

The Orthopedic Surgery Service, Ashkelon Government Hospital, Israel
and the Experimental Surgery Unit, Beit Dagan Veterinary Institute, Israel

THE EFFECT OF GROWTH HORMONE ON THE HEALING OF EXPERIMENTAL BONE DEFECTS

H. Z. HEROLD, A. HURVITZ & A. TADMOR

Accepted 26.v.71

Age is a major factor influencing the rapidity of fracture healing; yet the time required for consolidation of a fracture does not increase parallel to the age of the injured patient. In a previous study (Weissman, Herold & Engelberg 1966) we have found that the greatest difference in the time required for fracture union was between patients below fifteen years of age and those above twenty. It was also noteworthy that in the adult group itself there was practically no difference between the different age groups. This relation between skeletal growth and the accelerated rate of fracture healing suggested some influence of a somato-trophic hormone on the union of fractures.

Zadek & Robinson (1961) reported some success with bovine growth hormone in treating experimental long bone defects in dogs. The possibility of using such a substance clinically to increase the rate of bone healing was suggested by these authors. Koskinen (1963, 1964, 1967) reported the successful treatment of fractures with a combination of growth hormone and thyreotropine. Further trial of growth hormone and its effect on the healing of bone in rabbits and rats is reported here.

MATERIAL AND METHODS

Rabbits

Fifty rabbits served for the present study. One and a half centimeter segments were resected from the radii of every animal. During dissection care was taken to excise the bone with the periosteum in order to delay new bone formation. Following operation, serial X-rays were taken at monthly intervals and callus formation evaluated (Figures 1 and 2). The animals were divided into three groups. The first group consisted of twenty-six adult female rabbits with closed radial epiphyses



Figure 1. X-ray view of forelegs of an adult rabbit two months after resection of an osteo-periosteal segment of the radius. No callus is seen.

and an average weight 4 ± 0.25 kg. These served as controls and were given no medication. The second group consisted of twelve young rabbits with open radial epiphyses weighing 2 ± 0.2 kg each. The third group consisted of twelve adult female rabbits similar to those of the first group. They were treated with growth hormone after the bone resection. Six animals were given bovine growth hormone distributed by the Endocrinology Study Section, N. I. H., Bethesda, Md. (NIH-GH-B-7, 50 mg = 50 USP units). Each 100 mg of the dry bovine growth hormone powder was dissolved in 10 ml of saline, and 1 ml NaOH 0.1 N was added. Before injection this solution was diluted, and each rabbit was given 4 mg of the hormone intramuscularly twice a week for three weeks, thus receiving a total of 24 USP units. Six other rabbits from the treated group were given porcine lyophilised somatotrophic hormone supplied by the Mann Research Laboratories (Division of Becton Dickinson & Cot, N. Y.). The solvent for the lyophilised hormone consisted of glucose 0.25 g, phenol 0.025 g, HCL ad pH 4 and sterile water ad 5 ml. Ten I. U. dry hormone were dissolved per ml and each rabbit was given 1 ml injections twice weekly for a month after operation, thus receiving a total of 80 I. U. growth hormone.

Rats

Thirty adult albino rats were used for histological studies. A small 1.5 ml trephination hole was performed in both parietal regions symmetrically, halfway between the auditory meatus and the sagittal midline, according to a technique we

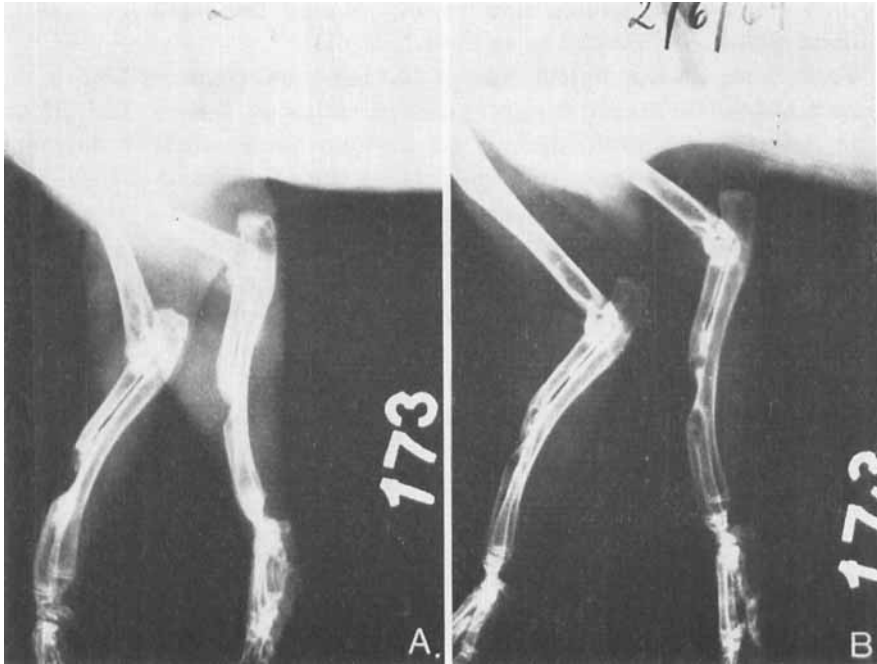


Figure 2. Forelegs of a young rabbit with open epiphyseal lines. A) A month after resection of a radial segment; new bone partially fills the gap. B) Two months after operation; the resected segment is practically replaced with new bone.

used in a previous experiment (Herold, Hurwitz & Tadmor 1970). The operated rats were divided into two groups. The first group consisted of eighteen rats which served as controls and were given no medication. The second group consisted of twelve rats treated with growth hormone after the trephination of their calvaria. They were given ovine growth hormone distributed by the Endocrinology Study Section, N. I. H., Bethesda, Md. (NIH-GH-S-5 Ovine). Each rat was given a one unit injection every other day, receiving a total of eight units during the sixteen days following operation. All the rats were killed seven weeks after operation and their calvaria decalcified with a solution of sodium citrate and formic acid in distilled water. The specimens were embedded in paraffin, cut in the sagittal plane and stained routinely with hematoxylin and eosin.

RESULTS

Rabbits

The mortality among the operated animals left us with a reduced number at the completion of follow-up, four months after the bone resection. Forty-four radial bone defects among the adult controls,

twenty among the young, and twenty among the hormone treated rabbits could be evaluated as to their healing.

Table 1 shows the distribution of the bone defects according to the time required for the first appearance of callus on X-rays. The difference between the adult animals on the one hand, whether hormone treated or not, and the young animals on the other hand is quite evident and statistically significant using the Chi square test ($\chi^2 = 21$ $p < 0.001$). Among the adult animals, though the appearance of callus seems at first view somewhat favoured by treatment with growth hormone, the difference with untreated controls is statistically insignificant ($\chi^2 = 1.3$ $p > 0.1$).

Table 1. Distribution of radial bone defects in rabbits according to the time required for the first appearance of callus on X-rays.

	Initial callus evident in four weeks	No callus whatsoever at four weeks	Total no. of bone defects
Adult controls	14	30	44
Young animals	19	1	20
Growth hormone treated adult rabbits	9	11	20
Total	42	42	84

Table 2. The incidence of non-union in untreated adult rabbits, in young rabbits and in growth hormone treated adult rabbits.

	Complete filling of bone defect by sixteen weeks	Non-union with persistent bone defect	Total no. of bone defects
Adult controls	13	31	44
Young rabbits	18	2	20
Growth hormone treated adult rabbits	12	8	20
Total	43	41	84

Table 2 shows the incidence of non-union in the three groups of rabbits. Here again the difference between the young and the adult animals is quite significant ($\chi^2 = 16.8$ $p < 0.001$). The difference between the hormone-treated adult rabbits and untreated controls is statistically insignificant ($\chi^2 = 4.87$ $p > 0.05$).

Table 3. Histological findings in calvarial defects of growth hormone treated rats and of untreated controls seven weeks after operation.

Histological aspect	Control animals	Growth hormone treated rats
Simple hematoma	...	1
Organised hematoma	11	5
Fibrotic callus	5	1
Osteoblastic reaction		
around bone defect: Weak	5	3
Important	3	5
Total	24	15

Rats

The specimens of the drilled calvaria were examined under the microscope and the healing process evaluated seven weeks after operation. The results of the histological examination are summarised in Table 3. For technical reasons, several slides were unreadable so that fifteen bone defects in the growth hormone treated group and twenty-four in the control group could be evaluated. The findings varied between simple hematoma and more or less intensive osteoblastic reaction (Figure 3). When viewed by an objective observer, not knowing which specimens represented growth hormone treated animals and which untreated controls, no significant difference was found between the two groups.

DISCUSSION

Koskinen (1963, 1964, 1967) has shown the effect of growth hormone and especially of its combination with thyreotropine on the formation of callus. A treatment with the latter combination led to a larger callus, and metabolic studies demonstrated a definite retention of calcium. A clinical trial on humans gave the impression that the administration of growth hormone led to accelerated fracture union and favoured the healing of established non-union. In this last point, the evidence is far from absolute since all cases had surgical treatment and the hormones were merely used as adjuvants.

In a recent report Zadek et al. (1961) describe a striking effect of growth hormone in preventing non-union of long bone defects in the dog. The series reported is very small, and the present experiment was

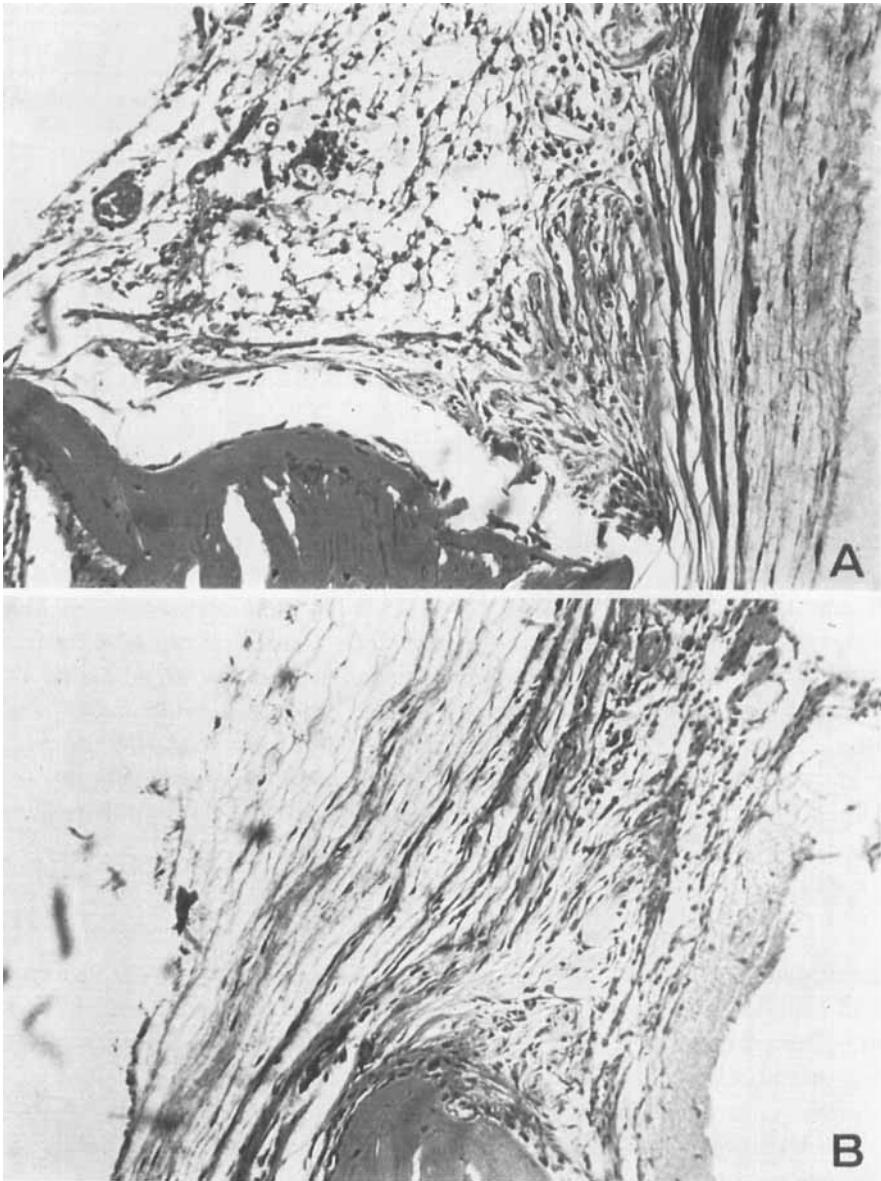


Figure 3. Histological aspects of defects in rats' calvaria seven weeks after operation. A) Untreated control. B) Growth hormone treated animal.

an attempt to find whether similar results could be found in other animals.

Hormonal treatment given in our series consisted of relatively large doses as compared to the experiments previously reported, so that any pharmacological effect would be evident. The influence on rabbits was far from clear, and though a minimal acceleration of callus formation might be possible, this was far from statistically insignificant.

The histological study of rats' calvaria with trephination defects showed no difference between growth hormone-treated animals and untreated controls. Such defects are never filled with cartilage, and bone formation, though minimal, is by direct apposition in the hematoma. Growth hormone, on the other hand, was shown to provoke hypertrophy of the cells of hyaline cartilage filling long bone defects. The hypertrophic cartilaginous callus would act as a bone inducer. Such a mechanism might explain why no influence of growth hormone could be found when studying the healing of calvarial trephination holes. It might be concluded from the experiments described above that growth hormone has no effect on the healing of bone of membranous origin. Its effect is limited to hypertrophy of fracture callus of long bones and an associated retention of calcium. The acceleration of fracture healing previously reported in dogs was not evident in rabbits. Further experimentation with growth hormone is necessary before its clinical use as adjuvant to fracture treatment is justified.

SUMMARY

The effect of growth hormone on fracture healing was tried on long bone defects in adult rabbits and on calvarial defects of rats with intact pituitaries. No statistically significant acceleration of healing was observed in either case. In the light of previous favourable reports on the action of this hormone, its failure in the present series might be due to the variation of animal species. The lack of influence of growth hormone on the healing of skull defects could be explained by the hypothesis that its action is limited to bones of enchondral origin. Further experimentation on the influence of growth hormone on bone healing seems mandatory before it can be recommended with certitude as an adjuvant in the treatment of fractures.

REFERENCES

- Herold, H. Z., Hurwitz, A. & Tadmor, A. (1970) Callus formation after acute brain damage. *Israel J. med. Sci.* **6**, 163-166.
- Koskinen, E. V. S. (1963) The effect of growth hormone and thyreotropine on human fracture healing. *Acta orthop. scand. Suppl.* **62**.
- Koskinen, E. V. S. (1964) Clinical and metabolic effects of hormonal treatment on bone repair. *Proceedings of the Second European Symposium on Calcified Tissues, Liege, April 1964*, pp. 157-166.
- Koskinen, E. V. S. (1967) Effect of endocrine factors on callus formation in experimental fractures. *Symp. biol. hung.* **7**, 315-322.
- Weissman, S. L., Herold, H. Z. & Engelberg, M. (1966) Fractures of the middle two thirds of the tibial shaft. *J. Bone Jt Surg.* **48 A**, 257-267.
- Zadek, R. E. & Robinson, R. A. (1961) The effect of growth hormone on healing of experimental long bone defects. *J. Bone Jt Surg.* **43 A**, 1261.