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SHORT COMMUNICATIONS

ACTIVITIES OF SOME ENZYMES IN THE PERIENTERIC FLUID OF ASCARIS SUUM

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Abstract. Activities of 12 enzymes (amylase, lipase, cholinesterase, nonspecific carboxyl esterase, lactate dehydrogenase (LDH), alkaline phosphatase, glutamate-oxalacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), γ-glutamyl transferase (γ-GT), leucine aminopeptidase (LAP), malate dehydrogenase (MDH) and peroxidase) were determined in the perienteric fluid and homogenate of *Ascaris suum*. With the exception of amylase, all activities were higher in the homogenate than in the perienteric fluid. The enzyme activities in the perienteric fluid were then compared with those in the human serum. Comparable activities were demonstrated for LDH, LAP, lipase and alkaline phosphatase, markedly higher activities in perienteric fluid were demonstrated for MDH, GOT, GPT and amylase, and much lower for cholinesterase. No γ-GT activity was detected in the perienteric fluid.

It is known that the perienteric fluid of *Ascaris* contains some enzymes. Lipase and carboxyl esterase were determined by Tan and Zam (1973), LAP by Rhodes et al. (1966) and some enzymes including amylase, lipase and phosphatase by Cavier (1951). The aim of this study was to compare the enzyme activities in the perienteric fluid with those in the homogenate of the whole body of *A. suum* and to compare the enzyme composition in the perienteric fluid of *A. suum* with that in the serum of higher animals, in this case human serum. For this purpose it is necessary to know the activity expressed quantitatively and to compare it with the total enzyme activity in the parasite demonstrated by the same method. Since the above authors studied the enzyme activity in the perienteric fluid only, and in some cases (Cavier 1951) the initial enzyme activity was not given, a quantitative evaluation could not be made. The enzymes and methods used in this study were chosen with regard to a possible comparison of the values obtained with the reference values given for human serum.

MATERIAL AND METHODS

Adult specimens of *A. suum* were collected at the abattoir at České Budějovice. They were thoroughly washed in saline and processed within 1 h after collection. The perienteric fluid was obtained from adult females in the following manner. The worms were hanged upright, carefully cut in the lowest part of body and the fluid was left to flow out for 10 min. The perienteric fluid was then centrifuged at 23 000 g for 10 min at 4 °C. The homogenate was prepared from female bodies homogenized by a MSE knife homogenizer (cca 8 000—10 000 rpm) for 2 × 2 min. The homogenization was carried out in PBS (30 g of worm body + 100 ml of PBS). The homogenate was then centrifuged under the same conditions as the perienteric fluid. The proteins were demonstrated by biuret method after Davídek (1977).

Demonstration of enzyme activities. The activities of individual enzymes were demonstrated by the following methods: amylase — Spofa test (Slovakofarma, Hlohovec) using starch with covalently bound stain at 37 °C, incubation time 15 min; lipase — the method after Tan and Zam (1973) using

Table 1. Enzyme activities in perienteric fluid and homogenate of *A. suum;* comparison with values given for human serum

Enzyme	Perienteric fluid activity	Homogenate activity	Human serum activity
Amylase	0.267 nkat/mg 11 417 nkat/l	0.0917 nkat/mg	0.0167—0.071 nkat/mg 1167—5000 nkat/l
Lipase	0.073 nkat/mg 3133 nkat/l	0.478 nkat/mg	< 0.033 nkat/mg < 2333 nkat/l
Cholinesterase	0.0283 nkat/mg 1217 nkat/l	0.1033 nkat/mg	0.452—0.905 nkat/mg 31 667—63 333 nkat/l
Nonspecific carboxyl esterase	0.3167 nkat/mg 13 367 nkat/l	1.383 nkat/mg	—
LDH	0.0417 nkat/mg 1800 nkat/l	0.463 nkat/mg	0.0095—0.038 nkat/mg 666.7—2666.7 nkat/l
Alkaline phosphatase	0.00467 nkat mg 200 nkat/l	0.0325 nkat mg	0.009 nkat/mg 633.3 nkat/l
GOT	0.0265 nkat/mg 1125 nkat/l	0.0766 nkat/mg	0.00086—0.002 nkat/mg 60—140 nkat/l
GPT	0.0367 nkat/mg 1550 nkat/l	0.162 nkat/mg	0.00086—0.002 nkat/mg 60—140 nkat/l
γ-GT	0 nkat/mg	0.0533 nkat/mg	0.0024—0.025 nkat/mg 167—1767 nkat/l
LAP	0.00204 nkat/mg 86.7 nkat/l	0.092 nkat/mg	0.0019—0.0052 nkat/mg 133—367 nkat/l
MDH	0.8 nkat/mg 34 333 nkat/l	10.18 nkat/mg	0.011—0.023 nkat/mg 800—1633 nkat/l
Peroxidase	0.175 nkat/mg 7438 nkat/l	1.47 nkat/mg	—

tributyrin as substrate, pH 7.4, incubation time 40 min; cholinesterase — Test-Fibel (1976) (Boehringer) using acetylcholiniodide as substrate; nonspecific carboxyl esterase — the method after Němec et al. (1969) using p-nitrophenylacetate and 405 nm wave length instead of 394 nm for absorbance measuring; LDH, alkaline phosphatase, GOT, GPT and γ-GT — Bio/la test (Lachema); LAP — Test-Fibel (1976) (Boehringer) using L-leucin p-nitroanilid as substrate, resulting concentration 0.1 M phosphate buffer, pH 7.2, 0.8 mM substrate; MDH — the method after Rotmans (1978) using pH 10.4 (which was found to be optimum) instead of pH 10.0; peroxidase — the method after Davídek (1977). In all cases the enzyme activities were expressed in nanokatals. The peroxidase activity was expressed in relation to O_2. The activities were counted for mg of protein or liter of biological material.

RESULTS AND DISCUSSION

The results are summarized in Table 1.

Protein content in perienteric fluid was determined for 42.5 ± 2.64 mg/ml perienteric fluid (5 determinations). Since the protein level was almost even, the mean value was used for calculating enzyme activity for mg of protein. In the homogenate the protein level ranged from 10 to 20 mg/ml and the activities were related to individually found amounts of proteins.

The reference values for human serum for amylase, alkaline phosphatase, GOT, GPT, γ-GT and LDH were taken from the data given by the producers of these sets (i.e., Spofa for amylase and Lachema for other sets). The activities of lipase, LAP and cholinesterase were taken from Test-Fibel (1976). The MDH activity was given by Holeček et al. (1979). The value 70 mg protein/ml serum was used for counting the activity for mg of serum proteins.

A comparison of the enzyme activities in the perienteric fluid with those in the homogenate showed that only in the case of amylase the activity was higher in the perienteric fluid than in the homogenate; in all other cases the activities in the perienteric fluid were lower. Rather high activities in the perienteric fluid were demonstrated for amylase, MDH, nonspecific carboxyl esterase and peroxidase.

A comparison of the perienteric fluid with the serum revealed that the LDH and LAP activities were comparable, lipase activity was somewhat higher in perienteric fluid and alkaline phosphatase was less active in perienteric fluid than in serum, but even this value was not very different. A markedly higher degree of activity in perienteric fluid was demonstrated for MDH, GOT, GPT and amylase and lower activity for cholinesterase. No γ-GT activity was detected. Consequently, some of the enzyme activities in the perienteric fluid were comparable with those in the serum, others were different.

The results obtained indicate that the enzyme activities in the perienteric fluid of *A. suum* are mostly low and due to the fact that there is only a small amount of perienteric fluid in the worm, the enzyme activities in the perienteric fluid represent only a very small portion of the total enzyme activity in the parasite. It may be therefore considered that these activities, like most of enzyme activities in the serum of higher animals, result only from natural disintegration of cells and release of enzymes from their contents and do not play any important role in the metabolism.

These studies on the enzyme activities in perienteric fluid and homogenate of *A. suum* and quantitative evaluation of the results will serve as a basis for further studies dealing with isolation and characterization of enzymes, including studies on their antigenic properties.

АКТИВНОСТЬ НЕКОТОРЫХ ФЕРМЕНТОВ В ОКОЛОКИШЕЧНОЙ ЖИДКОСТИ *ASCARIS SUUM*

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Резюме. Изучали активность 12 ферментов (амилазы, липазы, холинэстеразы, неспецифической карбоксилэстеразы, лактатдегидрогеназы (ЛДГ), щелочной фосфатазы, глутамат-оксалацетат-трансаминазы (ГОТ), глутамат-пируват-трансаминазы (ГПТ), γ-глутамил трансферазы (γ-ГТ), лейцинаминопептидазы (ЛАП), малатдегидрогеназы (МДГ) и пероксидазы) в околокишечной жидкости и гомогенате *Ascaris suum*. Кроме амилазы, все другие активности были выше в гомогенате, чем в околокишечной жидкости. Активности ферментов в околокишечной жидкости сравнивали с активностями ферментов в сыворотке человека. Сравнительные активности доказаны для ЛДГ, ЛАП, липазы и щелочной фос-

фатазы, значительно высшие активности в околокишечной жидкости обнаружены для МДГ, ГОТ, ГПТ и амилазы и более низкие для холинэстеразы. Активность γ-ГТ в околокишечной жидкости не выявлена.

REFERENCES

CAVIER R., L'équipement enzymatique du liquide coelomique de l'Ascaris du porc, *Ascaris lumbricoides*, Linné 1758. Bull. Soc. Chim. Biol. 33: 1391—1399, 1951.

DAVÍDEK J., Laboratory manual for food analysis. SNTL, Praha, 720 pp., 1977. (In Czech.)

HOLEČEK V., RACEK J., SLABÝ P., Clinical enzymology. SPN, Praha, 127 pp., 1979. (In Czech.)

NĚMEC V., SLÁMA K., HRUBEŠOVÁ H., Effect of hormones on nonspecific esterase activity in adult *Pyrrhocoris apterus* (L.) (Heteroptera). Acta entomol. bohemoslovaca 66: 87—92, 1969.

RHODES M. B., MARSH C. L., FERGUSON D. L., Studies in helminth enzymology. V. An aminopeptidase of *Ascaris suum* which hydrolyzes L-Leucyl-beta-naphtylamid. Exp. Parasitol. 19: 42—51, 1966.

ROTMANS J. P., *Schistosoma mansoni*: Purification and characterization of malate dehydrogenases. Exp. Parasitol. 46: 31—48, 1978.

TAN P., ZAM S. G., Isolation and characterization of nonspecific esterase enzyme of *Ascaris suum* perienteric fluid. Comp. Biochem. Physiol. 46B: 673—682, 1973.

Test-Fibel, Boehringer Mannheim GmbH, 1976.

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N. S. Motavkina (Ed.): Vliyanie khozyaystvennoy deyatel'nosti na strukturu prirodnykh ochagov kleshchevogo entsefalita v Primorskom kraye. (Influence of economic activities on the structure of the tick-borne encephalitis natural foci in the Primorye Territory.) *Dalnevostochn. nauchn. centr AN SSSR, Vladivostok 1982, 135 pp., 14 Figs., 29 Tables. Price 1.20 R.*

Human influence on nature involves great changes of environment which are also reflected in the character of diseases with natural foci. The literature devoted to these problems is not very numerous, although they are very pressing problems. The present monograph consists of 12 papers written by scientists of the Laboratory of Medical Geography of the Far East Scientific Centre of the USSR Academy of Sciences, summarizing the results obtained in their studies conducted in the model region of the Primorye Territory. Analyzed are the forest microclimate and its influence on the structure of tick population; the fauna of rodents, insectivores, birds and their role in the local natural foci of tick-borne encephalitis; the anthropogenic effects on ticks, rodents, birds and virus circulation; some epidemiological peculiarities of this disease in the given region and its clinics.

The publication contains many stimulative materials for similar studies in other parts of the area where tick-borne encephalitis occurs. It will surely find a keen response among parasitologists, zoologists, physicians, epidemiologists and geographers for whom it is intended.

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