EFFECT OF α-FETOPROTEIN ON REPARATIVE REGENERATION OF THE SKIN AND LONG BONES

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The effect of human, canine, and feline α-fetoprotein on reparative regeneration of the skin and long bones of mice and rats was studied. α-Fetoprotein has a stimulating action, through the formation of young connective tissue, on regeneration but without strict species-specificity. Of the various α-fetoproteins studied, a commercial preparation of human α-fetoprotein had the highest physiological activity.

KEY WORDS: α-fetoprotein; regeneration; biostimulation; connective tissue.

The solution to the problem of shortening the time required for the healing of skin wounds and fractures is closely bound up with the use of biostimulators of regeneration, among which the foremost place is occupied by embryonic tissues [7, 1]. Reports of the stimulating effect of fetal blood sera of various animals on growth of tissue cultures [12, 14] and also on the reparative regeneration of skin, long bones, and tissues of the bladder and stomach [5] have recently been published. With the discovery of α-fetoprotein – the chief protein component of fetal sera [1-3, 9, 10, 13] – it was possible to determine its influence on processes of reparative regeneration.

EXPERIMENTAL METHOD

Semipurified preparations of human, canine, and feline α-fetoprotein were used as biostimulators in the investigations, and a parallel series of control experiments was set up in which the serum α-globulin fraction of the corresponding adult animals and man, not containing α-fetoprotein, was used.

To isolate the α-fetoprotein the method of precipitation by ammonium sulfate followed by purification by preparative electrophoresis in agar [4] was used. In another series of experiments commercial human α-fetoprotein* was used. The α-globulin fractions were obtained by preparative electrophoresis of whole blood sera in agar [4]. The total quantity of protein injected daily in both the experimental and control series was 90 mg/kg body weight. The daily dose of α-fetoprotein was chosen empirically and averaged 0.2 mg/kg body weight. The protein preparations were injected into the thigh muscle daily until healing was complete.

Regeneration was studied on experimental models of graduated thermal burns of the dorsal skin and closed fractures of the radius without immobilization in 56 rats and 118 mice of different age groups. Thermal burns of the skin with an area of 2 cm^2 in the rats and 0.8 cm^2 in the mice were inflicted by a specially constructed instrument.† Fractures of the radius were produced by mechanical pressure on its middle third with surgical forceps.

The course of repair was assessed clinically, roentgenologically, by measuring the area of the wound and calculating the rate of contraction, by recording the strength of the bony callus by means of an apparatus of the writers' own design,‡ and by histological methods of investigation. Preparations obtained when the animals were sacrificed on the 2nd, 7th, 16th, 29th, and 46th days of the experiments were stained with hematoxylin–eosin, borocarmine, Bismarck brown, Lyon blue, and by Hart's method. The location of alkaline phosphatase, RNA, and DNA also was determined histochemically. Mucopolysaccharides were dissected by staining with

*Obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology as part of an immunodiagnostic kit for primary liver carcinoma.

†Efficiency Suggestion Certificate No. 109 dated May 24, 1975.

‡Efficiency Suggestion Certificate No. 136 dated June 24, 1976.

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TABLE 1. Duration of Healing of Skin Burns and Fractures of the Radius in Rats and Mice (M ± m)

Nature of injury	Preparation injected	Number of animals		Times of healing, days	
		rats	mice	rats	mice
Skin burns	Donor's blood serum	—	10	—	16.0±0.3
	Commercial human α-fetoprotein	—	10	—	9.0±0.5 $P<0.001$
	Human α-fetoprotein isolated by authors	—	10	—	10.0±0.4 $P<0.05$
	α-Fraction of adult canine blood serum	10	16	19.0±0.6	11.0±0.5 $P>0.05$
	Canine α-fetoprotein	21	33	13.0±0.5 $P<0.01$	8.0±0.3
Fractures of radius	α-Fraction of adult canine blood serum	7	12	21.0±0.9 $P<0.001$	16.0±0.5 $P<0.001$
	Canine α-fetoprotein	18	28	17.0±0.4	11.0±0.3

TABLE 2. Effect of α-Fetoprotein on Wound Microflora of Skin Burns in Mice (M ± m)

Preparation injected	Number of microbial colonies grown on different days per cm² nutrient medium			Composition of microflora isolated on 5th day of experiment, %				
	2nd-4th day	5th-7th day	8th-10th day	Proteus	Staphylococcus	Streptococcus	Escherichia coli	Bacillus pyocyaneus
α-Fraction of adult canine blood serum (control)	35,0±1,8	44,0±1,6	30,0±1,5	43,7	62,5	18,7	37,5	12,5
Canine α-fetoprotein (experiments)	19,0±0,9	21,0±0,8	18,0±0,8	24,2	39,3	6,0	75,7	3,0

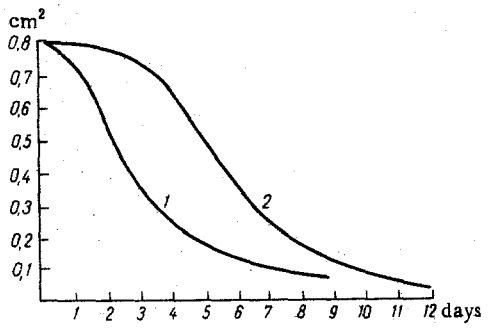

Fig. 1. Changes in area of burn during healing in mice: 1) experiment; 2) control. Abscissa, time (in days); ordinate, area of wounds (in cm²).

toluidine blue and identified with testicular and bacterial hyaluronidase. The bones were decalcified by Modyaev's method [6]. The burned surface was examined for the presence of a microflora by seeding its contents on nutrient media and determining the mean number of microbial colonies per unit area in the experimental and control series.* The white and blood cell counts and ESR, total protein (on the RLU refractometer), and serum protein fractions (by electrophoresis on paper) were determined in the blood of some of the animals periodically.

EXPERIMENTAL RESULTS

As Table 1 shows, parenteral injection of human and canine α-fetoprotein shortened the period of healing of skin burns in rats and mice on average by 30%. This was accompanied histologically by activation of proliferation of young connective tissue, accumulation of mucopolysaccharides in it, and the more rapid maturation of granulations and epithelization of the wound from the periphery toward the center.

*The microbiological part of the work was done in the Bacteriological Department of the Clinical Diagnostic Laboratory of the V. M. Bekhterev City Infectious Diseases Hospital, with the assistance of bacteriologists Dr. N. V. Evsyukova and Dr. A. G. Sorokina.

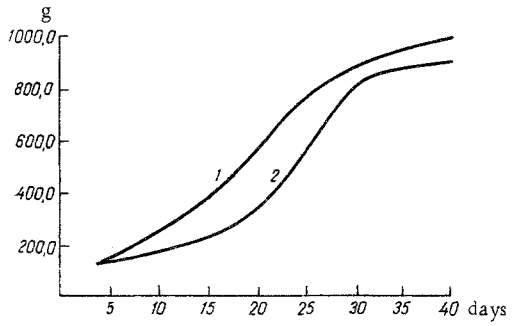

Fig. 2. Changes in strength of radius in rats during consolidation: 1) experiment; 2) control. Abscissa, time (in days); ordinate, force breaking bone (in g).

The reduction in the mean area of the burn took place irregularly, and more quickly in the experimental animals than in the controls (Fig. 1). The mean rate of contraction of the skin wound in the longitudinal and transverse directions was 0.45 ± 0.05 and 0.93 ± 0.05 mm/day respectively in the experimental animals, but significantly lower in the controls, namely 0.39 ± 0.05 and 0.52 ± 0.05 mm/day ($P < 0.05$). Indrawing of the edges of the dorsal skin wounds in both the experimental and control series was observed to take place more intensively in the transverse than in the longitudinal direction ($P < 0.01$). The newly formed scar in the skin of the experimental animals was much softer and less adherent to the underlying tissues.

α-Fetoprotein also affected the general resistance of the animal: after its injection growth of the wound microflora was inhibited and its qualitative composition was changed (Table 2); the period of normalization of the white and red blood cell counts, ESR, and the protein fractions also was reduced.

In the animals with experimental fracture of the radius injection of α-fetoprotein shortened the period of "nursing" of the injured limb by $25.0 \pm 1.0\%$ ($P < 0.05$) and the period of consolidation of the bone (Table 1). By the 10th-13th day after trauma the bony callus of the experimental rats was spongy in structure and consisted of delicate bone trabeculae, located in all zones of the reparative area. It contained a rich network of blood vessels and was surrounded by well-developed connective tissue, whereas in the control animals these processes did not develop until the 16th-18th day.

After injection of α-fetoprotein the strength of the bony callus also increased appreciably. The force required to fracture the knitting bones increased irregularly in the course of repair, but the type of curve was identical in the experimental and control series and was characterized by a logistic function (Fig. 2).

Since considerable accumulation of histiocytes with bright γ- and β-metachromasia, an increase in alkaline phosphatase activity, and an increase in the RNA and DNA contents were observed in the zone between the fragments during consolidation, this suggests that injection of α-fetoprotein stimulates regeneration in the bone tissue through the formation of young connective-tissue structures and shortens the stages of their maturation.

The stimulating action of human (isolated in the writers' laboratory, or a commercial preparation) canine, and feline α-fetoprotein on the healing of skin wounds in rats and mice was compared. The results showed that the α-fetoprotein, regardless of the species of animal and method of its isolation, accelerated regeneration (Table 1). The similarity between the results obtained by the use of human and animal α-fetoprotein indicates absence of strict species specificity of its stimulating action.

α-Fetoprotein is thus a physiologically active substance capable of stimulating processes of reparative regeneration.

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