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PHOSPHATASE ACTIVITY IN THE LYMPH DURING THE FEBRILE REACTION

M. M. Minnebaev, F. I. Mukhutdinova, and L. G. Popova

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In previous investigations we showed that the enzyme composition of the lymph in various pathologies (shock, allergy, terminal states, postresuscitation period, inflammation) reflects changes in cell membrane permeability and the degree and depth of cellular injuries more accurately than blood enzyme levels. On the other hand, we know that the febrile reaction (FR) is accompanied by considerable changes in enzyme activity in various organs and tissues and in the blood serum. Meanwhile the character of changes in enzyme activity in lymph flowing from different organs and regions of the body and their comparison with changes in blood enzyme activity during FR have not been studied.

We accordingly undertook a comparative investigation of activity of acid (AcP) and alkaline (AlP) phosphatases and of AlP isozymes in lymph and blood in the course of FR of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 64 chinchilla rabbits weighing 2.5-4.2 kg. FR was produced by injecting pyrogenal by the method described previously [8]. Animals receiving an injection of pyrogen-free physiological saline, made up in bidistilled water, served as the control. Lymph for determination of activity of AcP [11], AlP [10], and its isozymes — isolated from liver (AlP_{liv}), intestine (AlP_{int}), and bone (AlP_{bone}) was obtained from the thoracic lymph duct (TLD) and the hepatic lymphatic trunk, while blood for investigation was taken from the femoral vein. The experimental results were subjected to statistical analysis. The animals were killed humanely by injection of a lethal dose of general anesthetic.

EXPERIMENTAL RESULTS

The investigations showed (Tables 1-3) increased activity of total AlP in both types of lymph in the course of FR. With an increase in the duration of fever, the degree of activation of the enzymes increased, although the degree of increase of AlP activity in lymph flowing from the liver was lower than in lymph from TLD. The degree of the increase in the total AlP level in the blood was less, and its normal level was regained sooner, than in lymph (5-5.5 and 10 days after 1 and 3 injections respectively of pyrogenal).

The AlP isozyme spectrum in the biological fluids changed on the whole similarly to total activity of the enzyme. However, the increase in activity of the intestinal isozyme in the lymph was greater than that of the liver and bone isozymes. Meanwhile, whereas an increase in the content of AlP_{liv} and AlP_{int} in lymph from TLD was observed at all times of investigation, the AlP_{bone} level in the late stages after 3 and 5 injections of pyrogenal returned close to its original values. The increase in the content of individual isozymes in the blood was inconstant and was less than in the lymph.

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TABLE 1. Activity of Acid Phosphatase (in mmoles/liter·sec) and Alkaline Phosphatase and its Isozymes (in μmoles/liter·sec) in Lymph from TLD of Rabbits during FR ($M \pm m$, $n = 7$)

Name of enzymes	Control animals	Injections of pyrogenal							
		one		three		five		ten	
		after 2.5-3 h	after 5.0-5.5 h	4th day	6th day	10th day	6th day	10th day	11th day
Acid phosphatase	36,49±5,18	65,96±9,02*	63,10±7,22*	118,61±10,94*	102,50±12,07*	48,65±4,83	112,74±10,14*	90,35±9,85*	193,01±15,04*
Alkaline phosphatase (total)	0,35±0,03	0,60±0,05	0,76±0,04*	1,09±0,06*	1,19±0,08*	0,65±0,05*	1,76±0,15*	0,70±0,05*	1,80±0,18*
AlP_{liv}	0,29±0,03	0,44±0,05*	0,58±0,03*	0,85±0,05*	0,79±0,06*	0,46±0,06*	1,25±0,13*	0,54±0,06*	1,19±0,13*
AlP_{int}	0,03±0,01	0,09±0,01*	0,13±0,02*	0,17±0,01*	0,29±0,03*	0,15±0,02*	0,42±0,03*	0,11±0,03*	0,45±0,05*
AlP_{bone}	0,03±0,01	0,08±0,01*	0,05±0,01	0,07±0,02*	0,11±0,02*	0,04±0,01	0,09±0,01*	0,05±0,02	0,16±0,03*

Legend. Here and in Tables 2 and 3, $*p < 0.05$.

TABLE 2. Activity of Acid Phosphatase (in mmoles/liter·sec) and Alkaline Phosphatase and its Isozymes (in μmoles/liter·sec) in Hepatic Lymph of Rabbits during FR ($M \pm m$, $n = 5$-8)

Name of enzymes	Control animals	Injections of pyrogenal							
		after 2.5-3 h	after 5.0-5.5 h	4th day	6th day	10th day	6th day	10th day	11th day
		one		three		five		ten	
Acid phosphatase	44,82±7,40	64,96±6,82*	83,35±10,50*	99,85±15,39*	94,60±12,84*	40,56±6,60	146,58±7,84*	97,88±6,07*	175,46±17,44*
Alkaline phosphatase(total)	0,44±0,03	0,70±0,03*	0,66±0,04*	1,00±0,07*	1,07±0,04*	0,71±0,06*	1,38±0,09*	0,79±0,07*	1,88±0,31*
AlP_{liv}	0,36±0,03	0,58±0,03*	0,50±0,04*	0,70±0,04*	0,75±0,04*	0,49±0,04*	0,91±0,03*	0,52±0,08*	1,25±0,30*
AlP_{int}	0,05±0,01	0,05±0,01	0,11±0,01*	0,17±0,03*	0,23±0,02*	0,14±0,03*	0,38±0,07*	0,22±0,03*	0,49±0,06*
AlP_{bone}	0,03±0,01	0,07±0,01*	0,04±0,01	0,07±0,01*	0,07±0,01*	0,08±0,02*	0,11±0,03*	0,05±0,02*	0,12±0,02*

TABLE 3. Activity of Acid Phosphatase (in mmoles/liter·sec) and Alkaline Phosphatase and its Isozymes (in μmoles/liter·sec) in Blood Serum from Femoral Vein of Rabbits during FR ($M \pm m$, $n = 7$)

Name of enzymes	Control animals	Injections of pyrogenal							Ten
		one		three			five		
		after 2.5-3 h	after 5.0-5.5 h	4th day	6th day	10th day	6th day	10th day	11th day
Acid phosphatase	38,56±3,22	62,63±6,10*	77,68±10,57*	195,61±23,34*	160,34±15,50*	47,15±8,44	226,47±19,29*	232,67±23,07*	218,83±24,49*
Alkaline phosphatase (total)	1,14±0,08	1,74±0,19*	1,46±0,23	1,66±0,17*	2,13±0,17*	0,93±0,11	2,47±0,16*	1,99±0,14*	1,89±0,25*
AlP_{liv}	0,82±0,06	1,25±0,18*	0,91±0,16	1,25±0,15***	1,21±0,07*	0,61±0,09	1,70±0,17*	1,29±0,11*	1,04±0,18
AlP_{int}	0,26±0,07	0,40±0,03*	0,39±0,09*	0,32±0,04	0,81±0,11*	0,28±0,04	0,64±0,04*	0,59±0,06*	0,42±0,06*
AlP_{bone}	0,07±0,02	0,09±0,02	0,17±0,03*	0,09±0,02	0,12±0,02	0,04±0,01	0,14±0,02*	0,11±0,04	0,42±0,07*

The degree of activation of AcP in lymph and blood during FR was similar to that of AlP, but by the 10th day after the 3rd injection of pyrogenal its level in all fluids had returned to the original values.

Thus FR was accompanied by increased phosphatase activity in the body fluids. However, in lymph from TLD and hepatic lymph their changes were more marked in magnitude and duration than in blood.

We suggest that the increase in AlP activity in the body fluids during FR is evidence of damage to cell membrane structures. This view is in agreement with the important role which this enzyme is considered to play in functions of cell membranes and membranes of cell organelles [9, 12]. Meanwhile, activation of the sympathico-adrenal system observed during FR [3] may probably also lead to an increase in the AlP content in the body fluids. On the other hand, we know that adrenalin causes marked stimulation of AlP activity in the liver and its release from the blood cells and vascular endothelium [1, 5].

The earlier and more marked changes in activity of lysosomal enzymes in the lymph than in the blood can also be explained on the grounds that tissues of the intestine and liver are distinguished by a high content of lysosomes. On the other hand, during FR labilization of lysosomal membranes is observed, with release of their enzymes into the cell cytoplasm and extracellular space [6, 13], and this may evidently be another important factor in the cell damage and changes in the microcirculation in the splanchnic bed and the liver parenchyme. Finally, we know that high-molecular-weight compounds as a rule are

resorbed from the intercellular connective-tissue spaces by lymphatic capillaries, and that they subsequently pass through the thoracic duct into the general bloodstream.

The definite "normalizing effect" of glucocorticoids and *c*AMP, increased biosynthesis of which is observed during FR [4, 7], on increased permeability of extra-, and intracellular membranes must also be noted. It is evidently the relationship between these factors (i.e., the degree of "aggregation" of the factors of membrane labilization, and on the other hand, the degree of intensity of factors stabilizing them) which ultimately determines the intensity of release of lysosomal enzymes during FR. The raised level of lysosomal enzymes in the body fluids shows that the absolute and relative involvement of these pathogenetic factors during FR is shifted considerably in favor of membrane labilizing factors, and that depending on the duration of FR, this effect is more marked still.

The results thus indicate that an important role in the transport of phosphatases released from the tissues into the general circulation is played by the lymphatic system, whose resorption and transport functions largely determine the dynamics and level of their changes in the blood during the febrile reaction.

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