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URINARY ENZYME EXCRETION IN RATS DUE TO HEAVY PHYSICAL WORK: EFFECT OF THE ANTIOXIDANT PHENOSAN*

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Physical work has a significant effect on the hemodynamics of the kidneys and their excretory function. The renal blood flow is reduced during physical work in proportion to its heaviness (sometimes by 50-60%) due to the redistribution of blood in the body, caused by the increased proportion of it supplying working muscles [6]. Glomerular filtration also is reduced significantly (by 30%), although by a lesser degree. Thus, during heavy physical work, besides general hypoxemia, relative renal ischemia also develops. Of all the structures of the kidney, cells of the proximal tubules are considered to be most susceptible to hypoxia [10]. Damage to the tubular epithelium can evidently explain the increased urinary excretion of gamma-glutamyl transpeptidase (γ-GTP), a marker enzyme of renal ischemia, localized in the membrane of the brush border, which has been observed in athletes after long-distance running [8], and also the disturbance of tubular function after intensive exercises [7]. Activation of lipid peroxidation (LPO) in cell membranes is ascribed an important role in the mechanism of tissue damage during ischemia and hypoxia. The use of antioxidants in physical work has been shown to reduce the accumulation of LPO products in the blood [1], and to stabilize cell membranes, thereby preventing the release of cytosol enzymes into the blood stream [2].

The aim of this investigation was to study γ-GTP activity and the excretory function of the kidneys in rats made to do heavy physical work, and also the effect of the synthetic water-soluble antioxidant phenosan on enzyme and renal function.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing initially 190-250 g. The model of physical work consisted of making the rats swim to exhaustion (until they could swim no more) in water at a temperature of 30°C, carrying a load fixed to the base of the tail and equivalent to 10% of body weight. There were five sessions of swimming with intervals of 1-2 days between sessions. Before the rats began their heavily laden swimming sessions (HS) they were adapted to the experimental conditions by swimming for 15 min daily on 3 successive days, carrying a load equivalent to 2% of body weight. At the end of the series of adapative swimming exercises and after each HS the rats were kept for 18 h in metabolism cages in order to collect the urine, and using sodium azide as bacteriostatic agent. There were three series of experiments. In series I, on 47 rats (group 1) the effect of physical work on urinary enzyme excretion, diuresis, urinary creatinine excretion, and creatinine clearance was investigated. In the experiments of series

*Potassium salt of di-tert-butylhydroxyphenylpropionic acid – Translator

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TABLE 1. Urinary γ-GTP Activity, Parameters of Renal Excretory Function, and Duration of Swimming Sessions for Rats of Group 1 (M ± m)

Parameter	HS 10% of body weight					
	0 (n=47)	1st (n=47)	2nd (n=38)	3rd (n=41)	4th (n=47)	5th (n=47)
Urinary enzyme excretion, units/18 h	4,74±0,42	6,16±0,57*	6,56±0,57*	5,97±0,5	5,77±0,49*	7,46±0,49*
Diuresis, ml/18 h	7,73±0,62	10,0±0,72*	11,1±1,0*	10,1±0,74*	10,7±0,78*	12,5±0,88*
Creatinine excretion ml/18 h	5,38±0,26	6,12±0,26*	6,13±0,27**	6,54±0,27*	5,97±0,2**	7,27±0,26*
Creatinine clearance, ml/min	0,51±0,04 (n_1 = 21)	0,63±0,05 (n_1 = 21)	—	—	—	0,84±0,05* (n_1 = 21)
Duration of swimming, min	—	22,3±1,1	24,3±1,3	27,0±1,78**	24,9±1,43	21,8±1,0

Legend. *p < 0.01, **p < 0.05 compared with initial swimming (0); n) number of rats, n_1) number of determinations of creatinine clearance.

TABLE 2. Urinary γ-GTP Activity and Duration of Swimming Sessions in Rats Receiving (Group 2A) and Not Receiving (Group 2B) Phenosan (M ± m)

Parameter	Group of rats	HS 10% of body weight					
		0	1st	2nd	3rd	4th	5th
Urinary enzyme excretion, units/18 h	2A (n = 18)	5,17±0,49	4,46±0,47	5,48±0,72	5,22±0,55	6,04±0,65	6,1±0,65
	2B (n = 17)	4,69±0,39	5,5±0,67**	4,8±0,44	6,75±0,81**	6,0±0,57**	8,18±0,9* p_2<0,05
Duration of swimming, min	2A (n = 18)	—	28,5±1,2	39,7±0,55*	42,3±1,8*	40,6±0,4*	36,1±1,5*
	2B (n = 17)	—	28,7±2,1	32,9±1,6 p_2<0,01	38,2±3,0*	36,3±1,8** p_2<0,05	26,7±1,8 p_2<0,01

Legend. Asterisk indicates significance of difference between parameters when compared with initial level (0) or with 1st swimming session: *p < 0.01, **p < 0.05, p_2) comparison of groups 2A and 2B.

II the effect of phenosan was studied on these same parameters. The animals (group 2A, 18 rats) received phenosan dissolved in bidistilled water by intraperitoneal injection 30 min before HS, in a dose of 10 mg/kg initially (before the first two HS), and thereafter of 5 mg/kg. Control animals (group 2B, 17 rats) were given injections of bidistilled water. Rats receiving phenosan did not swim until exhausted but for the maximal time of swimming of the animals of group 2B in the corresponding HS. The reason was that phenosan, as preliminary observations showed, increased the duration of swimming by 1.5 times, and the experimental setup was such that the intensity of the physical work could be equalized to some degree. In series III (group 3), in order to test the opinion expressed in the literature [7] that the urinary enzyme excretion depends on the rate of flow of the urine, diuresis was stimulated in 10 rats, not undertaking physical work, by intraperitoneal injection of aminophylline in a dose of 30 mg/kg twice a day. Some of the urine collected from animals of all groups was analyzed [3] and γ-GTP activity was determined, using kits of reagents from "Chemapol" (Czechoslovakia). The results were expressed in units per liter per minute, and excretion of γ-GTP in 18 h was calculated. The creatinine concentrations in the blood and urine were determined by the method in [4]. The results of investigations after the end of adaptive swimming were taken as the initial data (0). The results were subjected to statistical analysis by Wilcoxon's paired T test, the signs test, Student's t test, and correlation analysis.

EXPERIMENTAL RESULTS

Tables 1 and 2 (group 2B) show a significant increase in γ-GTP activity in the urine starting with the first HS, and its highest values were noted during the 5th HS. Taking its increase above the initial level by 20% or more to be an increase in urinary enzyme excretion, hyperenzymuria was observed after the 1st-2nd HS in 51-52% of rats, after the 3rd-4th HS in 61-58% of animals, and after the 5th HS in 75% of rats. Physical work until exhaustion had a training action until the 3rd-4th swimming session. Toward the 5th HS, however, reduction of the duration of swimming and a more marked urinary enzyme excretion were noted.

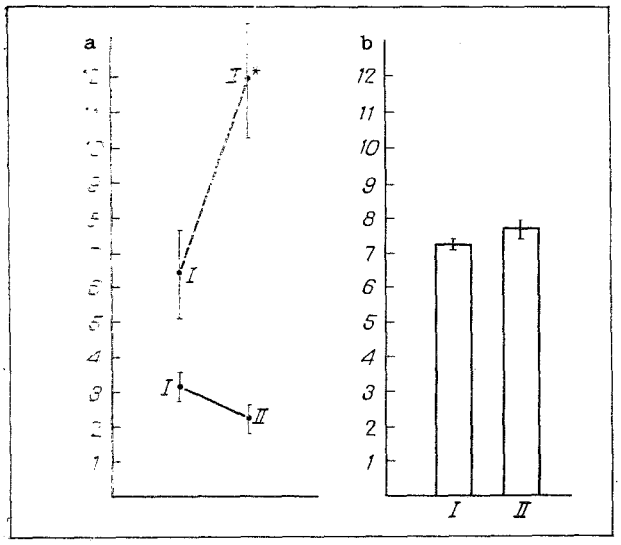

Fig. 1. Urinary excretion of γ-GTP and creatinine and diuresis in rats not subjected to physical work, before (I) and after (II) injection of aminophylline. a) Continuous line — urinary enzyme excretion, U/18 h; broken line — diuresis, ml/18 h; b) creatinine excretion, mg/18 h. *p < 0.01.

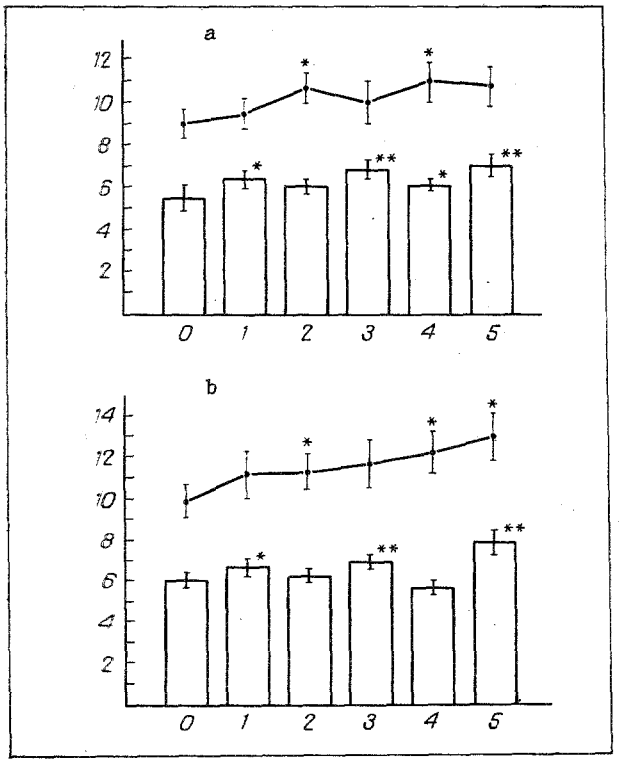

Fig. 2. Time course of diuresis (curve) and urinary creatinine excretion (columns) for rats receiving (a) and not receiving (b) phenosan. Abscissa: 0-5) serial numbers of swimming sessions; ordinate, diuresis (in ml/18 h) and creatinine excretion (in mg/18 h). *p < 0.05, **p < 0.01 compared with initial level (0).

Analysis of data on the excretory function of the kidneys during HS showed that instead of reduction of glomerular filtration, which some workers observed [5, 6], it was increased, and there was also a significant increase in the diuresis and urinary creatinine excretion. However, when these results are interpreted it must be recalled that in this case they re-

flect the total sum of the changes taking place both during physical work and in the immediate recovery period, for the urine was collected for 18 h, so as to obtain a sufficient quantity of it. During this period the phase of relative ischemia of the kidneys with depression of their function could be replaced by recovery or even by enhancement of the renal blood flow and glomerular filtration.

Correlation analysis of the initial data (0, Table 1) revealed significant correlation between the urinary enzyme excretion and diuresis ($r = +0.58$, $p < 0.01$) and also between the urinary enzyme excretion and creatinine excretion ($r = +0.38$, $p < 0.05$). These correlations were preserved during HS also: moreover, correlation between enzyme excretion and diuresis was found more often (in four of five HS) and it was closer than between enzyme excretion and creatinine excretion. It was therefore worthwhile testing whether the increase in enzyme excretion during HS was caused by an increase in diuresis in the recovery period, more especially because of the view expressed in the literature that enzymes of the brush border are "flushed out" by any considerable diuresis [7]. We showed in rats not subjected to physical work and receiving aminophylline to stimulate diuresis, that the twofold increase in urinary excretion caused by aminophylline did not affect γ-GTP excretion (Fig. 1). The correlation revealed between urinary enzyme excretion and diuresis was evidently not determined by any relationship of cause and effect between them.

The use of the antioxidant phenosan (Table 2, group 2A) prevented any significant increase in urinary enzyme excretion during all HS and maintained it at close to its initial level, despite the fact that the mean duration of physical work in the 2nd and 4th and, in particular, in the 5th HS was significantly longer than in control group 2B. In these rats, which did not receive the preparation, the urinary enzyme excretion during four of the five HS was higher than initially. In the 5th HS it was almost doubled and was significantly higher than the γ-GTP excretion in experimental group 2A. It will be noted that the increase of diuresis in the rats of group 2A in response to HS was smaller and less constant, whereas the increase in creatinine excretion was more stable than in group 2B (Fig. 2).

Intensive physical work thus leads to a significant increase in γ-GTP activity in rat urine. Hyperenzymuria and the number of animals in which it developed were maximal by the 5th HS, when working capacity was reduced and adaptation to work disturbed. The use of phenosan limits the outflow of γ-GTP from the membrane of the brush border, evidently on account of its ability, which is typical of antioxidants, to depress activation of LPO during hypoxia, and thereby to exert a membrane-stabilizing action.

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