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Effects of Exercise on Plasma and Tissue Levels of Lactate Dehydrogenase and Isoenzymes in Rats. (32260)

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In recent years there has been a growing interest in the effects of stress, especially physical exertion, on untrained individuals. The rat has proved to be a useful animal for studying these effects in the laboratory. Marked rises in serum lactate dehydrogenase (LDH) activity and in other enzyme levels have been reported in untrained rats subjected to different types and severity of exercise (1-5). No explanations were given in these studies for the high levels of serum LDH seen in the control animals nor for the marked variation in levels in both the control and exercised groups(1-3). Studies in our laboratory showed similar discrepancies and investigations were undertaken which resulted in the elucidation of several factors contributing to high control values and variability between determinations. To control these factors, a modified method was evolved for collecting and preparing rat blood for LDH analysis. This method was then employed along with an electrophoretic procedure for determination of LDH-isoenzymes, recently developed by one of the authors(6), in order to better evaluate serum and tissue LDH changes resulting from exercise in rats.

Experimental procedure. Twenty untrained male rats of the Walter Reed strain, weighing 150 to 250 g, were randomly divided into an exercised group and an unexercised control group. Both groups were fasted overnight prior to the procedure. The exercise group was subjected to 4 hours of swimming with a 5-minute rest every 30 minutes, using a 30 gallon, 52 × 52 × 92 cm tank of water at 30-32°C temperature. Within 20 minutes after completion of exercise, the animals were anesthetized with ether, the abdomen opened, blood was removed from the inferior vena cava using a plastic syringe, and placed in a plastic tube containing heparin (50 μg/3 ml blood). Immediately after removal it was centrifuged for 3 minutes in a microfuge (12000 rpm), to separate the plasma which was subsequently analyzed for LDH and isoenzymes.

Following the collection of blood, tissue samples were excised from the liver, cardiac ventricles and thigh muscle, and the animal was sacrificed. These tissues were rinsed in saline, blotted and weighed. Ten per cent tissue homogenates were prepared in a glass homogenizer with buffered saline pH 7.5. The homogenates were then centrifuged at 15000 rpm for 10 minutes and the supernatants were analyzed. Histological sections were prepared from the excised tissues fixed in 10% Formol-Ringers solution and stained with H & E, PAS, and Oil Red O. Histological sections were also prepared from other animals 3 days post exercise.

Red blood cell and platelet preparations were made by centrifuging heparinized blood from control rats at 600 rpm for 10 minutes. An aliquot was removed from the supernatant

TABLE I. LDH Activity in Control and Exercised Untrained Rats.

Exercise		LDH (units/ml/min ± SEM)			
		Serum		Plasma	
Type	Duration (hr)	Control	Exercised	Control	Exercised
Rotating cage(1)	16	1140 ± 170	2740 ± 404	—	—
Idem(2)	6	1540 ± 139	2116 ± 247	—	—
	12	"	3523 ± 187	—	—
	16	"	2859 ± 96	—	—
" (3)	6	730 ± 192	1109	—	—
	16	"	2270 ± 162	—	—
Swimming (this study)	4	181 ± 36	1190 ± 368	62 ± 3	196 ± 6

platelet rich plasma for isolation of platelets and a sample of red blood cells was transferred from the bottom of the centrifuge tube into another centrifuge tube. The red blood cells were washed twice in saline and then hemolyzed by suspension in 2 volumes of water and freezing and thawing twice. The ghosts were precipitated by centrifugation and appropriate dilutions were made from the supernatant for analysis of isoenzymes. The platelet rich plasma was centrifuged at 2500 rpm for 15 minutes and the platelet button was processed the same way as the red blood cells.

Total LDH was determined on the serum, plasma, and tissue extracts by the spectrophotometric method described in Bergmyer (7); LDH-isoenzymes by an electrophoretic procedure recently reported(6); and hemoglobin by the method of Crosby(8).

Results. Originally blood was obtained by cardiac puncture and serum was analyzed for LDH and isoenzymes as reported by previous investigators(1-3). The results are shown in Table I along with the results reported by these investigators. Extremely variable levels of serum LDH are shown in control animals with mean values ranging from 181-1540 units, which led us to investigate possible causes of these variations. When aliquots of blood from the same animal were allowed to clot and centrifuged at different time intervals, progressive increase of LDH activity in the serum was observed as shown in Fig. 1.

Three possible sources of the variations of LDH seen in Table I and Fig. 1 were considered and tested: 1, hemolysis; 2, disruption of platelets; and 3, heart injury due to the cardiac puncture. Hemoglobin determination of the examples shown in Fig. 1 showed that some hemolysis is inevitable and the extent of hemolysis increased with time. Further evidence of hemolysis and platelet disruption was noted by the association of increased LDH activity with increase in density of the isoenzyme-5 zone. As shown in Fig. 2, isoenzyme-5 is associated with red blood cells and platelets. Variation of isoenzymes 1 and 2 was also observed in the same control experiments. As shown in Fig. 2, these isoenzymes are present in high concentration in heart indicating that during cardiac puncture heart LDH may be released into the serum.

The plasma LDH values in control and exercised rats, obtained as described in the experimental procedure, are shown in Table I. Values of under 100 units for LDH in control animals are reported for the first time; a 3-4-fold increase of LDH was found in exercised animals.

Typical LDH-isoenzyme patterns of control and exercised rats are shown in Fig. 3. Both patterns were developed simultaneously,

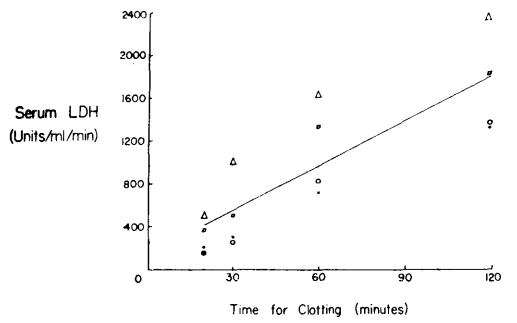

FIG. 1. Effects of blood clotting on serum LDH activity. Aliquots of blood obtained from 4 control rats were allowed to clot for various time intervals before centrifuging and analyzing the serum for LDH. Solid line is the regression line for values obtained.

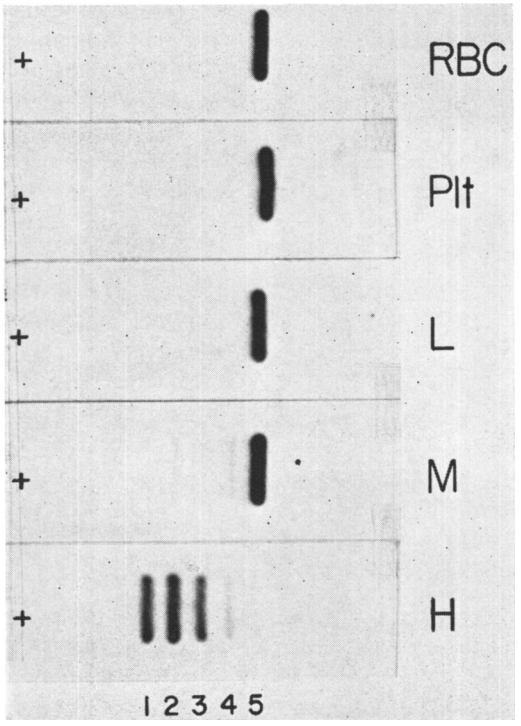

FIG. 2. LDH-isoenzyme patterns of rat tissues by agar gel electrophoresis; RBC, red cells; Plt, platelets; L, liver extract; M, skeletal muscle; H, heart.

using the same amount of plasma; this facilitated comparison and detection of changes. In the control, 5 isoenzymes were detected and the intensities of the zones were $5>1>2>3>4$. Significant change in the experimental animals occurred in isoenzyme 5, as shown by the increased density of this zone. Isoenzymes 1 and 2 also increased, the isoenzyme 2 zone increasing more than the 1, and the two zones appear of equal intensity. A comparison of these isoenzyme patterns with those of the various tissues shown in Fig. 2 indicates that isoenzyme 5 is associated with red blood cells, platelets, liver, and skeletal muscle. Therefore, the increase in plasma may have been derived from these tissues, while the increase of isoenzymes 1 and 2 may be influenced by the heart.

In order to correlate the increased plasma LDH activity with possible LDH changes in the heart, liver, and muscle, LDH and isoenzymes were determined in these tissues before and after exercise; no changes were detectable. The high LDH concentration in these tissues and the comparatively small increase in the plasma after exercise, does not exclude the possibility of small changes beyond the sensitivity of the method.

Histological examination showed that in tissue sections obtained immediately after exercise, numerous fine fat droplets were present in the myocardial fibers and in the hepatic parenchymal cells of the centrilobular zones. In animals sacrificed 3 days after exercise, small zones of focal necrosis were present in the myocardium.

Discussion. Several possible reasons have been uncovered to account for elevated and poorly reproducible values of serum LDH found in control rats. These are the use of cardiac puncture which apparently introduces cardiac LDH into the blood; disruption of platelets and hemolysis of red blood cells, both resulting in a release of LDH. Allowing clotted blood to stand before centrifuging off the serum results in a progressive elevation of LDH, probably by means of the latter two mechanisms. Cohen and Larson(9) also reported variations in LDH levels of human blood due to various factors including glass contact and clotting of blood.

The plasmas obtained by the experimental procedure described here, were clear and the hemoglobin values under 5 mg%. The preparation of rat blood for analysis is more difficult than the human because of the relatively higher platelet and red cell count and greater lability of red cells in rats than in humans.

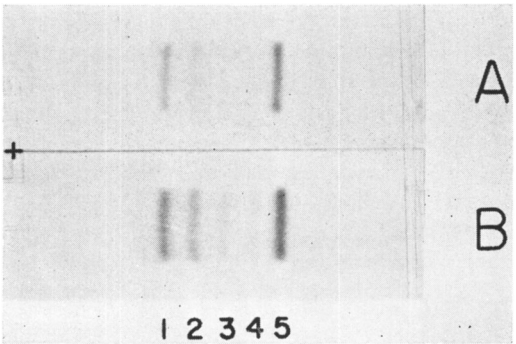

FIG. 3. LDH-isoenzyme patterns of rat plasma by agar gel electrophoresis: A, unexercised control; B, exercised.

Subjecting untrained rats to swimming for 4 hours resulted in about a 3-fold increase in plasma LDH which is in the same range as the serum LDH increases reported by other investigators(1-3) with 6 to 16 hours of exercise in rotating cages. The increased levels apparently resulted from escape of LDH from heart, skeletal muscle, liver, and possibly red cells and platelets. This is further substantiated by the demonstration of histopathological changes in this study and others, and by the finding of elevations of other enzymes in the blood by other investigators(1-5).

Summary. Elevated and variable serum levels of LDH in control rats were found to result from the commonly used method of collecting the blood by cardiac puncture and from the preparation of serum for analysis. Evidence is presented that LDH from heart, red blood cells, and platelets are contributing factors to this discrepancy. When blood was collected from the inferior vena cava and plasma was immediately prepared in plastic tubes for analysis instead of serum, the extraneous factors were largely eliminated. These modifications were employed for the determination of LDH and isoenzymes in untrained rats subjected to 4 hours of swimming, and in untrained controls. The data indicated a 3-fold increase of LDH activity in the plasma of exercised rats. LDH-isoenzyme determination and histological examination indicated that probable sources of the increased LDH activity were the heart, liver, and skeletal muscle.

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Responses of Cell Cultures to Insecticides. IV. Relative Toxicity of Several Organophosphates in Mouse Cell Cultures.* (32261)

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The proven toxic effect of organophosphorus insecticides in animals is primarily due to the inhibition of acetylcholinesterase in the central nervous system. The toxicity of malathion and possibly of other organophosphorus compounds is dependent on at least 2 metabolic reactions which occur in the liver microsomes(1,2,3,4,5): the oxidative activation of malathion to malaoxon, a more potent anticholinesterase than the original compound(1), and the hydrolysis of malathion and malaoxon by carboxyesterase, which leads to the detoxification of both compounds(6,7,8). It is this detoxification reaction of malathion which is responsible for its remarkably low mammalian toxicity.

In contrast to this low mammalian toxicity, malathion is relatively toxic to established human-derived Chang liver and HeLa strain cells(9) as well as to primary cell cultures derived from chick embryos(10). Since the activities of esterases(11), of liver glutamate dehydrogenase(12), and of trypsin(13) are affected by organophosphorus compounds, cytotoxicity in cell cultures may also be induced by an effect on other enzyme systems. Because of the mouse's high resistance to malathion, we chose to investigate the suscepti-

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