on the 46th day of action of MTU.

This investigation thus shows that rats kept in practically a state of athyreosis, do not form endogenous growth hormone: the width of the cartilaginous plate of the tibia, as shown above, is $84-157\mu$, while in hypophysectomized rats, according to Evans and his co-workers [5], it is about 160μ . The cartilaginous plate in the tibia of rats with blocked thyroid gland function was found to be sufficiently sensitive to growth hormone injected for a period of 4 days, and consequently this feature in these animals may be used as an index in the testing of the activity of the preparation.

SUMMARY

It was proved by the tibial test that the endogenic growth hormone is not produced in rats with blocked function of the thyroid gland. It was demonstrated that tibial epiphyseal cartilage is sufficiently sensitive to the corresponding preparations.

Preparations of growth hormone may be tested on rats in which the function of the thyroid gland is blocked by administration of methylthiouracil. This method is more convenient than the one used formerly. The latter was based on utilization of hypophysectomized rats.

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