EFFECT OF EXOGENOUS HOMOLOGOUS RNA AND HYDROCORTISONE ON THE CONTENT OF COLLAGEN AND GLUCOSAMINOGLYCANES IN REGENERATING RAT BONE

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The effect of a single injection of hydrocortisone and of injections of exogenous homologous RNA preparations on fracture healing was studied. Exogenous homologous RNA was found to stimulate reparative osteogenesis considerably, while hydrocortisone inhibits bone formation.

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Protein biosynthesis by the osteogenic cells of the regenerating bone is one of the principal metabolic processes ensuring the normal course of reparative osteogenesis.

Earlier investigations [1, 3] showed that injection of exogenous homologous RNA preparations into animals stimulates reparative osteogenesis, mainly through protein biosynthesis in the bone tissue [2]. Hydrocortisone, if given frequently, is known to inhibit reparative processes in bone tissue by suppressing protein biosynthesis in osteogenic cells [10-12]. However, it has not yet been discovered whether this inhibition of osteogenesis can be produced by a single injection of hydrocortisone. If under these circumstances the regenerative process is substantially unchanged, injection of hydrocortisone in this way could be used to increase the rate of repair of tissues by increasing the concentration of breakdown products of protein and mucoprotein complexes in the fracture zone [7, 8].

Hydrocortisone can thus act as a distinctive "indirect" stimulator of bone regeneration.

Having regard to the stimulating role of homologous RNA preparations in osteogenesis and the facts concerning the action of hydrocortisone described above, it was decided to compare their effect on the healing of experimental fractures.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180 g, with an experimental fracture of both bones of the forearm. A preparation of total RNA was obtained from the bone tissue of young rats [4, 6].

The RNA was injected intramuscularly into the rats as follows: 0.7 mg/100 g body weight 24 h after the fracture, and 0.5 mg/100 g body weight on the 3rd-4th and 5th days after.

Hydrocortisone (Richter) was injected intramuscularly into the rats in a dose of 1 ml, containing 25 mg hydrocortisone.

The content of collagen (as hydroproline [9]) and of glucosaminoglucans (as hexosamines [5]) in the regenerating bone was determined. Roentgenograms were taken on the M-125 apparatus after 6-12 and 20 days.

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TABLE 1. Hydroxyproline and Glucosaminoglucan Content in Regenerating Bone of Rat

Days	Injections of RNA				Injections of hydrocortisone			
	n	hydroxyproline	n	glucosaminoglucans	n	hydroxyproline		glycosaminoglucans
6-th	5	52,9±8,256	5	2,10±3,002	4	11,92±1,918	5	1,04±0,1913
12-th	4	63,9±5,776	5	1,70±0,2995	5	31,70±8,418	5	0,60±0,1425
20-th	5	63,02±3,377	5	1,10±0,2412	5	34,07±5,254	5	0,20±0,04

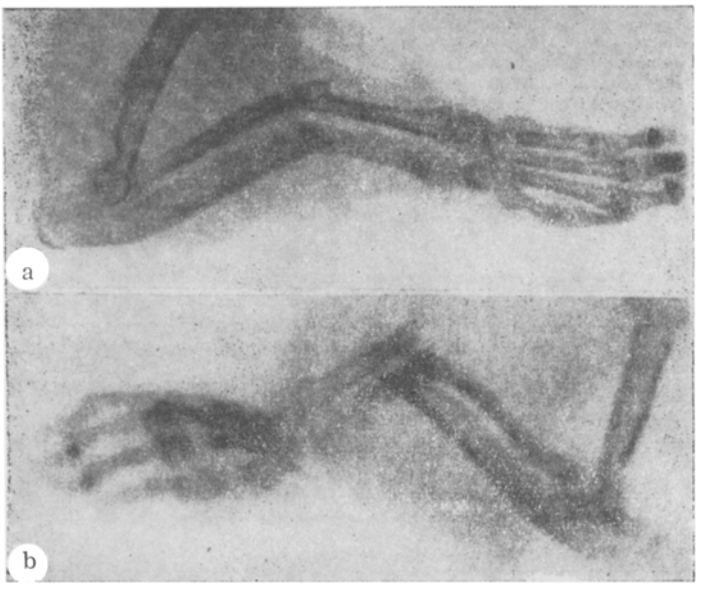

Fig. 1. Roentgenogram of right forearm of rat receiving injection of hydrocortisone: a) on 6th day after fracture reparative changes are absent at the ends of the fragments; b) on the 20th day after fracture regeneration of bone tissue is absent.

EXPERIMENTAL RESULTS

The experiments showed definite differences in the course of fracture healing in the rats receiving RNA and hydrocortisone. On the 6th day after the fracture, no signs of regeneration could be seen on the roentgenograms of animals receiving hydrocortisone (Fig. 1a), whereas in animals injected with homologous RNA, cloud-like deposits could be seen on the periosteal and endosteal surfaces (Fig. 2a). Significant differences were also detected in the hydroxyproline and glucosaminoglucan contents. The hydroxyproline content in regenerating bone of rats receiving injections of homologous RNA was 52.9 mg, but in corresponding bone from rats receiving hydrocortisone it was 4.4 times smaller (Table 1).

A similar pattern was observed in relation to the glucosaminoglycan content: this was twice as high in the rats receiving exogenous RNA.

By the 12th day the hydroxyproline content in the rats after receiving exogenous RNA was increased by 12%, but in those receiving hydrocortisone it was increased by 266.3%, i.e., in these animals a much more marked increase in the hydroxyproline content was observed than at the earlier periods.

Hence, despite the appreciable lowering of the protein and mucoprotein level in the early periods, by the 12th day some degree of equalization of the collagen content was observed in the regenerating bone of rats receiving hydrocortisone and those receiving RNA, although in general the collagen content in the former was only half that in the latter.

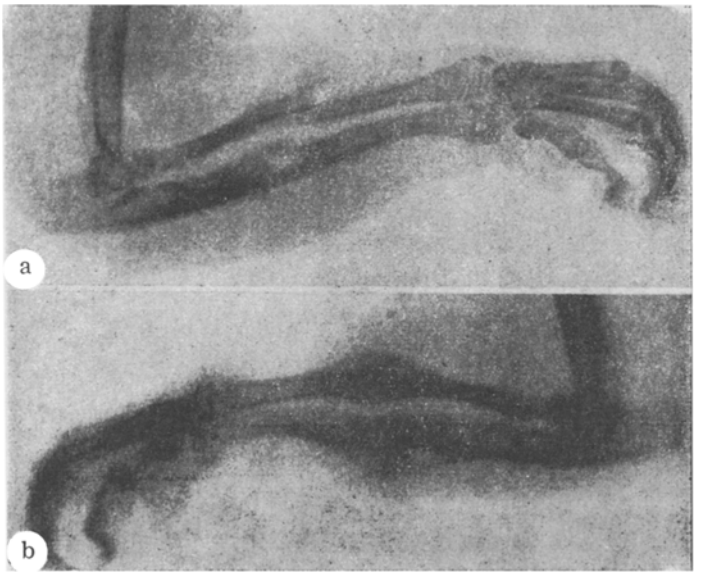

Fig. 2. Roentgenogram of right forearm of rat receiving homologous RNA: a) 6th day after fracture, cloud-like periosteal deposits are visible on the proximal and distal fragments; b) complete consolidation of the fracture on the 20th day after injury.

Characteristically, by the 12th day after injection of hydrocortisone, the content of glucosamines in the regenerating bone was also appreciably reduced.

No reparative changes were observed in the fracture zone of the rats receiving hydrocortisone by the 12th day compared with their appearance on the 6th day, while in rats receiving homologous RNA the intensity of the periosteal shadows of bone callus was considerably increased. By the 20th day the content of glucosaminoglucans in the group of animals receiving hydrocortisone was lower than in the rats receiving homologous RNA. When both groups were compared at this period, a difference in the collagen content in the regenerating bone of the rats could be clearly seen after injection of exogenous RNA, on the one hand, and also of hydrocortisone, on the other.

The roentgenograms also showed a significant difference in fracture healing in the two groups of animals. In the rats receiving hydrocortisone, no signs of consolidation could be seen just as before (Fig. 1b), while in the rats receiving an injection of homologous RNA, complete consolidation of the bone fragments occurred, due to the formation of a considerable mass of periosteal and endosteal bone callus, and the commencing formation of a medullary canal could be seen (Fig. 2b).

The results show that exogenous homologous RNA, injected intramuscularly into animals at a point remote from the fracture, considerably accelerates the process of fracture healing, while hydrocortisone, even if injected only once, inhibits osteogenesis. The suggestion that a single high dose of hydrocortisone might be given to stimulate the early phase of regeneration of bone tissue was thus not confirmed.

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