The first embryoid-like structures to appear in the cultures were usually abnormally large and often showed no signs of cotyledon formation. This type of structure was disregarded as were rhizogenic structures when determining the time of appearance of normal embryoids. Cell counts were performed on these abnormal embryoids. The data indicate a faster cell doubling time than that occurring during 'normal' development. This abnormal growth is possibly due to the initial lack of some conditioning factor from the medium. Some support for this view comes from an experiment in which a sample of globular embryoids separated as eptically[3], was transferred to fresh Murashige and Skoog medium lacking 2,4-D. Large abnormal embryoid structures were produced.

To obtain cell counts for the very young globular stage we used a geometrical method which assumed that the structures were spherical and that the cells packed within the embryoid approximated to cubes all of a similar size. From a determination of the peripheral cell number of a cross-section we were then able to calculate the number of cells in the embryoid. Cell counts for later stages in embryoid development were obtained by more conventional means. Individual cells were freed from the embryoids by treatment with 1% cellulase in acetate buffer pH 4.6 for 3 h at 37 °C. The separated cells were then suspended in 50% glycerol and counted using a haemocytometer slide.

Results and discussion. Rates of cell division (i.e. cell doubling times) were calculated assuming that embryoids develop from single cells[4]. Thus, average cell doubling times are tiven by h/D where h is the time required for appearance of the embryoid stage and D is equal to the number of doublings in cell number. D is defined by the expression $\log N - \log N_0/\log_2$ where N = the cell number after h hours and N_0 is the original cell number. The values obtained provide a minimum estimate for the maximum rate of cell division and assume that all the cells in the embryoid are dividing and at a similar rate. The lack of polarized growth in the young globular stage points to this 'ideal' situation.

Table 1 shows the average number of cells in each embryoid stage together with the time from the initiation of a culture to when examples of each stage may first be observed. In table 2 we have presented the average cell doubling times for each stage in embryoid development.

The initial development of embryoids in culture appears to be much more rapid than occurs in vivo. E.g. in cotton[7] the initial cell doubling time is 20–22 h as compared with 14–15 h in carrot cultures. This may reflect the modification to, or absence of, important control systems in culture as compared with the in vivo situation. The formation of large abnormal embryoids is probably a further manifestation of this, representing a situation yet more remote from the controlled environment of the ovule.

Values for the doubling time of small meristematic (embryogenic) cells of carrot growing in a nondifferentiating stage (td 65 h, calculated from Jones[8]) indicate that the removal of auxin from the culture medium results in a dramatic reduction in doubling time for cells destined to develop into embryoids (see table 2 for comparison). Data of Bayliss[9] also indicate that doubling times for carrot cells growing in the absence of auxin are much reduced, however it is not clear to what extent embryoids were included in the cell counts.

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Effect of beta-adrenergic blockade on intramuscular triglyceride mobilization during exercise[1]

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Summary. It was shown in the rat that mobilization of intramuscular triglyceride during exercise is controlled by the adrenergic system.

It was repeatedly shown that intramuscular triglycerides are mobilized during exercise[2–8]. However, the mechanism controlling this process has not been understood yet. In the present work it was found that the adrenergic system is responsible for activation of intramuscular triglyceride breakdown during exercise.

Materials and methods. The experiments were carried out on male Wistar rats weighing 200–250 g, fed ad libitum with commercial pellet diet for rodents. The animals were divided into 2 groups: I the control group, II the propranolol-treated group. Propranolol (Inderal, Galenika) in a dose 2.5 mg/kg was injected s.c. 15 min before exercise. Half of each group was subjected to the exercise, while the other half was kept at rest and deprived of the food at the same time. The exercising rats were loaded with a weight equivalent to 1% of their b. wt attached to base of the tail and then they were forced to swim in a metal barrel 58 cm in diameter filled to a level of 40 cm with water at temperature 33–35 °C. 6 rats were made to swim simultaneously. It was found in preliminary experiments that the control rats were able to swim for not much more than 5 h, and the propranolol-treated rats for not much more than 3 h. According to these data, to avoid stressfull drowning, rats were subjected to exhaustive swimming which was ceased after 5 h in the

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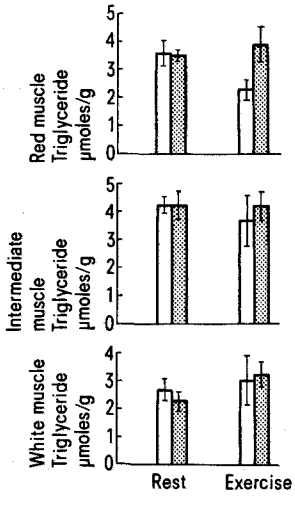
control group and after 3 h in the propranolol treated group. Additionally, in the control group measurements were made after 3 h of swimming. Rats were anaesthetized with urethan given i.p. Blood from abdominal aorta and than samples of different types of skeletal muscle were taken. The muscle samples were taken according to the identification of Baldwin et al.[9] in the following order: 1. the most superficial layer of the vastus lateralis which consists of white fibres (the white muscle), 2. the deepest layer of the same muscle which consists of red fibres (the red muscle) and 3. the soleus muscle which is composed in 70% of intermediate fibres and in 30% of red fibres (the intermediale muscle). Levels of glucose[10] and free fatty acids (FFA)[11] in the blood were determined. Intramuscular triglycerides were extracted according to the method of Carlson[12] and then determined quantitatively according to the method of Galletti[13]. The results obtained were evaluated statistically by means of Student t-test for unpaired data.

Results. In the red muscle of the control group, both after 3 and 5 h of swimming, the levels of triglyceride were significantly lower than at rest (at rest 3.57 ± 0.44, after 3 h − 2.37 ± 0.47, after 5 h − 2.29 ± 0.34 μmoles/g; $p < 0.001$ and $p < 0.001$ respectively). In the white and intermediate muscles, no decrease of triglyceride levels occurred during the exercise. Propranolol fully prevented the reduction of triglyceride level in the red muscle (figure). In the control group only 5 h swimming lowered significantly ($p < 0.001$) blood glucose level (at rest − 5.08 ± 0.45, after 3 h − 4.98 ± 0.55, after 5 h − 3.84 ± 0.56 μmoles/ml). In propranolol-treated group blood glucose level was significantly ($p < 0.05$) lower than in the control group only at rest but not after 3 h exercise (at rest − 4.62 ± 0.21, after 3 h − 4.53 ± 0.69 μmoles/ml).

There was marked rise of blood FFA level during exercise without the blockade (at rest − 239.3 ± 41.5; after 3 h − 801.7 ± 135.1; after 5 h − 955.3 ± 144.2 μEq/l). Propanolol insignificantly increased FFA level at rest and partially prevented its rise after 3 h exercise (at rest − 287.0 ± 90.8; after the exercise − 559.5 ± 84.4 μEq/l, $p < 0.001$ vs. the value after 3 h without the treatment).

Discussion. The results of the present study showing that, during physical exercise in rats, only the red skeletal muscle utilizes to a marked extent the triglycerides stored in its cells confirmed results obtained by others[2,5,7]. Data concerning regulation of intramuscular triglyceride mobilization are only scarce. In man, prolonged i.v. infusion of noradrenaline lowered triglyceride level in m. vastus lateralis[14] but similar treatment in dog resulted in increase of intramuscular triglyceride level[15]. It was shown in vitro that adrenaline and growth hormone stimulated lipolysis in diaphragm muscle[16,17]. Activity of the adrenergic system[18–21] and blood growth hormone level[22–24] increase during exercise. According to these data, it could be assumed that lipolysis of intramuscular triglycerides during exercise might be stimulated both by catecholamines and growth hormone. However, the results obtained in the present work have shown that blockade of beta-adrenergic receptors fully prevented the intramuscular triglyceride mobilization. These results strongly suggest that catecholamines acting through beta-receptors are primarily responsible for mobilization of intramuscular triglycerides during physical exercise in rat. Growth hormone does not seem to be involved in this process.

Therefore, in rat, there are marked differences in regulation of triglyceride breakdown in fat tissue and skeletal muscle during exercise. Neither beta-adrenergic blockade nor 6-hydroxydopamine thoroughly prevented exercise-induced rise of blood FFA level[25–28]. It was postulated that mechanisms other than the adrenergic system, most probably of hypophyseal origin, might be responsible for the increased lipolysis in fat tissue[25,28]. Contrary to fat tissue, the accelerated breakdown of intramuscular triglycerides during exercise seems to be controlled exclusively by the adrenergic system.



Effect of beta-adrenergic blockade on intramuscular triglyceride mobilization in different types of skeletal muscle during exhaustive exercise. Open bars-the control group; hatched bars-the propranolol-treated group. Each result presented on the figure and in the text is an average of determinations performed on 8 rats.

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