To characterize the cell-virus interactions at different ages of the embryo, 7-day and 19-day NR were infected with Sindbis virus. In 7-day NR, the virus production was first detected at 2–3 h and increased rapidly until 12 h after adsorption. In contrast, slight reduction of the virus infectivity was observed in 19-day NR. No significant loss of infectivity of the virus incubated with MEM and 10% FBS was detected during the incubation period (figure 2).

In a parallel experiment, NR-cells were infected with Sindbis virus RNA employed as a probe to examine the cellular capability of the viral translation. Sindbis virus purified according to the method of David[8] was suspended in 0.01 M Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl and 1 mM EDTA, extracted for its RNA with phenol containing 1% SDS. NR cells were washed three times with phosphate-buffered saline (PBS) and incubated with the sample of RNA (equivalent to MOI about 100) in diluted PBS (×4) containing 1 mg/ml of DEAE-dextran at room temperature for 20 min. The virus yield determined after 10 h incubation was 9.7×10^8 and 1.7×10^8 PFU/ml in 7-day and 19-day NR, respectively. This difference might be attributed to the reduced level of the cellular protein synthesis of the 19-day NR which is about 50% of that of 7-day NR (unpublished observation).

It may therefore be concluded that NR from 19-day-old embryo still retained the cellular capability to support viral translation and maturation. The restricted multiplication of the virus observed is thus due to the modulated inability of the cell to process the adsorbed virus for subsequent replication. To understand the nature of biochemical events associated with the restricted cycle of virus infection in the cell, further analyses of the pathways of uncoating and initiation of replication will be necessary.

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Effects of the spinal cord section and of subsequent denervation on the mechanical properties of fast and slow muscles

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Summary. The Soleus muscle of the rat, 3–6 months old, becomes significantly faster than in the controls, if the spinal cord is cut at birth. Mechanical properties of Extensor Digitorum Longus (EDL) muscle are not altered by spinal cord section. In cordotomized animals Soleus muscle always remains slower than EDL muscle. Denervation, performed 3–6 months after birth, has the same slowing effects in the Soleus and EDL muscles, both in cordotomized and in the control animals.

The contraction speed of striated muscles in mammals is at birth uniformly slow. During the 1st few weeks after birth, the contractions become faster, and more so in the muscles subjected to a phasic activity than in the muscles with a tonic activity, with the consequent differentiation into the fast and the slow types[1–2].

The process of muscle differentiation appears to be altered, if normal motor activity at birth is either reduced by spinal cord section or completely abolished by nerve section. In the 1st case, slow muscles in the cat are reported to become nearly as fast as normal fast muscles[1]; in the other case, the differentiation of the slow muscle seems to be unaffected, while the speeding of the fast muscle does not occur, so that after 3 weeks the contraction times of the 2 muscles are reported to be, in the rat and in the rabbit, much about the same[3].

It must be observed that denervation, when performed in adult animals, does not abolish the mechanical differences between fast and slow muscles[4], although the differences are reduced in the rabbit[5]. Thus, it seems possible that muscle differentiation affects the changes following denervation. In the present work, we repeated in the rat the experiments of spinal cord section at birth, partially confirming the results obtained by Buller et al.[1] in the cat; subsequently, with a view to investigate whether the degree of muscle differentiation is of some importance for the effects of denervation on the dynamic properties of muscles, we compared the effects of nerve section in the cordotomized and in the control animals.

Methods. Spinal cord section was carried out at the mid-thoracic level, under ether anaesthesia, in albino rats 2 days old. After 3–6 months, the sciatic nerve was cut unilaterally, always under ether anaesthesia, near the trochanter. In a group of normal animals of the same age, the sciatic nerve was also cut unilaterally, as a control. 3 weeks after denervation, contractile properties of Soleus and EDL muscles were examined in vitro. The muscles, immersed at 37 °C in the Krebs solution (pH 7.2–7.4) aerated with a mixture of 95% O_2 and 5% CO_2, were connected to an isometric transducer and stimulated through platinum electrodes, with the massive stimulation method[6]. The stimuli were supramaximal, of 0.2 msec duration. Mechanical responses were recorded by a storage oscilloscope (Tektronic 5103 N/D11), and contraction time (CT), half relaxation time of the twitch (1/2RT), tetanic fusion frequency (TFF), Tetanus: twitch ratio (T:t ratio) and maximum rate of rise of tetanus were measured. The muscles were weighed at the end of the experiments.

Results. Soleus muscle (table 1). In Soleus muscles from the animals with spinal cord section, CT and T:t ratio were significantly smaller ($p < 0.05$), TFF and the rate of rise of tetanus significantly greater than in the control muscles. When denervated after spinal cord section,

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Soleus muscle showed, in comparison with the contralateral muscle, a significant increase of CT and of $^{1}/_{2}$ RT and a significant decrease of TFF and of the T:t ratio. Although not significantly, the maximum rate of rise of tetanus resulted decreased. The weight loss of denervated muscle was great. In the control animals, denervated muscles compared to the contralateral ones showed the same changes observed in the cordotomized animals.

EDL muscle (table 2). After spinal cord section, there were no significant changes in mechanical properties of EDL muscles with respect to the controls. EDL muscles of previously cordotomized animals showed, after denervation, a significant increase of CT and of $^{1}/_{2}$ RT, and a significant decrease of TFF and of the T:t ratio. Also the rate of rise of tetanus was decreased. A great loss of muscle weight was also observed. The same changes after denervation were observed in the muscles of the control animals.

Discussion. In agreement with the data of Buller et al.[1], from the present work it results that the spinal cord section alters the development of the dynamic properties of Soleus muscle, so that it becomes faster than in normal animals; such an effect reflects probably a reduction in the tonic impulse activity to the muscle, following the suppression of the supraspinal influences. Actually, the pattern of motor activity has a strong influence on the dynamic properties of muscles, as shown by the experiments of nerve cross-union[7] and of in vivo electrical stimulation[8–10]. It should be noted, however, that in our experiments the increase in the speed of contractions, though statistically significant, was not so marked as it appears from the results of Buller et al.[1]; in our experiments, in fact, the differences between fast and slow muscles were clearly maintained, although to a lesser extent than in the controls.

Table 1. Changes in contractile properties of Soleus muscle

	CT (msec)	$^{1}/_{2}$ RT (msec)	TFF (stim/sec)	T:t ratio	max. rate of rise (g/msec)	weight (g)
Control	41.11 ± 0.99 (4)	41.62 ± 2.03 (4)	63.75 ± 2.39 (4)	6.73 ± 0.11 (4)	0.30 ± 0.16 (4)	0.140 ± 0.01 (4)
Cord section	33.22 ± 1.83 (6)	37.96 ± 2.87 (5)	78.33 ± 4.77 (6)	5.52 ± 0.44 (6)	0.50 ± 0.09 (5)	0.125 ± 0.09 (6)
Changes (%)	– 19.19*	– 8.79	+ 22.87*	– 17.98*	+ 66.67*	– 10.71
Control	41.11 ± 0.99 (4)	41.62 ± 2.03 (4)	63.75 ± 2.39 (4)	6.73 ± 0.11 (4)	0.30 ± 0.16 (4)	0.140 ± 0.01 (4)
Sciatic nerve section	54.33 ± 2.54 (4)	62.49 ± 5.14 (4)	56.25 ± 3.14 (4)	3.37 ± 0.39 (4)	0.15 ± 0.01 (4)	0.099 ± 0.01 (4)
Changes (%)	+ 32.16*	+ 50.14*	– 11.76	– 56.12*	– 50.00*	– 29.28*
Cord section	33.22 ± 1.83 (6)	37.96 ± 2.87 (5)	78.33 ± 4.77 (6)	5.52 ± 0.43 (6)	0.50 ± 0.09 (5)	0.125 ± 0.09 (6)
Cord section and sciatic nerve section	44.72 ± 2.57 (6)	58.08 ± 5.33 (5)	60.83 ± 4.73 (6)	3.04 ± 0.37 (6)	0.36 ± 0.05 (6)	0.079 ± 0.01 (6)
Changes (%)	+ 34.62*	+ 53.00*	– 22.34*	– 44.93*	– 28.00	– 36.80*

CT, contraction time; $^{1}/_{2}$ RT, half relaxation time; TFF, tetanus fusion frequency; T:t ratio, Tetanus: twitch ratio; max. rate of rise, maximum rate of rise of tetanus; numbers in parentheses refer to the number of experiments; values are mean ± SE; * $p < 0.05$.

Table 2. Changes in contractile properties of EDL muscle

	CT (msec)	$^{1}/_{2}$ RT (msec)	TFF (stim/sec)	T:t ratio	max. rate of rise (g/msec)	weight (g)
Control	14.20 ± 0.22 (4)	10.11 ± 0.46 (4)	176.25 ± 2.39 (4)	5.70 ± 0.38 (4)	0.80 ± 0.12 (4)	0.148 ± 0.01 (4)
Cord section	15.25 ± 0.65 (6)	11.36 ± 0.42 (6)	162.50 ± 6.29 (4)	5.19 ± 1.10 (6)	0.76 ± 0.20 (4)	0.157 ± 0.14 (6)
Changes (%)	+ 7.39	+ 12.36	– 7.80	– 8.95	– 5.00	+ 6.08
Control	14.20 ± 0.22 (4)	10.11 ± 0.46 (4)	176.25 ± 2.39 (4)	5.70 ± 0.38 (4)	0.80 ± 0.12 (4)	0.148 ± 0.01 (4)
Sciatic nerve section	20.83 ± 0.53 (4)	17.37 ± 0.57 (4)	142.50 ± 6.29 (4)	4.32 ± 0.28 (4)	0.52 ± 0.04 (4)	0.097 ± 0.14 (4)
Changes (%)	+ 46.69*	+ 71.81*	– 19.15*	– 24.21*	– 35.00	– 34.46*
Cord section	15.25 ± 0.65 (6)	11.36 ± 0.42 (6)	162.50 ± 6.29 (4)	5.19 ± 1.10 (6)	0.76 ± 0.20 (4)	0.157 ± 0.14 (6)
Cord section and sciatic nerve section	21.08 ± 0.57 (6)	19.41 ± 1.11 (6)	135.00 ± 5.63 (6)	2.41 ± 0.14 (6)	0.47 ± 0.07 (6)	0.102 ± 0.09 (6)
Changes (%)	+ 38.23*	+ 70.86*	– 16.93*	– 53.56*	– 38.16	– 35.04*

For explanation see table 1.

This would suggest that the pattern of motor activity, physiologically applied to the muscles, is not a basic factor in determining the differentiation of muscles into the fast or slow types, or at most, that it is of relevance for the differentiation into the fast type, since according to Brown[3] denervation in the rat and in the rabbit, when performed at birth, prevents the differentiation of EDL, but not of Soleus muscle.

Denervation performed 3–6 months after birth, caused the same marked slowing effects both in the control and in cordotomized animals; moreover, the changes in dynamic properties of the muscles were of the same order of magnitude in the fast and in the slow muscles. Thus it appears that the effects of denervation are not related to the mechanical properties of the muscles and to the degree of speeding which is reached after birth. Furthermore, from the parallel slowing of Soleus and EDL muscles after denervation, such that the contraction time ratio of the 2 muscles was not significantly changed, it can be inferred that motor innervation, according to the observation made by Lewis in the cat[4], is not essential for maintaining the differences already developed in muscles.

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The relationship between membrane potential and ATP content in rat liver during ischemia

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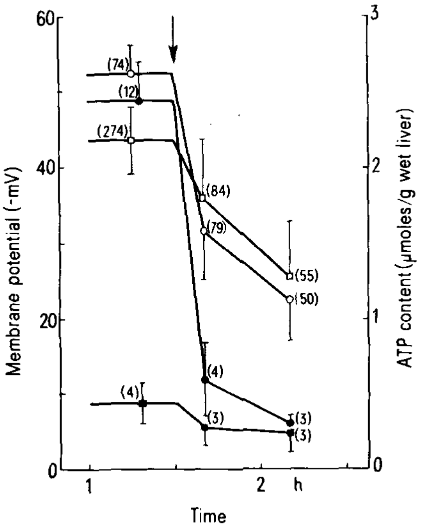
Summary. Both ischemia and ethionine decreased the membrane potential and ATP content in rat liver. In ethionine-treated rat liver, ischemia brought about a further decrease in membrane potential without significant decrease in ATP content.

The transmembrane potential of liver cell is very sensitive to ischemia: it decreases far more quickly than that of muscle cell during ischemia[1]. In addition, the ATP content in liver decreases also during ischemia[2]. From these facts, it can be supposed that an ATP supply is required for maintenance of the membrane potential. In fact, ethionine, which decreases intracellular ATP content by its adenine trapping effect[3], decreases both the membrane potential and ATP content in rat liver, and the decreased membrane potential and ATP content are restored by adding adenine to the ethionine-treated rat[4].



Effects of ischemia on the membrane potential and ATP content in the ethionine-treated and untreated rat livers. ○ and ●, membrane potential and ATP content in untreated rat livers, respectively. □ and ■, membrane potential and ATP content in ethionine-treated rat livers, respectively. Anesthetic injection was carried out at time 0. Ischemia was started at the arrow. Each point shows the mean ± SD. The number of measurements is shown in parentheses.

In the present experiments, the effects of ischemia on the membrane potential and ATP content in the livers of ethionine-treated and untreated rats were investigated. The results obtained suggest that the decrease in the membrane potential by ischemia cannot be simply explained as a result of its ATP depleting effect.

Materials and methods. Female donryu-rats (130–200 g b. wt) were anesthetized with an i.p. injection of pentobarbital (4 mg/100 g b. wt; Somnopentyl, Pitman-Moore Inc.) and midline laparotomy was carried out 20 min after the anesthetic injection. Ischemia was carried out by occluding both the portal vein and common hepatic artery 90 min after the anesthetic injection. DL-ethionine (75 mg/100 g; Katayama Chemical Co.) was injected i.p. 4 h before the anesthetic injection. Membrane potentials were measured from the left lateral lobe in situ in the conventional way using microelectrodes filled with 3 M KCl solution[4]. In each of 7 ethionine-treated and 6 untreated rats, the potential measurements were carried out 10–30 min before the onset of ischemia, and 5–15 min, and 30–50 min after it. In each measurement period, 55–274 measurements were performed in the ethionine-treated rats and 55–74 measurements in the untreated rats. For the investigation of the effect of ischemia on liver ATP content, 10 ethionine-treated rats (4 for control, 3 for the effect of 10-min ischemia, and 3 for the effect of 40-min ischemia) and 19 untreated rats (12 for control, 4 for the effect of 10-min ischemia, and 3 for the effect of 40-min ischemia) were used. A tissue sample of about 0.2 g wet weight was removed from the left lateral lobe of each animal and frozen in liquid nitrogen as soon as possible. The ATP determinations were carried out according to the method of Lamprecht and Trautschold[5].

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