Table 1. Cholinesterase activity determined in homogenates of the plant nematode *Aphelenchoides ritzema-boosi* with 3 different substrates

Substrate concentration (M)	nmoles SH/min/mg protein produced with ASCh	PSCh	BSCh
1.25×10^{-4}	0.74	0.71	1.41
2.50×10^{-4}	1.16	1.13	2.11
5.00×10^{-4}	1.54	1.94	3.17
1.00×10^{-3}	2.22	2.96	4.05
2.00×10^{-3}	3.34	4.50	4.89

The total volume of the reaction mixture was 3 ml, the incubation temperature 20 °C. The amounts of sulfhydryl groups produced during an incubation period of 10 min were obtained from a calibration curve in which absorbance (412 nm) was plotted against amount of reduced gluthathione.

Table 2. I_{50}-values obtained with purified bovine erythrocyte acetylcholinesterase (AChE), human plasma cholinesterase (ChE), and nematode cholinesterase (N-ChE) using different carbamates and organophosphates as inhibitors

Inhibitors	I_{50}-values (M) with AChE	ChE	N-ChE
Eserine	7×10^{-8}	1×10^{-7}	8×10^{-5}
Aldicarb	2×10^{-6}	2×10^{-6}	$>10^{-4}$
Carbaryl	3×10^{-6}	1×10^{-5}	$>10^{-4}$
Dioxacarb	5×10^{-6}	1×10^{-5}	$>10^{-4}$
Carbofuran	5×10^{-8}	4×10^{-6}	6×10^{-5}
Monocrotophos	2×10^{-5}	8×10^{-7}	$>10^{-4}$
Dichlorvos	8×10^{-7}	7×10^{-8}	3×10^{-7}
Paraoxon	5×10^{-8}	1×10^{-8}	4×10^{-8}

The I_{50}-values were determined by an automated cholinesterase inhibition procedure (for details see G. Voss[3]). Enzymes and inhibitors were pre-incubated at 37 °C for 20 min and then reacted with the preferred substrates.

concentration of 2×10^{-3} M ASCh. Again, this finding indicates that the cholinester hydrolysing enzyme of *Aphelenchoides* is not a typical acetylcholinesterase.

In combination with the optimum substrate BSCh, a further characterization of the nematode enzyme was carried out by comparing its inhibition with that of 2 other types of cholinesterases, e.g. erythrocyte acetylcholinesterase and human plasma pseudocholinesterase, using a number of well-known carbamate and organophosphorus insecticides as inhibitors. The results (Table 2) demonstrate that the nematode enzyme is extremely insensitive to many inhibitors when compared with the better known types of cholinesterases from mammals. Even eserine which is a potent inhibitor of most cholinesterases in vitro requires a concentration of approximately 10^{-4} M to inhibit the enzyme by 50%. Only paraoxon resulted in inhibition values comparable to those obtained with the two other enzymes.

It is too early to conclude from the first studies on the cholinesterase of a plant nematode species that the entire group of these organisms contains a type of enzyme unique with regard to substrate specificity and inhibition properties. The present findings, however, may well initiate further investigations on additional species, since a modified enzyme often constitutes a target for more selective control agents.

[3] G. Voss, in *Advances in Automated Analysis* (Technicon Int. Congr., Chicago 1969; Mediad Inc., White Plains, N. Y. 1970).

Activity of Two Components of Serum Ribonuclease under Conditions of Physical Exercise

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Summary. Serum ribonuclease activity before and after physical exercise in healthy persons was estimated. It is found that a work load of 6000 kgm/5 min increased ribonuclease activity measured at pH 8.5 and decreased the activity of the same enzyme measured at pH 7.0 in the presence of $ZnSO_4$. The observed changes were more pronounced in untrained than in trained persons.

It is commonly known that physical activity causes a variety of biochemical changes in the blood. For example, it has been demonstrated that the activity of several enzymes in the blood serum was affected by exercise[1]. Changes in activity of AlAT, AspAT, CPK, MDH, LDH following physical exercise have been reported[2–5]. As these changes were usually less pronounced in trained than untrained subjects, the estimations of enzyme activities before and after exercise may provide information about the physical fitness of individuals.

Recently Albanese et al.[6,7] have suggested that the measurement of serum ribonuclease activity before and after exercise may indicate to what extent an organism

[1] The abbreviations used are: AlAT, alanine aminotransferase; AspAT, aspartate aminotransferase; CPK, creatine phosphokinase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; RNA, ribonucleic acid; RNAase, ribonuclease.

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Effect of short term submaximal work on Serum ribonuclease activity measured at different pH

	RNAase activity pH 8.5			RNAase activity pH 7.0			Heart rate		
	Before exercise	After exercise	P	Before exercise	After exercise	P	Before exercise (min^{-1})	After exercise (5 min^{-1})	P
Control ($n = 20$)	35.9 ± 6.5	50.3 ± 6.7	< 0.001	11.9 ± 1.7	8.2 ± 1.2	< 0.001	85 ± 12	781 ± 32	< 0.001
Athletes ($n = 20$)	36.3 ± 7.0 [a]	39.8 ± 5.9	n.s.	9.9 ± 1.6 [b]	8.9 ± 1.4	n.s.	68 ± 6 [b]	640 ± 38	< 0.001

Enzyme activity is expressed as units per ml serum. One unit corresponds to the amount of enzyme activity which the absorption for 0.1 unit of absorption.
All values are means $\pm$ SEM. Statistical significance was analyzed using Students t-test for mean values before and after exercise. Where p is greater than 0.05 the values are quoted as n.s. (not significant). [a] Difference of values against control not significant. [b] Difference ($p < 0.001$).

is adapted to physical work. Following this suggestion, serum ribonuclease activities before and after exercise in both trained and untrained male subjects were examined and compared.

Materials and methods. Serum ribonuclease activity was studied in 20 healthy untrained subjects aged 18–22 (control group) and 20 trained athletes of the same age (trained group).

Blood samples (400 µl) were drawn from capillaries of the finger before and immediately after exercise. The work load consisted of 1200 kgm/min during 5 min of continuous work on a bicycle ergometer. In addition, the heart rates were noted during the 5 min of exercise. The blood was centrifuged and the serum obtained was used for estimations of ribonuclease activity. Control estimations of protein content in serum before and after exercise showed that there was no change in protein concentration.

Ribonuclease activity was estimated according to the procedures described by ROTH[8] and modified by BARDOŃ et al.[9].

Estimation of ribonuclease activity at pH 8.5. Ribonucleic acid (RNA highly polymerized from yeast, BDH Chemicals, England) was dissolved in water in a concentration of 1 mg/ml. 20 µl of this substrate solution was mixed with 100 µl of DAVIES[10] pH 8.5 buffer and supplemented with 75 µl of water. The reaction was initiated by addition of 5 µl of serum, and allowed to continue for 30 min at 37 °C.

Estimation of ribonuclease activity at pH 7.0. 20 µl of substrate solution was added into 100 µl of DAVIES buffer pH 7.0 supplemented with 20 µl of 0.01 M $ZnSO_4$ and 40 µl of water.

The reaction was started by addition of 20 µl of serum and incubation continued for 30 min at 37 °C. The reaction was stopped by addition of 200 µl of precipitating reagents containing 1 M HCl in 76% ethanol. The samples were kept at 0 °C for 30 min, centrifuged and absorptions in supernatants were read at 260 nm using a Unicam SP 500 Spectrophotometer. Samples in which precipitating reagent was at zero time were used as a blank.

Results. The Table shows the effect of short term submaximal work on serum ribonuclease activity measured at different pH. It can be seen that at pH 8.5 in untrained subjects the activity of this enzyme significantly increased 40.1%, whereas in the group of athletes, the same work load increased enzyme activity only 9.6%. It can be noticed that the heart rates measured during 5 min of exercise were faster in the group of untrained individuals.

When the same enzyme activity was measured at pH 7.0 and in the presence of $ZnSO_4$[11,12], the direction of changes in ribonuclease activity after exercise was opposite to that observed at pH 8.5 and in the absence of $ZnSO_4$. It can be seen that exercise decreased serum ribonuclease activity and that the decrease observed was more pronounced in the group of untrained individuals. The average decrease in untrained individuals was 31.1%, whereas in the group of athletes exercise lowered the ribonuclease activity only 10.1%.

Discussion. Blood serum shows ribonucleolitic activity which is dependent on the presence of enzymes originating mainly from 2 sources: a) ribonuclease [E.C.3.1.4.16] from pancreas, showing a maximal activity at pH 8.5 and sensitive to $ZnSO_4$[11,12], b) ribonuclease from other tissues, mainly from spleen and liver, which shows a maximal activity at pH 7.0 and which is insensitive to $ZnSO_4$.

Data presented in this paper indicate that the activity of the pH 8.5 enzyme was raised by exercise, whereas at pH 7.0 enzyme activity (estimated in the presence of $ZnSO_4$) declined under the same conditions. The direction of changes in enzyme activities was the same in untrained and trained individuals, but in untrained subjects changes in enzyme activities were more pronounced than in athletes. Moreover, it would appear from these data that changes in serum RNAase activity at pH 8.5 after exercise were associated with large increases in heart rates. Assuming that the changes in serum enzyme activities are symptoms of metabolic disorders caused by physical exercise, it can be concluded that the degree of this disorder is less pronounced in subjects who are adapted to work by training. In addition, it is of interest that depending on their origin, enzymes catalyzing the same reaction may increase or decrease their activity at the same time under conditions of physical exercise.

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