STIMULATION OF THE REGENERATION PROCESSES AFTER AMPUTATION OF EXTERNAL ORGANS OF MAMMALS, BY TREATING THE WOUND SURFACE WITH TRYPSIN AND CALCIUM CHLORIDE

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It is known that amputated extremities and tails of mammals do not regenerate [1]. Certain investigators have been able to stimulate regeneration processes after amputation of toes and parts of extremities of young animals. Thus, offspring have been obtained from white rats that were kept for a long time on a diet free of vitamins A and D. Finger-like outgrowths consisting of one segment occurred in 5 of the 34 newborn rats after amputation [3]. In other investigations [7] the fourth and fifth toes of the right forefoot were amputated, and the wound surface was repeatedly treated with crystalline trypsin and a solution of calcium chloride. As a result, in some of the experimental animals the investigators noted regeneration of atypical digits whose skeleton consisted only of one long phalanx. According to certain observations [8], in 12- to 15 day-old rats, after amputation of the extremity in the distal part of the diaphysis of the femur, the epiphyseal cartilage is regenerated in some cases on the end of the bone stump, but it is appreciably smaller than that removed and does not have a marrow cavity. The formation of such an epiphysis provided elongation of the stump. In the present work, we investigated the stimulation of regenerative processes by local treatment with trypsin and calcium chloride after amputation of the hind leg and tail of young white rats.

EXPERIMENTAL

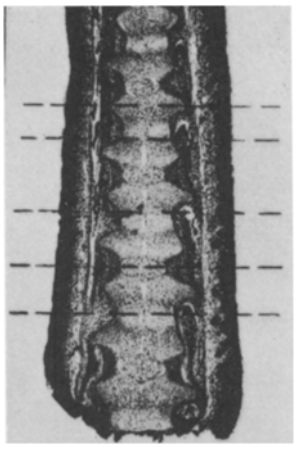

Fig. 1. Part of a longitudinal section through the amputated portion of the tail. The dashed lines indicate the different variants of tail amputation in (series of experiments) I and II. Objective 3.7 ×, ocular 4 ×.

The experiments were carried out on 52 two day old rats which had been housed together with the mothers and had received the usual full ration. We left no more than 6 rats in each brood since it is known that with a greater number of animals in the brood poor growth and a low postoperation survival rate are observed. In the first two series of experiments we studied the effect of treating the amputated surface of the tail with trypsin and calcium chloride, on the regenerative processes. In series III and IV we investigated the effect of this treatment on the regenerative processes after amputation of the leg. In the study of tail regeneration we amputated 1 cm distal section. The left hind legs were amputated slightly above the distal epiphysis of the femur. All amputated parts were fixed for subsequent exact determination of the place of amputation.

On the day after amputation the wound surfaces of the tail and leg of the animals in series I and III were treated with a warm 0.95% solution of calcium chloride and crystalline trypsin. Treatment began with submergence of the amputation surface into the calcium chloride solution for 3-5 min. Then we applied crystalline trypsin to the surface, also for 3-5 min. The amputation surface was treated with each of these substances 5-6 times, during which we observed softening of the scab and its removal from the surface. The stumps were thus treated daily for 15 days. The control animals

Fig. 2. Longitudinal section through regenerated portion of tail. Objective 3.7 ×, ocular 4 ×.

of series II and IV were not subjected to treatment after amputation of the tail and leg. All animals were killed 35 days after amputation. For the histological investigation the stumps and all amputated parts of the leg and tail were fixed in a 20% solution of formalin, then transferred to a 10% solution of formalin, decalcified by the electrolytic method, embedded in celloidin–paraffin and sliced into a series of sections 7-8 μ thick. The sections were stained with Boehmer's hematoxylin and eosin.

RESULTS

After amputation of the tail it was established that the amputation line passed through different sections of the vertebra (Fig. 1). In some cases we removed a part of the distal epiphysis of the vertebral body or the epiphysis completely, and in other cases the line of amputation passed along the diaphysis of the vertebral body or in such a way that the distal epiphysis and the entire diaphysis were removed. In several cases this line was found between the vertebrae.

A study of the histological sections of the tail stump of the control animals showed that in the overwhelming majority of cases no appreciable regeneration of the last vertebra was observed (see table). Only in those cases where during amputation of the tail we removed a small section of the distal epiphysis of the vertebral body did regeneration take place. Regeneration as a rule was absent when the entire epiphysis was removed. In all cases of the experimental animals whose wound surface was treated with trypsin and calcium chloride we noted regeneration of the missing section of the last vertebra. In one experiment after amputation of the tail a cartilaginous outgrowth developed between the vertebrae on the articular surface of the last vertebra. We emphasize that we observed complete regeneration of the vertebra even in those experiments where in the stump only its proximal epiphysis was left. In these cases regeneration of the soft tissues occurred along with regeneration of the skeletal elements of the stump, which in the aggregate led to regeneration of a small section of the tail (Fig. 2). Thus in the experimental series, in comparison with the control series, we noted an appreciable stimulation of the regeneration processes.

In series III and IV we investigated regeneration processes after amputation of the hind leg. The control rats were not subjected to additional effects after the operation. A study of the stump sections of these animals on the 35th days after amputation showed that in the overwhelming majority of them there was no appreciable regeneration

Effect of Trypsin and Calcium Chloride on Regeneration of Last Vertebra After Amputation of the Tail of White Rats

Experimental conditions	Control		Experimental	
	total	number of cases of regeneration	total	number of cases of regeneration
Removal of part of distal epiphysis of vertebral body	2	1	2	2
Removal of distal epiphysis of vertebral body	2	–	1	1
Amputation along middle of vertebral body	2	–	–	–
Removal of distal epiphysis and entire diaphysis of vertebral body	2	–	3	3
Amputation between vertebrae	6	–	5	1

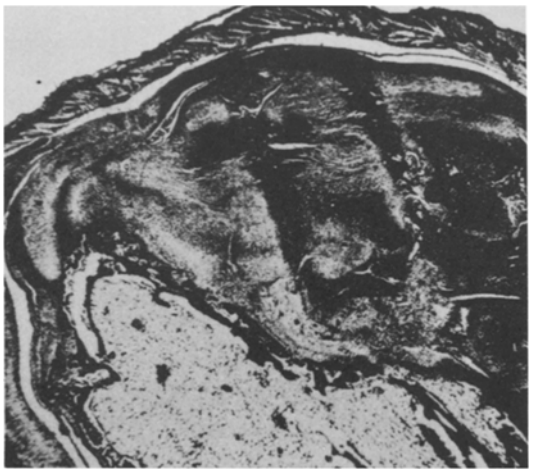

Fig. 3. Part of a longitudinal section through leg stump. The newly formed epiphysis of the femur is seen. Objective 3.7 ×, ocular 4 ×.

of the femur or stump. Only in 2 of the 15 animals (13.3% of the cases) was the sawed surface of the bone covered with a layer of cartilage which had formed like an epiphysis. Among the experimental animals, cartilage formation on the end of the bone stump in 4 of the 12 animals (33.3% of the cases) and the newly formed epiphyses reached larger sizes than those of the control animals (Fig. 3). The formation of a marrow cavity began in one of the epiphyses. We note that for most of the animals in the experimental series we observed in the distal part of the stump an accumulation of little-differentiated cellular elements which had participated in the formation of cartilage. In connection with this we can assume that if the experimental animals had been under observation for a longer time than in our experiments, regeneration would have taken place in more animals and in a greater volume.

The following considerations can be expressed about the mechanism of the regenerative stimulating effect of treating the amputation surface with trypsin and calcium chloride. In a number of investigations the significance of the stage of destruction and dedifferentiation of tissues in the wound area for regeneration processes has been demonstrated [2, 4]. It is known that trypsin destroys certain intercellular substances and in connection with this is used for disaggregation of tissues [6]. The calcium ions stabilize solutions of trypsin, preventing the autolysis of the enzyme, and can have a favorable effect on their conversion of proteins [5]. It is quite probable that it is precisely these properties of trypsin and calcium chloride that underly their stimulating effect on the regeneration processes in our investigations. At the same time, the repeated removal of the scab from the amputation surface as a consequence of its specific treatment, promoted traumatization of the tissues and enhancement of the destructive processes in the wound area.

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