(fractional turnover rate of intralipid) of circulating triglycerides in this group of population. This suggested an inverse correlation between plasma triglycerides and removal rate of this fraction.

The results on the metabolic studies of lipids in mice maintained on high sucrose diet are given in table 2. The triglycerides were found elevated both in males and females but the rise in males was significantly more than females. The possible reasons for higher levels in males could be either due to higher secretion rate from liver to plasma, or due to lower removal rate by extrahepatic tissues in comparison with females. However, there seemed to be no difference in the secretion rates of triglycerides as seen from the results of table 2. The fractional turnover of the triglycerides by extrahepatic tissue was significantly reduced in males when compared to that of females, indicating slower removal rate in males. Furthermore, there was higher rate of conversion of U-^{14}C-glucose into liver triglycerides as well as higher levels of triglycerides in liver of male mice than females.

Thus, the present studies in humans and in mice suggested higher plasma triglycerides in males, possibly due to slower removal rate. Macdonald[8] concluded in his study that the sex difference observed in plasma triglycerides in response to dietary carbohydrates could be due to the preventive effect of estrogen and progesterone in elevation of fasting serum glyceride. This was later disproved because administration of estrogens increased the serum triglyceride concentration, and this was associated with decrease in PHLA (postheparin lipolytic activity)[16,17]. The sex difference in triglyceride metabolism could, nevertheless, be due to effects of other hormones on the PHLA or clearance rates, the clarification of which needs a detailed investigation.

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Effect of exercise on ribonuclease activity in rat skeletal muscle

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Summary. Distribution of ribonuclease activity (measured at pH 7.6) in subcellular fractions of homogenates of rat skeletal muscle was investigated in sedentary animals and after 8 weeks running program. Training increased ribonuclease activity (expressed as units of enzyme per g of muscle protein). There was no increase in nuclear fraction, but in both cytoplasmic and mitochondrial fractions the RNA-ase activity increased 42% and 45% respectively.

It is commonly known that the adaptation of skeletal muscles to physical effort is closely connected with changes in metabolism, and to a high degree is the result of the regulation of the synthesis of biologically active proteins.

The results of several authors' studies make it possible to ascribe the regulation of protein biosynthesis at the stage of translation[2-5] and transcription[6-8] to ribonucleases. Until recently, there was no convincing proof that ribonucleases may fulfil the function of endonucleases described in degradation processes pre-m RNA to the form of cytoplasmic m-RNA.

The studies of Bardoń[9] and Libonati[10] would seem to confirm the role of intracellular alkaline ribonucleases in the processes of transformation of pre-m RNA into m-RNA. The biological role of RNA-ase, and our earlier results concerning changes in RNA-ase activity in blood serum following physical effort[11], led us to study the effect of physical exercise on the activity and distribution of alkaline ribonuclease in the skeletal muscles of rats.

Materials and methods. Training program. Investigations were carried out on male Wistar rats, weight 200–250 g. The animals were divided into 2 groups: a sedentary-control group, and a group subjected to running exercise on a motor-driven treadmill. All the animals were fed a standard diet.

The exercised animals ran at intervals for 8 weeks on a treadmill at an 8° incline. In the exercise cycle, the effort was increased from 4 2-min runs at a speed of 24 m/min, with 2-min intervals breaks, to 12 2-min runs at a speed of 48 m/min, with 2-min intervals breaks.

The joint power load each training day was increased from 4.5 to 27 W/kg and was kept at this level for the last 5 weeks of the experiment.

Preparation of subcellular fraction. Both groups of animals were sacrificed by decapitation (those exercised were killed 24 h after the last run). The femoral muscles were rapidly prepared, cleaned of accompanying tissues, weighed and chilled in ice-cold 175 mM KCl with 0.1 mM EDTA (pH 7.4). Tissues were minced with scissors and portions about 8 g of the muscle were homogenized in 30 ml of 175 mM KCl by means of an electric homogenizer at 4 °C for 40 sec. The homogenate was neutralized by adding 1 M KOH and was centrifuged at 15,000 × g for 20 min. The supernatant was considered as cytoplasm, the sediment was resuspended in 175 mM KCl and homogenized in a glass hand homogenizer. The suspension was centrifuged again at 800 × g for 15 min. The supernatant was treated as a mitochondrial fraction and the sediment as a nuclear fraction. The resulting fractions were assigned for measurement of the RNA-ase activity and protein levels. Noncollagen protein was determined by the biuret procedure[12,13].

Enzyme assay. Acid stable RNA-ase was extracted from fractions by treatment with 0.250 M H_2SO_4. The homogenate and cellular fractions were treatment with cooled H_2SO_4 and kept at 4 °C for 48 h. Then the fractions were centrifuged at 3800 rev./min at 4 °C for 25 min. The supernatant was adjusted to pH 7.6 with 1 M KOH. The supernatant was used to determine RNA-ase activity. The ribonuclease assay medium contained[14]: substrate - highly polymerized RNA 2 g/l, buffer Tris-HCl 0.1 M at pH 7.6, bovine serum albumine 4 g/l and RNA-ase extract in total volume of 0.5 ml. In order to control linear relation between enzyme activity and the amount of product formed, incubation was performed for 2, 5 and 10 min. Precipitation of the residual RNA was performed with precooled precipitating reagent (1 M HCl in 76% ethanol). The samples were stored at 0 °C for 30 min and centrifuged at 4 °C. The clear supernatant was diluted 5-fold with water and absorbances determined at 260 mm spectrophotometrically in relation to the control assay in which the precipitation reagent was added at 0 time. Enzyme activity was expressed as a μmole acid-soluble product/1 h/g protein using extinction coefficients of 11×10^3.

Results and discussion. Distribution of ribonuclease activity in subcellular fractions of untrained and trained rats skeletal muscles are shown in figures 1 and 2. The results obtained indicate that RNA-ase activity measured at pH 7.6 is present in all subcellular fractions of rats skeletal muscles. The bulk of RNA-ase activity was found in mitochondrial and cytoplasmic fractions (figure 2). The RNA-ase activity values obtained in muscles of untrained rats provided the control, making it possible to determine the effect of exercise on RNA-ase activity. The physical effort caused an increased RNA-ase activity in the muscles. The average increase of RNA-ase activity in the homogenate was 40.6%, in cytoplasmic fraction - 42% and in mitochondrial fraction - 45,3%. At the same time the protein contents in the muscles was assayed (figure 1). The results obtained show only a very slight increase in amount of protein (a mean of about 5.7%) in muscles of trained rats. The greatest changes in RNA-ase activity were found in cytoplasmic and mitochondrial fractions, i.e. in cellular compartments, in which translation processes occur. We may conclude, therefore, that RNA-ase measured in pH 7.6, which is in muscles together with a natural inhibitor, may take an active part in adaptation to physical effort.

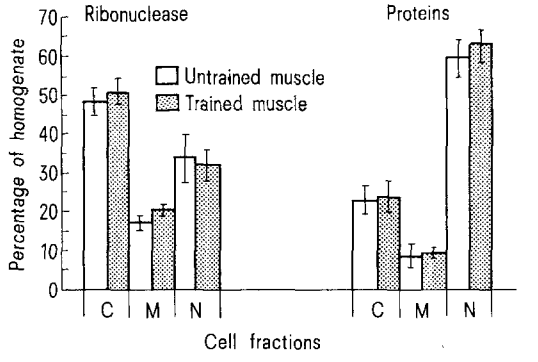

Fig. 1. Effect of training on the percentage distribution of the ribonuclease activity and protein in rat skeletal muscle. All values are means±SEM. Changes are not significant ($p > 0.05$). C - Cytoplasmic; M - Mitochondrial fractions; N - Nuclear fractions.

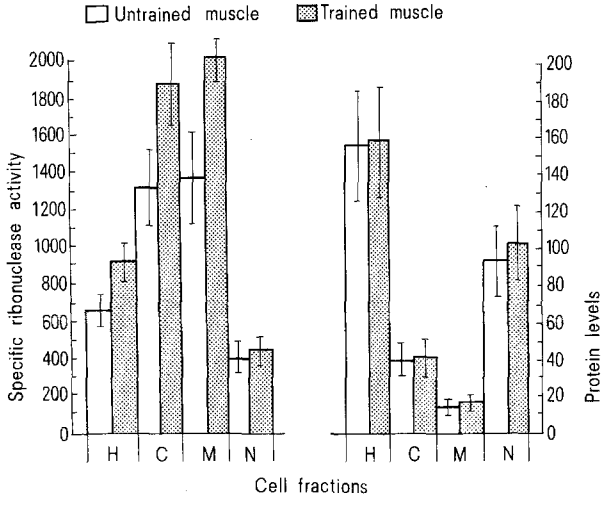

Fig. 2. Effect of training on the intracellular distribution of specific activity of alkaline ribonuclease and protein levels. RNA-ase specific activity in cell fractions are expressed in μM acid-soluble product/1 h/g protein. Protein levels are expressed in mg/g wet tissue. All values are means ± SEM. The changes of RNA-ase activity are statistically significant ($p < 0.001$). H - Homogenates; C - Cytoplasmic; M - Mitochondrial fractions; N - Nuclear fractions.

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