rather better than the latter. Medium 8c can be used as resuspending medium for blood preserved at a low temperature under protection by 1,2-PD. The most favorable times for transfusion are the first 24 h after rewarming of the frozen erythrocyte suspension. Glycerol and 1,2-PD in residual amounts, had no significant effect on Na^+ and K^+ metabolism in the course of hypothermic keeping for 5 days, whereas 1,2-PD has a more favorable action on ATP and 2,3-DPG metabolism than glycerol.

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LACTATE DEHYDROGENASE ISOZYME SPECTRUM OF FAST AND SLOW MUSCLES DURING DISTURBANCES OF INNERVATION

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The problem of neurotrophic control of skeletal muscle has attracted many experimental investigations [3, 8, 11]. The factors involved in its realization, of which the most important are acknowledged to be the flow of impulses along the nerve and the necessity for substances synthesized in the perikarya of the motoneurons and transported along axons to muscle fibers to be present [2], have been discussed. It has been shown that denervation, causing cessation of spike activity, and application of colchicine, blocking axoplasmic transport, are manifested as denervation-like changes in muscles. Studies of development of skeletal muscles have shown that early myogenesis takes place in the absence of nervous influences, but later differentation would be impossible without interaction between muscle tubules and motoneurons [10].

The question whether blockade of axoplasmic transport affects the differentiation of skeletal muscles still remains unstudied. The writers have investigated the effect of application of colchicine to the nerve and of denervation on differentiation of fast and slow muscles.

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TABLE 1. LDH Isozyme Spectrum (in %) in Fast and Slow Muscles at Different Times of Development, Denervation, and Application of Colchicine to Sciatic Nerve (M ± m)

LDH isozyme	Time of investigation				
	adult rat	newborn rats	rats aged 1 month		
			control	denervation	colchicine
			Extensor digitorum longus		
LDH_1	17,8±3,1*	39,3±3,2	37,3±1,8	8,9±1,3*	28,7±2,8*
LDH_2	36,3±3,6*	27,4±2,6	10,5±2,6	13,1±2,4	27,2±1,7*
LDH_3	21,9±5,2	16,4±2,3	20,5±5,3	22,1±3,5	18,4±2,6
LDH_4	27,6±1,2*	10,8±1,2	18,4±5,7	30,3±2,3*	16,8±2,6
LDH_5	32,4±9,3*	7,7±1,3	20,3±7,0	38,8±3,8*	12,9±4,1
			Soleus		
LDH_1	28,5±1,3*	34,9±2,4	34,9±2,4	18,9±4,9*	24,3±2,2
LDH_2	13,1±6,8*	21,6±2,6	21,6±2,6	13,6±2,6*	25,3±3,8
LDH_3	20,3±1,4	19,7±2,5	19,7±2,5	23,0±2,7	19,7±3,4
LDH_4	15,8±3,2	12,2±3,5	12,2±3,5	32,4±6,8*	18,3±2,8*
LDH_5	19,0±4,3	14,3±4,3	14,3±4,3	24,3±2,1*	18,8±3,7

Legend. *) Differences significant when isozymes compared in muscles of mature and newborn rats, and for rats aged 1 month, difference between experimental and control.

EXPERIMENTAL METHOD

The extensor digitorum longus (fast) and soleus (slow) muscles, which differ in their morphological and functional characteristics, were studied in 37 Wistar rats. In series I, on newborn animals (1-2 days old), under ether anesthesia and sterile conditions the sciatic nerve was divided; in series II 0.5 mM colchicine (from Ferak, West Germany) was applied to the sciatic nerve for 5 min. Rats of series III served as the control: Physiological saline was applied to the sciatic nerve of five rats. Weighed samples of muscles from newborn rats and rats aged 2 and 3 weeks and 1 month, and mature animals were homogenized in a glass homogenizer with 0.2 ml distilled water and centrifuged at 8000 g; the supernatant was subjected to polyacrylamide gel disc electrophoresis. Activity of lactate dehydrogenase (LDH) isozymes was determined in the gels [4]. The gels were photographed and scanned on the IFO-451 microdensitometer. The results were subjected to statistical analysis [7] at the 0.5 level of significance. Succinate dehydrogenase (SDH) activity was determined in frozen transverse sections to the test muscles [6].

EXPERIMENTAL RESULTS

Histochemical demonstrations of SDH in muscles of intact newborn animals and also after application of physiological saline to the nerve showed that all muscle fibers had the same level enzyme activity, that differentiation of muscle fibers into types began after the 2nd week of postnatal development, and by the 3rd week two types (B and C) could be identified in the soleus muscle and three types of muscle fibers (A, B, C) in extensor digitorum longus.

After division of the nerve the animals' limb at all times of observation appeared atrophied, none of the muscle fibers differed in their level of SDH activity, i.e., differentiation into types did not take place.

After application of colchicine to the sciatic nerve the experimental limb was outwardly indistinguishable at all times of observation from the contralateral limb. Demonstration of SDH, just as in the series with nerve division, showed the muscle fibers to be equally stained at all times of the experiment.

Extensor digitorum longus of the mature rat has a "muscular" type of LDH isozyme spectrum with predominance of activity of LDH_4 and LDH_5, whereas the soleus muscle is cardiac in type, with predominance of activity of LDH_1 and LDH_2. However, since the relative content of type C muscle fibers in this slow muscle is about 10%, and since the remaining 90% consists of type B fibers, a deviation from the "cardiac" type was observed in the LDH isozyme spectrum in the form of a high LDH_3 level [9].

In both muscles of newborn animals the "cardiac" type of LDH isozyme spectrum predominated (Table 1). The LDH isozyme spectrum in extensor digitorum longus 1 month after division of the nerve differed significantly from the control, but was closer to the spectrum for the mature rat. After application of colchicine the isozyme spectrum differed significantly from

the control but still remained "immature" and close to the spectrum for the newborn rat. Colchicine inhibited "maturation" of the soleus muscle, but not by the same degree as denervation. The fact that changes in postnatal myogenesis differed in fast and slow muscles will be noted. These changes can perhaps be explained on the grounds that according to changes in the velocity of contraction of the developing muscles and their relative content of muscle fibers of different types, fast, slow, and intermediate muscles are formed in the extensor digitorum longus from fibers that are relatively homogeneous with respect to these features, whereas in the soleus muscle the velocity of contraction during the first week of development increases, but then decreases [12], coinciding with a change in the program of synthesis of muscle myosin and troponins from the fast type to the slow [13, 14]; the possibility cannot be ruled out that the LDH spectrum is "adjusted" at the same time.

The muscles chosen differ in their LDH isozyme spectrum, which was used to estimate differentiation of the muscles. The results are evidence of the necessity for nervous control during muscle differentiation. Arresting the fast phase of axoplasmic transport by application of colchicine to the nerve has been used as a model with which to study the mechanism of neurotrophic control of the mature muscle [2]. The present experiments showed that if this model is used differentiation of developing fast and slow muscles is clearly inhibited.

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