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CARTILAGE EXTRACT IN TREATMENT OF FRACTURES IN RABBITS

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Various biological product have been tested for their possible accelerating effect on bone-healing. For the past two years we have been using cartilage and its extracts in the treatment of experimental bone defects. The choice of this product was empirical, after failure of other materials. The use of cartilage seemed somewhat logical, it being the main constituent of the early callus. Recent reports by Prudden (1963) on the favorable influence of cartilage on wound healing further encouraged our experiments.

In a series previously reported by us (1967), animals with experimental bone defects were treated with cartilage in a combined way, *i.e.* both locally and with subcutaneous cartilage extract injections. No statistically significant difference in the time of healing was observed when comparing the treated animals and controls. Nevertheless, in those cases where the local cartilage did not provoke an inflammatory reaction or an abscess formation, union seemed accelerated. The present series consists of animals treated exclusively with parenteral cartilage extract, thus avoiding any local inflammatory reaction at the fracture site.

MATERIAL AND METHODS

To study the possible action of cartilage extracts on bone-healing, a series of standard bone defects were produced in the radii of rabbits. Each defect consisted of a three millimeter segment removed from the midshaft of the radius, the ulna being left intact. These standard defects have the advantage that no fixation or splinting are necessary and the incidence of healing is predictable and reproducible.

The delay required for the first appearance of callus and that required for full union was determined by serial roentgenograms at weekly intervals (Figure 1). The histology of the callus a fortnight after operation was compared in a group of treated animals and untreated controls.

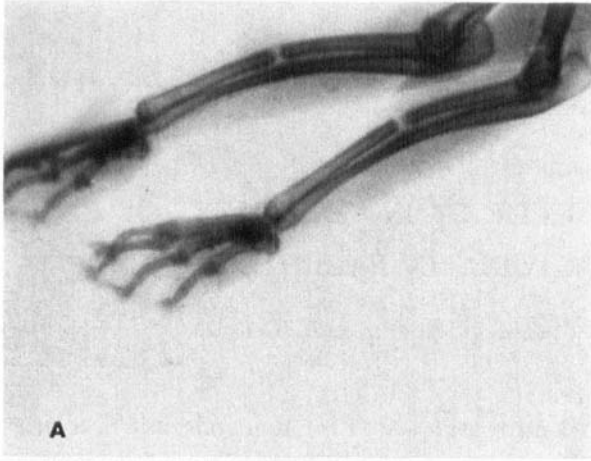
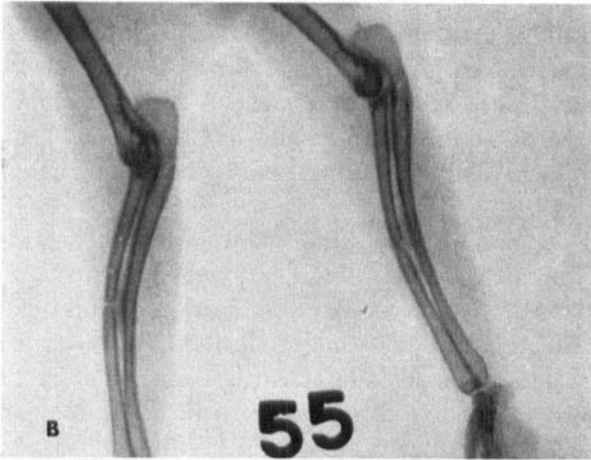
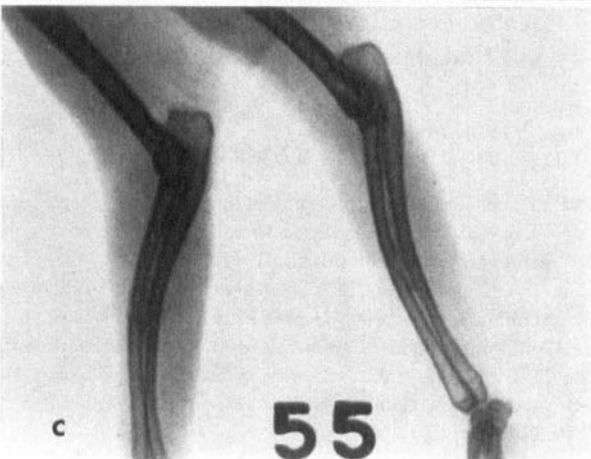


Figure 1. Radial bone defects of the rabbit as seen on roentgenograms. A. Three weeks after the operation, no callus evident.



B. At five weeks, appearance of callus.



C. At eight weeks, complete union.

The cartilage extract used in this experiment was prepared from cartilage powder supplied by the Nutritional Biochemicals Company. This was mixed with a physiological saline solution so as to obtain a suspension containing 12.5 per cent cartilage. After mixing in a shaker for three hours at room temperature, the liquid was centrifuged and filtered, resulting in a clear transparent fluid. This was bottled and kept in the freezer till use, when mild heating to about 30° facilitated its use for injections.

Controls

Eighteen adult female rabbits weighing 4 ± 0.2 kilograms each had both their forelegs operated, producing a series of thirty-six standard three-millimeter radial defects. One animal sustained a subsequent fracture of the ulna, thirty-five legs being left for evaluation of the healing time.

Cartilage-Treated

Twenty-three female rabbits of similar weight were operated to produce the same bone defects as in the controls. Each animal in this group was given subcutaneously ten milliliters of the 12.5 per cent cartilage extract in saline every other day for a fortnight and twice in the third week after the operation. One animal died subsequently before bone healing and one sustained a fracture of the ulna, forty-three bone defects being left for follow-up in this group.

Pathology

Ten rabbits were operated as above and five treated with cartilage extract. One rabbit expired of coccidiosis, and the remaining nine were sacrificed fourteen days after the operation, their eighteen operated forelegs being used for histological examination of the early callus.

RESULTS

X-Rays

The time necessary for the first appearance of callus and the time required for complete filling of the defects were studied. Table 1 compares the mean healing time in treated rabbits and in untreated controls. It is evident that both the appearance of callus and union were quicker in the cartilage-treated rabbits.

The distribution of cases according to healing time revealed that in six out of thirty-five control fractures, the first appearance of callus took more than four weeks. Among the cartilage-treated animals this delay occurred only in one case out of forty-three. The difference is statistically significant ($\chi^2 = 5.74$ $p < 0.05$). In five of thirty-five controls union was complete by five weeks, whereas among the treated animals twenty out of forty-three fractures were solidly united by this time interval. Here again the difference is statistically significant ($\chi^2 = 5.74$ $p < 0.05$).

Table 1. Mean healing time.

	No. of legs	First appearance of callus on X-rays (weeks)	Complete union (weeks)
Control group	35	3.8 $\pm$ 0.9	7.4 $\pm$ 2.3
Cartilage-treated	43	3.4 $\pm$ 0.4	6.3 $\pm$ 2
t (Student's test)		2.73	2.22
P		< 0.005	< 0.05

Table 2. Histopathological evaluation of callus two weeks after operation.

	No. of bone defects examined	Bone reparation with:			Non-specific results
		Strong cartilage formation	Medium cartilage formation	Poor cartilage formation	
Controls	10	0	2	7	1 (abscess)
Cartilage-treated	8	6	2	0	0

Pathology

Upon dissection at two weeks after operation, the bone defects were filled with soft tissue masses protruding from the neighboring bone, thus having the aspect of local tumefactions (Figure 2). Microscopic evaluation of the cartilage formation and the progress of healing in treated animals and controls is presented in Table 2. It is evident that callus formation was more advanced at this early stage among the treated animals.

A description of two extreme findings will give a fair representation of the reparative process observed. In the control group the bone defects were filled with loose connective tissue amply vascularized in places. Some fibrous strands were found traversing this connective tissue. Reparation was seen especially subperiostally as well as intramedullarily, but was slight in extent, with no tendency to unite with the opposite bone end. In the treated group copious cartilage formation with incipient calcium deposits and ossification was observed around each stump. The interval between the sawed bone ends was occupied largely by newly formed cartilage tissue, with progressive ossification. The few strands of connective tissue had begun to undergo cartilage metaplasia (Figure 3).

Figure 2. Gross appearance of the pathological specimens.

A. Dissected mounted forelegs of the rabbits; left leg immediately after operation, right leg two weeks after operation.

B. The defect of the radius and the intact ulna.

C. The macroscopic appearance of the callus.



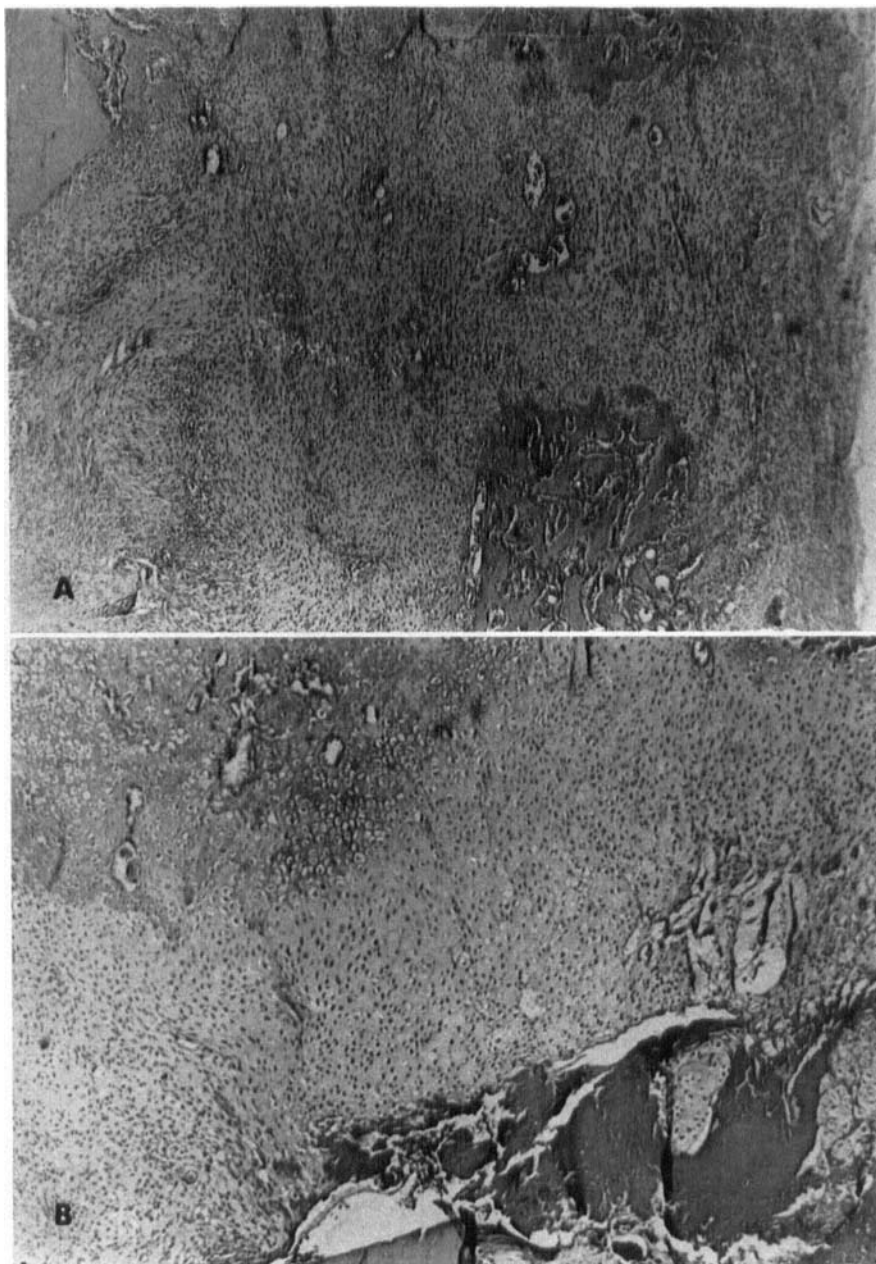


Figure 3. Microscopic appearance of the callus on low magnification. A and B = treated animal. Strong cartilage formation and beginning ossification in operated area. A $\times 10$, B $\times 16$.

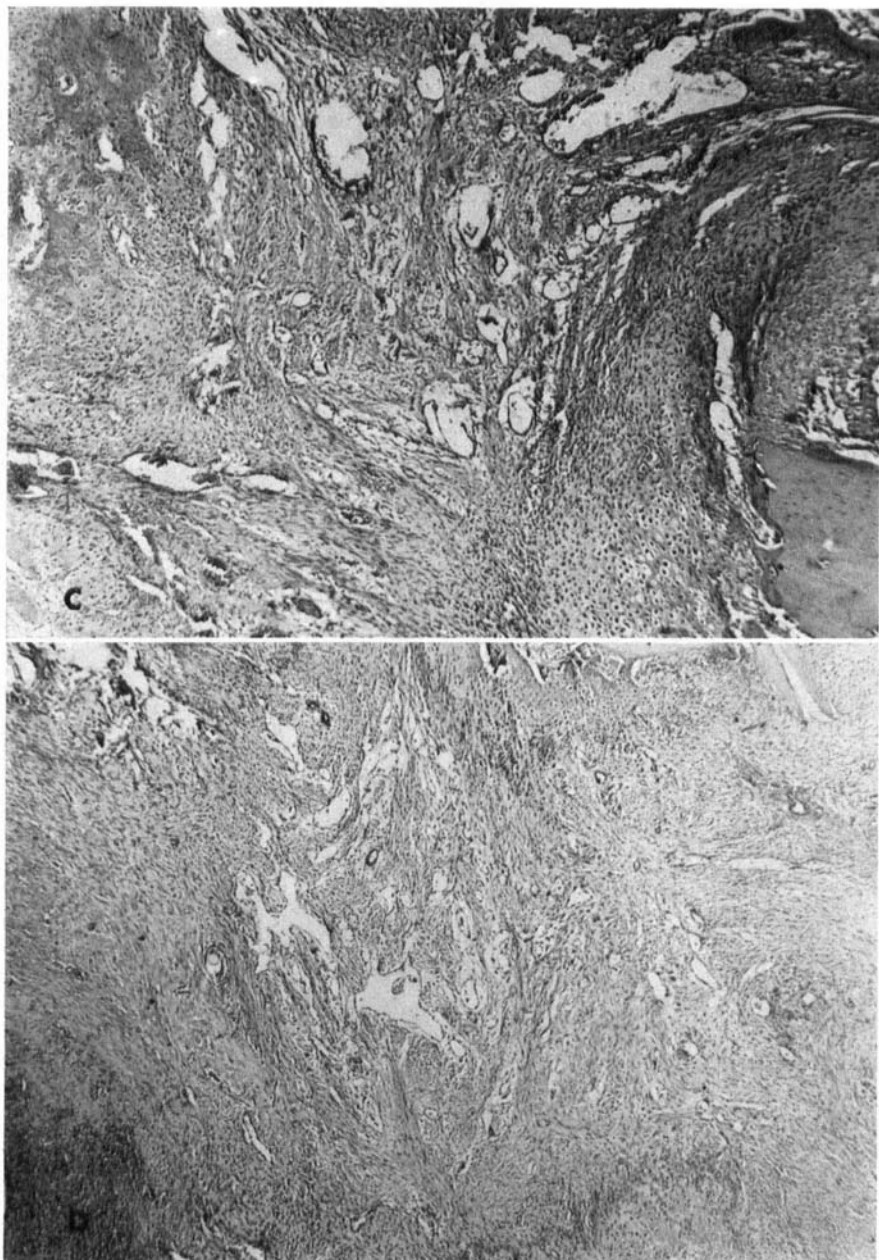


Figure 3. Microscopic appearance of the callus on low magnification. C and D = control. Connective tissue and poor cartilage formation in the callus. C $\times$ 10, D $\times$ 10.

DISCUSSION

Saline extract of cartilage powder appears to have a favorable influence on the healing of soft tissue wounds when administered locally or parenterally; parenteral administration of the extract seems to accelerate bone healing in the rabbit.

It is as yet impossible to determine the mechanism of action and the active components of cartilage. Histological study of fracture callus in the rabbit indicates that the extract administered has some influence on the early stages of union, before calcification.

Chondroitin sulphate was reported by Moss (1958) to play a role in inducing ossification. On the other hand, it was reported by Wolarsky (1965) to have no influence on soft tissue wound healing. Its role as the main active component of cartilage extract is improbable since any observable effect of this extract on bone seems to take place before ossification.

The possible action of saline cartilage extract on bone-healing is far from clear. The encouraging findings in the present series justify further research in this field.

SUMMARY

Experimental bone defects were produced in the radii of rabbits. A series of operated animals was treated with parenteral saline cartilage extract and the healing time compared with untreated controls. An earlier appearance of callus and accelerated union were observed in the treated rabbits.

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