lymphoid follicles, and in the number of lymphocytes in the red pulp. The splenic follicles lose their configuration and change into random clusters of lymphocytes, and most of them lose their reactive center. Condensation of the capsule and connective-tissue stroma is observed.

After the end of hormone injections the absorptive function of the RES increases again, the general condition of the animals improves, the atrophic changes in the spleen cease, the number of lymphocytes in the organ increases, and the normal splenic lymphoid follicles are restored (reappearance of the reactive centers and an increase in the number of pyroninophilic cells in them).

Administration of a regeneration stimulator after the end of hormone injections appreciably accelerates both the recovery of the animal's general condition and restoration of the atrophied spleen.

One month after the end of administration of hydrocortisone, the spleen which had partially atrophied under the influence of the hormone is almost completely restored. If colamine hydrochloride is given under these conditions, regeneration of the organ takes place more rapidly and the weight of the spleen regains the control level during the same period of observation.

LITERATURE CITED

1. Ya. L. Germanyuk and A. G. Minchenko, Byull. Éksp. Biol. Med., No. 2, 53 (1972).
2. K. P. Zak and B. M. Khomenko, in: Problems in Endocrinology and Metabolism [in Russian], No. 1, Kiev (1970), pp. 62-71.
3. K. P. Zak and N. I. Naumenko, Vrach. Delo, No. 3, 129 (1971).
4. L. D. Liozner, G. V. Kharlova, and B. M. Levitina, Trudy Moskovsk. Obshch. Ispyt. Prirody. Otdel Biol., 2, 17 (1961).
5. L. D. Liozner, Arkh. Patol., No. 7, 66 (1974).
6. A. A. Mezhlumyan, Byull. Éksp. Biol. Med., No. 5, 103 (1964).
7. D. S. Sarkisov, Arkh. Patol., No. 7, 70 (1974).

HUMORAL MECHANISMS OF REGULATION OF REPARATIVE OSTEOGENESIS

V. I. Filimov, N. P. Lepekhova, V. O. Nedospasov, K. S. Desyatnichenko, and V. L. Teterin

UDC 612.753-06

The effect of the blood serum from animals with active osteogenesis on the biosynthesis of nucleic acids and protein and on mineralization of regenerating bone tissue was studied in experiments in vivo and in vitro. Incorporation of labeled precursors of DNA ([^{3}H]thymidine) and protein ([^{14}C]proline) in the recipients was intensified and mineralization of bony callus (incorporation of ^{85}Sr) was accelerated. Comparison of the order of stimulation of nucleic acid and protein synthesis suggests that the active principle of the serum promotes more rapid cell proliferation in the fracture zone.

KEY WORDS: reparative osteogenesis; humoral regulation; radioactive isotopes

One of the aspects of the problem of the regulation of repair processes that has received the least study is the control of the natural course of reparative osteogenesis. The only information available is concerned with the stimulating effect of breakdown products of bone [4] or of the blood serum of animals with fractures [1, 8] on this process. The present writers [7] have also found that during the period of intensive osteogenesis the blood serum of animals acquired the property of stimulating fracture healing.

Laboratory of Pathophysiology and Laboratory of Biochemistry, Research Institute of Experimental and Clinical Orthopedics and Traumatology, Kurgan.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 725-727, December, 1977. Original article submitted April 6, 1977.

TABLE 1. Incorporation of [^{3}H]Thymidine and [^{14}C]Proline (in cpm/mg) into Areas of Bone Adjacent to Fracture Zone in Mice under the Influence of Serum (M ± m)

Isotope	Time after injection of dog's serum, h					
	12		24		48	
	experiment	control	experiment	control	experiment	control
[^{3}H]Thymidine	115,3±8,9	80,9±8,6*	222,5±20,7	135,6±13,4*	168,5±11,7	151,0±10,6
[^{14}C]Proline			160,9±14,8	207,6±14,5*	179,5±18,2	206,7±20,7

Legend. Here and in Table 2, asterisk indicates $P < 0.05$ relative to Student's criterion.

TABLE 2. Incorporation of [^{14}C]Proline and ^{85}Sr into Areas of Bone Adjacent to Fracture Zone and into Bony Callus of Mice under the Influence of Serum (M ± m)

Isotope	Time after injection of dog's serum, h							
	2		4		7		9	
	experiment	control	experiment	control	experiment	control	experiment	control
[^{14}C]Proline, cpm/mg	171,3±17,2	207,1±12,6	408,3±24,2	373,3±23,2	608,2±37,9	483,2±33,9*	—	—
^{85}Sr	1,12±0,08	1,12±0,07	2,16±0,14	1,67±0,12*	—	—	3,45±0,2	2,63±0,2*

Legend. Incorporation of ^{85}Sr expressed as ratio of activity (in cpm) of 1 mg injured bone to activity of 1 mg intact bone.

As donors of such a serum in the present investigation the choice fell on dogs with protracted reparative osteogenesis in the course of limb lengthening by Ilizarov's method [3]; consequently, greater accumulation of the stimulator can be expected.

EXPERIMENTAL METHOD

The effect of the blood serum of animals with active bone regeneration (2 weeks after bone lengthening) was investigated in vivo on mice with a fracture of the diaphyses of the bones of the leg and in vitro on minced tissue of rabbit bony callus. The serum was injected subcutaneously into mice weighing 20-22 g in a dose of 0.3 ml once daily for 3 days, starting on the day after fracture. The animals were killed 2, 4, 7, and 9 days after the first injection of serum. In another series of experiments the animals were given one injection of serum 48 h after the fracture and killed 12, 24, and 48 h after the injection. Activity of repair processes in the bone was judged by incorporation of radioisotopes: [^{3}H]thymidine was injected 4 h before sacrifice in a dose of 5 μCi, [^{14}C]proline 24 h before in a dose of 4.5 μCi, and ^{85}Sr 24 h before sacrifice in a dose of 5 μCi. Areas of the tibial diaphysis adjacent to the zone of fracture or bony callus when it had been able to form, were chosen for investigation. For comparison, areas of bone from the symmetrical part of the contralateral limb were used. The specimens labeled with ^{3}H and ^{14}C, after the necessary preparation, were examined on the Nuclear Chicago liquid scintillation counter, and specimens labeled with ^{85}Sr on the B-2 apparatus with an end-window counter. In addition, the total protein concentration as amino nitrogen [5] and the collagen contents as hydroxyproline [6] were determined.

For the experiments in vitro, 7-day bony callus was taken from rabbits, minced, and incubated at 37°C for 6 h in Hanks's medium containing 20% dog serum and 4 μCi [^{3}H]thymidine. After repeated washing of the tissue with ice-cold physiological saline, its DNA concentration was determined as in [2] and its radioactivity was measured on the liquid scintillation counter.

For each series of experiments a control was set up with blood serum from intact dogs. To ensure standardization of the conditions with respect to biological rhythms the experiments were so planned that the animals were always killed at the same time of day.

EXPERIMENTAL RESULTS

On the first day after a single injection of the test serum incorporation of [^{3}H]thymidine into the bone fragments of the recipients was appreciably increased in the immediate vicinity of the fracture line, whereas incorporation of [^{14}C]proline, on the other hand, was reduced compared with recipients of blood serum from intact dogs (starting from this time – the third day after fracture – the level of all isotopes used in the investigation was higher in the bone fragments than in analogous areas of intact bone in animals of both the experimental and control groups). Incorporation of [^{14}C]proline in the recipients of the test serum rose later, not until the fourth to seventh day after the first injection of serum (Table 2).

Parallel with this (7th day after injection of serum) the concentration of amino nitrogen in the mice of the experimental group was found to be increased: 54.6 ± 2.5 mmoles/g dry weight of bone compared with 42.8 ± 2.5 mmoles in the control ($P < 0.05$); the hydroxyproline concentration also was increased in these mice, from 22.3 ± 1.6 mg/g dry weight of bone in the control to 28.4 ± 1.9 mg ($P < 0.05$). Incorporation of ^{85}Sr in the mice of the experimental group was increased from the 4th day after injection of the serum (Table 2).

In the experiments in vitro increased incorporation of the DNA precursor was found under the influence of the test serum: The [^{3}H]thymidine level in the experimental group was 866.1 ± 65.9 compared with 668.9 ± 26.9 cpm/μg DNA in the control ($P < 0.05$).

The mechanism of action of the active principle of the test serum can be represented as follows. The point of application of the regulator is evidently the mechanisms of proliferation of cells of the bony callus. This is shown by the increased incorporation of [^{3}H]thymidine during the days immediately after a single injection of the test serum and by the experiments in vitro. The smaller protein production at this time was possibly due to the fact that cells starting mitosis do not synthesize functionally specific proteins. Later, when the proliferative wave evoked by the action of the exogenous stimulator had subsided a little, the gross increase in the number of cells makes itself felt, and protein biosynthesis is then increased in the animals of the experimental group.

This hypothesis is also confirmed by the fact that the active principle contained in the serum does not change the structure of protein synthesis: The levels of total protein and collagen in the experimental animals

were increased proportionally. More active mineralization is the result. Experiments in vitro showed that the test serum acts directly on proliferation of the bony callus cells. In the writers' view, the regulator discovered is also essential for proliferation of osteogenic cells in dogs undergoing bone lengthening. Activation of osteogenesis in mice and rabbits by dog's blood serum shows that the regulator is not species specific.

LITERATURE CITED

1. P. V. Dunaev and G. S. Solov'ev, in: Cell Differentiation in Histogenesis and Organogenesis (Proceedings of the 3rd All-Union Symposium) [in Russian], Kiev (1975), pp. 112-116.
2. I. B. Zbarskii and S. S. Debov (editors), Chemistry and Biochemistry of Nucleic Acids [in Russian], Leningrad (1968), pp. 80-83.
3. G. A. Ilizarov, A. M. Khelimskii, A. A. Devyatov, et al., Éksp. Khir., No. 2, 37 (1975).
4. A. A. Korzh, A. M. Belous, and E. Ya. Pankov, Reparative Regeneration of Bone [in Russian], Moscow (1972).
5. V. K. Leont'ev and Yu. A. Petrovich, Biochemical Methods of Investigation of the Enamel of Teeth [in Russian], Omsk (1976).
6. L. I. Slutskii, Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969).
7. V. I. Filimonova, G. A. Chalobyan, V. K. Kamerin, et al., in: Reactivity of the Organism in Trauma and Orthopedic Diseases [in Russian], Saratov (1975), pp. 136-139.
8. M. Yu. Épshtein, Nov. Khir. Arkh., 6, 449 (1925).