4. O. E. Vyazov, The Immunology of Embryogenesis [in Russian], Moscow (1962).
5. O. E. Vyazov, A. I. Murashova, L. I. Orlova, et al., in: Proceedings of the 10th Scientific Conference on Age Morphology, Physiology, and Biochemistry, [in Russian], Vol. 1, Moscow (1971), pp. 92-93.
6. K. S. Lobyntsev, Yu. I. Savchenkov, and V. P. Tereshchenko, Abstracts of Proceedings of the 9th International Congress of Anatomists [in Russian], Moscow (1970), p. 112.
7. A. I. Murashova, Arkh. Anat., No. 8, 43 (1966).
8. A. I. Murashova, "An Experimental study of humoral connections between homonymous organs of mother and embryo in rats," Candidate's Dissertation, Moscow (1969).
9. E. I. Novikova and Yu. I. Barashnev, in: New Problems in Pediatrics, [in Russian], No. 5, Sofia (1968), pp. 29-37.
10. L. K. Romanova, Proceedings of a Scientific Conference on Regeneration and Transplantation of Organs and Tissues [in Russian], Gor'kii (1965), pp. 79-85.
11. L. K. Romanova, in: Conditions of Regeneration of Organs in Mammals [in Russian], Moscow (1972), p. p. 149.
12. L. K. Romanova, in: Problems in Establishment of Hygienic Standards During the Study of Late Effects of Exposure to Industrial Substances [in Russian], Moscow (1972), pp. 78-82.
13. I. V. Sanotskii, V. N. Fomenko, G. N. Zaeva, et al., Zh. Vsesoyuz. Khim. Obshch. im. D. I. Mendeleeva, 29, No. 2, 146 (1974).
14. N. G. Stepanova, Lab. Delo, No. 5, 49 (1962).
15. M. Ya. Studinikin and G. P. Borisova, Pediatriya, No. 8, 5 (1970).
16. N. I. Shumskaya and N. M. Karamzina, in: The Toxicology of New Industrial Chemicals [in Russian], No. 8, Moscow (1966), pp. 14-27.
17. B. Kauneliené and S. Suksteriené, Sveikatos Apsauga, No. 4, 20 (1974).

EFFECT OF CORTISONE ON POSTTRAUMATIC REGENERATION OF MAMMALIAN SKELETAL MUSCLES

T. M. Kovalenko

UDC 616.74-001-003.93-02:615.357.453

Prolonged administration of cortisone to sexually immature rabbits and rats after previous injury to the tibialis anterior muscle inherits the regeneration of skeletal muscle tissue, as manifested by delay in growth of the myosymplasts and muscle tubes. In rabbits by the 15th day after the operation the area of the muscular components of the regenerating focus was less than their area in the control. Analysis of the intensity of methionine-^{3}H incorporation into the regenerating elements of the muscle tissue showed a significant decrease in uptake of the label into nuclear and cytoplasmic proteins of the myosymplasts of rats receiving cortisone. Inhibition of protein synthesis in the early stage of differentiation of muscle tissue was less marked than in mature differentiated muscle fibers of the intact muscle.

KEY WORDS: Regeneration of skeletal muscles; incorporation of methionine-^{3}H; area of components of regenerating focus.

The investigation of the hormonal regulation of morphogenetic processes and, in particular, of processes of regeneration is an urgent problem of practical as well as theoretical importance [1, 7-9]. Hormones are known to control the synthesis of specific proteins in the tissues [6, 10, 11-13] and they can thus determine the direction of growth and differentiation of cells [2, 4].

Department of Biology, I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 7, pp. 868-871, July, 1976. Original article submitted May 30, 1975.

TABLE 1. Areas of Muscle and Connective-Tissue Components of Focus of Regeneration in Rabbits (in units of area of paper models)

Muscle components				Connective-tissue components				Ratio between muscle and connective-tissue components	
No. of rabbit control	area	No. of rabbit (expt.)	area	No. of rabbit (control)	area	No. of rabbit (expt.)	area	control	experiment
1	79,0	7	46,0	1	16,7	7	11,9	4,73	3,87
2	50,0	8	29,7	2	15,3	8	7,7	3,27	3,86
3	44,5	9	21,0	3	18,5	9	8,3	2,41	2,53
4	73,0	10	43,5	4	16,1	10	7,3	4,53	5,96
5	68,0	11	43,0	5	17,2	11	11,1	3,95	3,87
6	65,0	12	25,0	6	15,9	12	8,3	4,09	3,01
$M \pm m$	63,2±5,8	$M \pm m$	34,7±4,6	$M \pm m$	16,6±0,5	$M \pm m$	8,8±0,9	3,8±0,9	3,9±0,5
	$P<0,01$				$P<0,001$				

TABLE 2. Incorporation of Methionine-^{3}H into Nuclei and Cytoplasm of Myosymplasts and Muscle Fibers of Rats on Tenth Day after Operation

Test object	Number of muscle fibers (fragments and myosymplasts)	Total area of cytoplasm (in μ^2)	Mean number of grains of silver per 100 μ^2 area of	
			cytoplasm	nuclei
Muscle fibers				
control	270	324 161	5,6±0,4	17,4±1,4
			$P<0,01$	$P<0,01$
experiment	270	241 483	2,6±0,2	7,5±0,6
Myosymplast				
control	77	49 913	12,2±0,9	18,8±0,8
			$P<0,05$	$P<0,05$
experiment	73	35 411	8,2±0,5	12,7±1,4

The object of this investigation was to study repair processes in the skeletal muscles during a change in the glucocorticoid balance of the body. Data on this problem at present available are few in number and contradictory in nature [3, 5].

EXPERIMENTAL METHOD

The test material consisted of the tibialis anterior muscle of 48 sexually immature male rabbits weighing 650-800 g and 16 Wistar rats weighing 95-100 g. The operation was performed under strictly aseptic conditions. After a linear incision in the skin and fascia the left tibialis anterior muscle was exposed and a hole 5 mm in diameter was made in its center in the rabbits by means of a special punch; in the rats the muscle was cut transversely for a distance of 3 mm. The tibialis anterior muscle of the right limb remained intact. From the first day after the operation some animals received cortisone acetate by intramuscular injection, the others physiological saline. The dose of the hormone was calculated by the equation: $a = a_0 (P/P_0)^{2/3}$ (a_0 is the dose for man, P_0 the human body weight, P the body weight of the animal), on the basis of a daily therapeutic dose of cortisone of 0.1 g. At various times after the operation the rabbits were killed, the material was fixed with Zenker-formol, and usual histological treatment carried out. For outline drawings of the areas of the focus of regeneration preparations stained by Mallory's method were used. The areas of paper models were measured with a planimeter.

To study the effect of cortisone on the intensity of protein synthesis in the muscle tissue, methionine-^{3}H was injected in a dose of 3.5 μCi/g into the rats 4 and 16 h before decapitation (on the 10th day after the operation). Material was fixed in Carnoy's mixture. Sections through the muscle were coated with type R emulsion. The duration of exposure of the autoradiographs was 40 days. The number of grains of silver above the cytoplasm and nuclei of the myosymplasts (region of the wound) and in 100 fragments of muscle fibers of intact muscle from each animal was counted. Meanwhile the corresponding histological structures were drawn

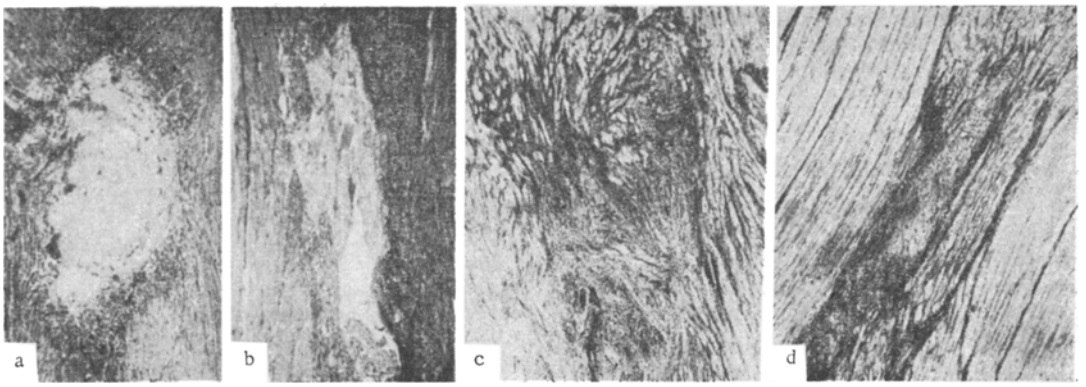

Fig. 1. Region of injury to tibialis anterior muscle in rabbits: I) 7 days after operation: a) control, b) experiment (Carazzi's hematoxylin, 24 ×); II) 15 days after operation: c) control; d) experiment (Mallory's method, 56 ×).

and the mean number of tracks determined per 100 μ^2 area of nuclei and cytoplasm. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

By the fifth day after the operation the resorption of debris in the zone of injury was finally complete in the control rabbits. On the seventh day granulations developed in the region of the wound, and in the peripheral areas they had already come to resemble young connective tissue (Fig. 1a).

Injection of cortisone sharply inhibited autolysis and phagocytosis, with the result that the fragmentation of the damaged muscle fibers and resorption of the debris thus formed were delayed (Fig. 1b). The central parts of the region of injury were filled with inflammatory exudate and fibrin and no granulation tissue developed, so that proliferation of the fibroblasts was sharply depressed. Not until 10 days after injury was the zone of damage freed from muscle debris. The developing connective tissue showed intensive differentiation accompanied by rapid reduction of blood vessels and more rapid collagenization than in the control. On the fourth and seventh days after the operation no appreciable decrease in the intensity of formation on young muscle elements could be observed in animals receiving cortisone. Later growth of myosymplasts and muscle tubes was delayed, and this was especially marked on the 15th day of the repair process. By the 15th day the area of the muscle components of the regenerating focus was smaller in the experimental than in the control rabbits. The area of the connective-tissue regions of the regenerating focus was reduced correspondingly (Fig. 1c and d; Table 1). Analysis of the autoradiographs of the control animals showed intensive incorporation of methionine-^{3}H into the cytoplasm and nuclei of the muscle tissue as early as 4 h after injection; the intensity of incorporation of the label into the nuclei was much higher than into the cytoplasm (Table 2). Incorporation of methionine-^{3}H into nuclear and cytoplasmic proteins of the myosimplasts was inhibited in the experimental rats and the concentration of label in the cytoplasm and nuclei of the intact muscle fibers also was reduced (Table 2). Comparison of the intensity of incorporation of methionine-^{3}H into the intact muscle fibers and regenerating muscle elements showed that in the early stage of differentiation of muscle tissue the inhibition of protein synthesis in the myosymplasts under the influence of cortisone was less marked than in the differentiated muscle fibers (different significant according to the criterion of signs; $P = 0.025$).

Cortisone, in the doses tested, thus inhibits regeneration of muscle tissue; the delay in growth of the myosymplasts correlates with the inhibition of protein synthesis. It can also be concluded from these results that at different levels of differentiation the antianabolic effect of cortisone is exhibited to a different degree.

LITERATURE CITED

1. A. A. Voitkevich, Repair Processes and Hormones [in Russian], Leningrad (1965).
2. T. M. Kovalenko, L. P. Tyurlikova, and L. V. Nemilova, Byull. Éksp. Biol. Med., No. 12, 77 (1971).
3. A. V. Negovskaya, "Reparative regeneration of skeletal muscle with the use of a framework of heteroperitoneal filaments and cortisone," Author's Abstract of Candidate's Dissertation, Kishinev (1970).
4. Yu. M. Olenov, Cellular Heredity, Cell Differentiation, and Carcinogenesis as Problems in Evolutionary Genetics [in Russian], Leningrad (1967).

5. M. B. Rapoport, K. V. Mosketi, and P. I. Gladunets, Byull. Éksp. Biol. Med., No. 4, 108 (1962).
6. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
7. V. S. Sil'vestrov, Klin. Khir., No. 12, 63 (1966).
8. G. M. Solov'ev, V. V. Men'shikov, I. N. Usvatova, et al., Adrenal Hormones in Surgery [in Russian], Moscow (1965).
9. G. V. Kharlova, in: Conditions of Regeneration of Organs in Mammals, edited by L. D. Liozner [in Russian], Moscow (1972), p. 103.
10. M. A. Yudaev, Probl. Endokrinol., No. 1, 112 (1967).
11. G. Bullock, A. M. White, and I. Worthington, Biochem. J., 108, 417 (1968).
12. I. I. Ferguson, Ann. Intern. Med., 60, 925 (1964).
13. P. Feigelson, in: Mechanisms of Hormone Action (ed. by P. Karlson), Academic Press, New York (1965), p. 246.

CYTOLOGICAL FEATURES DISTINGUISHING GROWTH OF THE RAT LIVER IN THE EMBRYONIC AND NEONATAL PERIODS AND DURING REGENERATION

Z. A. Ryabinina and E. A. Lisnevskaya

UDC 612.65'35.014.1

Embryonic and neonatal growth of the liver was studied in August rats and compared with growth of the regenerating liver of adult animals in the early stages after partial hepatectomy (22, 48, and 72 h after removal of two thirds of the organ). The mitotic activity and area of the nuclei of the hepatocytes were determined and the number of binuclear cells counted. Several general principles were discovered in the dynamics of the changes in the cytological parameters of embryonic and neonatal growth of the intact liver and growth of the regenerating liver of adult rats in the early stages after resection.

KEY WORDS: Embryonic and neonatal liver; regeneration; hepatocytes.

Studies of the growth and development of the liver in the early stages of ontogeny have recently been published [2,3]. However, many aspects of the cytological processes taking place under these circumstances remain inadequately explained and require clarification and further study. Meanwhile, regeneration of the liver after resection takes place to some extent through cytological processes which are the same as those found in the organ during its normal growth [1].

It was therefore thought worthwhile to make a comparative cytological study of growth of the embryonic and neonatal liver and of the regenerating adult liver in the early stages after resection.

EXPERIMENTAL METHOD

Rats of the August strain were used. The liver of embryos (of the 19th-20th day of gestation), newborn rats (until 3 h after birth), and day-old animals and the regenerating liver of adult rats (weighing 191 ± 29 g) from which two thirds of the liver had been removed, was investigated. The hepatectomized rats were killed 22, 48, and 72 h after the operation. At each time of study four to six animals were chosen. The liver (intact and regenerating was weighed. Material was fixed in Carnoy's fluid; the number of mitotically dividing hepatocytes in 6000 cells was counted in histological sections 5 μ thick, stained with hematoxylin and eosin. The mitotic index (MI) was expressed in promille. The number of binuclear hepatocytes was counted in 2000 cells and

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 7, pp. 871-872, July, 1976. Original article submitted July 10, 1975.