THE EFFECT OF RNA AND ITS NUCLEOTIDES ON REGENERATION OF THE NERVE FIBERS

A. M. Chernukh,* I. L. Razumova, and N. N. Alekseeva

UDC 612.818.92-06:612.398.145.1

The effect of intramuscular injection of total cytoplasmic and nuclear RNA and of their hydrolysis products on the rate of regeneration of nerve fibers of the divided sciatic nerve was studied. Injection of RNA was found to stimulate regeneration of the nerve fibers, especially in the early periods of regeneration. The greatest effect was observed by the use of nuclear RNA. A high degree of polymerization of the RNA plays an extremely important role in its stimulant effect. The complete assortment of nucleotides composing RNA has only a very slight stimulant action of no practical importance.

After it had been postulated that the synthesis of specific proteins can be induced in animal tissues by the action of organ-specific RNAs [15-18] investigators found that RNA can stimulate tissue regeneration. The work of Belous et al., for example, showed that injection of RNA from regenerating bone tissue considerably stimulates osteogenesis in rabbits [3-8] by accelerating proliferation and differentiation of regenerating bone cells [11], mainly on account of protein biosynthesis in the bone tissue [2, 9]. Akhabadze [1] and Skuba [14] described the possible stimulation of regeneration of heart muscle by injection of organ-specific RNAs.

On the assumption that degeneration in denervated muscle is closely connected with a disturbance of nucleic acid metabolism, the effect of homologous RNA from normal muscles was studied on a course of degeneration in denervated muscle and on the rate of regeneration of the divided sciatic nerve. It was found that injection of RNA slightly reduces the degree of atrophy of muscle fibers developing as a result of degeneration and considerably accelerates the growth of regenerating nerve fibers [12, 13].

The object of the present investigation was to study whether stimulation of regeneration is possible only by means of high-polymer RNA or whether the nucleotides composing it have the same action.

EXPERIMENTAL METHOD

Experiments were carried out on 98 male albino rats weighing 200-220 g. Under strictly aseptic conditions and under general ether anesthesia an operation was performed to divide and suture the right sciatic nerve. The skin was incised approximately in the middle of the thigh, the muscles were retracted with sharp hooks, and two parallel sutures were inserted into the epineurium of the sciatic nerve 5 mm above its division into the tibial and peroneal nerves. The nerve was then divided between the sutures and the ends immediately placed in apposition.

The animals after the operation were divided into seven groups with 14 animals in each group. The animals of group 1 acted as the controls while the remaining six groups of rats received RNA or its hydrolysis products in 0.1 ml physiological saline. The rats of group 2 received injections of cytoplasmic RNA, group 3 received a heated preparation of cytoplasmic RNA, group 4 an alkaline digest of RNA in a dose of

*Academy of Medical Sciences of the USSR.

Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 7, pp. 101-105, July, 1973. Original article submitted June 15, 1972.

TABLE 1. Length of Nerve Fibers (in mm) 10 Days after Division and Suture of Sciatic Nerve

Preparation injected	No. of animals	Length of nerve fibers	P
Control	4	3,75±0,41	
Cytoplasmic RNA	4	11,97±1,05	<0,001
Heated cytoplasmic RNA	4	8,45±0,86	<0,01
Alkaline digest of cytoplasmic RNA	4	4,14±0,28	>0,05
Nuclear RNA	4	18,37±0,5	<0,001
Heated nuclear RNA	4	10,65±0,86	<0,001
Alkaline digest of nuclear RNA	4	5,25±0,76	>0,2

Fig. 1. Graph of intensity of growth of nerve fibers of regenerating sciatic nerve in control (1) animals and after injection of cytoplasmic RNA (2), of a preparation of heated cytoplasmic RNA (3), of a digest of cytoplasmic RNA (4), of nuclear RNA (5), of a preparation of heated nuclear RNA (6), and of a digest of nuclear RNA (7). Ordinate, number of nerve fibers; abscissa, length of fibers (in mm).

0.125 mg/100 g body weight, group 5 received nuclear RNA, group 6 a heated preparation of nuclear RNA, and group 7 an alkaline digest of nuclear RNA in a dose of 0.03 mg/100 g body weight. The control rats received an injection of the same volume of physiological saline.

RNA was prepared from skeletal muscles of the limbs of intact rats by the method of Georgiev and Mant'eva [10] with the addition of dodecylsulfate. The fraction of nuclear RNA was obtained at 65°C from unpurified phenolic nuclei. Heating was carried out on a boiling water bath for 30 min. The RNA hydrolysis products were obtained by keeping the RNA with a 0.6 N solution of NaOH for 24 h at 37°C. The RNA preparations were kept as solutions for not more than four days at −20°C. All preparations were injected into the right gastrocnemius muscle 3 h after the operation and then every 72 h for 30 days.

The animals were killed by ether anesthesia on the 10th, 25th, 30th, 35th, and 40th days. The material was treated by the Bielschowsky–Gros method. The rate of regeneration on the 10th day was judged from the number and length of regenerating fibers of the sciatic nerve, and later by the state of the nerve endings in the gastrocnemius muscle. The length and number of nerve fibers in every case were determined by means of a screw-operated ocular micrometer in four sections with the farthest-growing nerve fibers.

EXPERIMENTAL RESULTS

Ten days after division and suture of the sciatic nerve in the control rats 451-554 nerve fibers were counted at a distance of 1 mm from the site of division (Fig. 1). The longest fibers had grown 4.9 mm into the peripheral segment.

Injection of cytoplasmic RNA considerably accelerated regeneration of the nerve fibers. At a distance of 1 mm from the site of division 700-900 nerve fibers were counted, 5 mm away they numbered 386, and the longest fibers reached 17.2 mm.

The effect of injection of nuclear RNA was greater still. At a distance of 6 mm from the site of division of the sciatic nerve 495 fibers were counted, and the longest fibers had grown 19.9 mm into the peripheral segments of the nerve, i.e., they were four times longer than in the control animals.

Preparations of heated nuclear and cytoplasmic RNA had almost identical effects on the rate of regeneration of the nerve fibers, rather less marked than the growth-stimulating action of cytoplasmic RNA. In both cases the longest nerve fibers had grown by 13.1 mm, and 450-460 fibers were counted 1 mm away from the site of division.

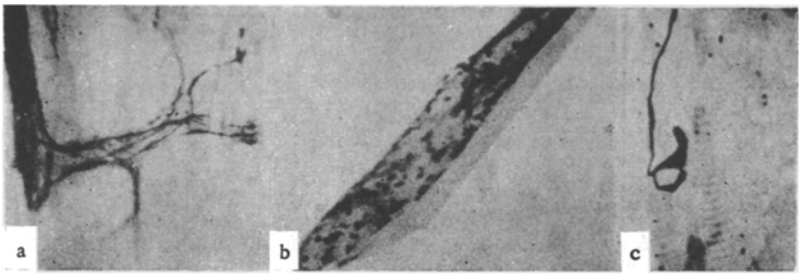

Fig. 2. Right gastrocnemius muscle of control rat 25 days after operation (treated by Bielschowsky–Gros method); a) few nerve fibers in bundle (20× 6); b) sensory neuromuscular spindle with two fibers (20× 6); c) pools of neuroplasma on preterminals (40× 6).

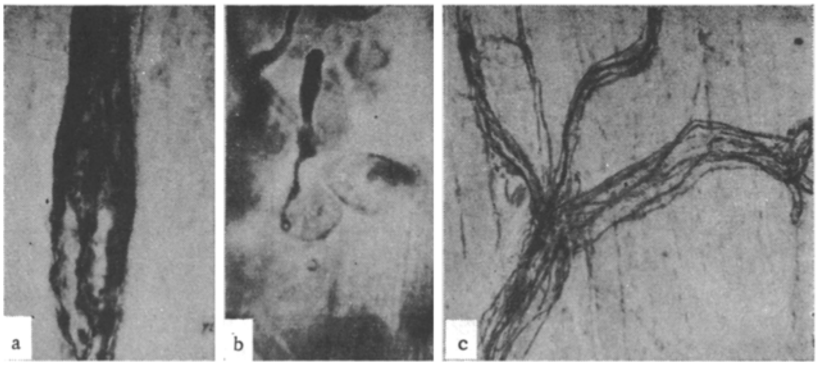

Fig. 3. Right gastrocnemius muscle of experimental rat (receiving nuclear RNA) 25 days after operation (Bielschowsky–Gros): a) well innervated neuromuscular spindle (20 × 6); b) motor nerve ending shaped like a loop (90 × 6); c) many nerve fibers in bundle (20 × 6).

The effect of alkaline digest of cytoplasmic and nuclear RNA was less marked still. At a distance of 1 mm from the site of division the number of nerve fibers was indistinguishable from the control and was 450-500, while the longest nerve fibers had grown only to a distance of 7-7.5 mm from the site of division (Table 1).

A few growing nerve fibers could be found 25 days after division of the sciatic nerve in large and medium-sized bundles of nerve fibers in the control animals (Fig. 2a). Only in 50% of the bundles did the number of fibers reach half of that existing before division of the nerve. However, side by side with the "empty" bundles, sensory neuromuscular spindles with one or two nerve fibers (Fig. 2b) and single, well innervated spindles were frequently found. No motor endings were seen, but from time to time preterminals with pools of neuroplasm were visible (Fig. 2c).

At this time after the injection of cytoplasmic RNA solitary motor endings could be seen and most of the neuromuscular spindles were well innervated.

After injection of nuclear RNA the gastrocnemius muscle was innervated considerably better. Up to 80% of the control number of fibers could be counted in the large and medium-sized bundles of nerve fibers (Fig. 3a), and around nearly every small bundle there were motor endings (Fig. 3b). As many as three or four neuromuscular spindles could be seen in a section, and all were well innervated (Fig. 3c). Sometimes excessive growth of nerve fibers in the spindle was observed. Occasionally nerve fibers could be seen to terminate on the nuclei of the spindle.

After injection of the alkaline digest of both cytoplasmic and nuclear RNA no stimulant effect of RNA was observed on the formation of nerve endings in the gastrocnemius muscle.

After 30 days the differences between the innervation of the gastrocnemius muscle in the control group and in the animals receiving RNA began to diminish and by the 35th day they had disappeared completely.

The results of this investigation show that administration of RNA stimulates regeneration of divided nerve fibers and that this stimulation is particularly marked in the early stages of regeneration. The greatest effect was obtained by the use of high-polymer nuclear RNA. Injection of heated preparations of cytoplasmic and nuclear RNA had an equal stimulant effect on growth of the regenerating nerve fibers and it was weaker than the growth-stimulating action of cytoplasmic RNA.

The stimulant effect of an alkaline digest of cytoplasmic and nuclear RNA on regeneration of nerve fibers also was identical, but weak and was observed only in the early stages of regeneration.

Consequently, a high degree of polymerization of RNA plays an extremely important rôle in its stimulant effect on regeneration of nerve fibers. The complete assortment of nucleotides composing RNA, if given in the dose used, had a weak stimulant action on the rate of growth of the regenerating nerve fibers, of no practical importance.

LITERATURE CITED

1. L. V. Akhabadze, in: Conditions of Regeneration of Organs and Tissues in Animals [in Russian], Moscow (1966), p. 3.
2. G. A. Baoiichuk and A. M. Belous, Byull. Éksperim. Biol. i Med., No. 10, 82 (1969).
3. A. M. Belous and E. Ya. Pankov, Ortoped. Travmatol., No. 18, 14 (1966).
4. A. M. Belous and I. M. Todorov, in: Traumatology [in Russian], No. 3, Kiev (1968), p. 178.
5. A. M. Belous, Ortoped. Travmatol., No. 11, 28 (1968).
6. A. M. Belous, E. Ya. Pankov, V. A. Gusakova, et al., Byull. Éksperim. Biol. i Med., No. 5, 85 (1969).
7. A. M. Belous, E. D. Gusakov, and E. Ya. Pankov, in: Proceedings of the Ninth International Congress of Anatomists [in Russian], Izhevsk (1970), p. 17.
8. A. M. Belous, A. K. Gulevskii, T. F. Klyueva, et al., in: Mechanisms of Regeneration and Cell Division [in Russian], Moscow (1971), p. 14.
9. A. M. Belous and I. M. Todorov, in: Nucleic Acids [in Russian], Moscow (1966).
10. G. P. Georgiev and V. L. Mant'eva, Biokhimiya, No. 5, 27 (1962).
11. E. Ya. Pankov and V. A. Gusakova, in: Mechanisms of Regeneration and Cell Division [in Russian], Moscow (1971), p. 117.
12. I. L. Razumova, Pat. Fiziol., No. 5, 78 (1970).
13. I. L. Razumova and N. N. Alekseeva, General Neurophysiology and Experimental Pathology of the Nervous System [in Russian], Moscow (1970), p. 68.
14. N. D. Skuba, in: Problems in Theoretical and Clinical Medicine [in Russian], Kiev (1969), p. 94.
15. I. M. Todorov and A. N. Blok, Vestn. Akad. Nauk Ukr. SSR, No. 10, 32 (1964).
16. H. Emanuelson, Acta Physiol. Scand., 48, 352 (1960).
17. P. Malpoix, Nature, 203, 520 (1964).
18. J. Schaeffer, Biochim. Biophys. Acta, 80, 247 (1964).