

CYTOCHEMICAL INVESTIGATION OF THE ALKALINE  
PHOSPHATASE ACTIVITY OF THE LEUKOCYTES  
AFTER INJECTION OF PYROGENAL

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The alkaline phosphatase activity of the neutrophilic leukocytes is an accurate, although nonspecific, indicator of the functional state of these cells [6, 10, 12-14, 21-23, 30].

Because of the known action of the new therapeutic preparation pyrogenal (bacterial lipopolysaccharide) on the number of neutrophilic leukocytes in the peripheral blood and on their phagocytic function [1, 2, 4, 5, 7, 9] the effect of pyrogenal on the alkaline phosphatase activity of the leukocytes was investigated.

EXPERIMENTAL METHOD

A single intravenous or intramuscular injection of pyrogenal was given in doses of between 12 and 50  $\mu\text{g}$  to volunteers and also to patients with schizophrenia receiving pyrogenal for therapeutic purposes [3]. All the subjects were physically healthy, and showed no signs on hematological diseases. The alkaline phosphatase activity was investigated by means of the azo-coupling reaction using a technique described earlier [14]. The activity of the enzyme was estimated quantitatively by calculating the index of phosphatase activity of the neutrophils (PAN) as described by Kaplow [20], counting only the neutrophils with segmented nuclei. The estimation of the total number of leukocytes and the study of the leukocyte formula were carried out by the usual method [11]. A statistical analysis was made of the numerical results [8].

EXPERIMENTAL RESULTS

After intramuscular injection of pyrogenal into the healthy subjects the PAN index ( $M \pm m$ ) after 3 h was  $20.0 \pm 9.2$  ( $P > 0.05$ ), after 24 h it was  $40.0 \pm 9.7$  ( $P < 0.05$ ), and after 72 h  $59.6 \pm 11.9$  ( $P < 0.05$ ). Before injection of pyrogenal the value of this index was  $16.0 \pm 3.6$ .

The PAN index in a healthy woman 6 h after intravenous injection of 33  $\mu\text{g}$  pyrogenal was 2, after 24 h - 195, after 4 days - 317, after 5 days - 265, after 6 days - 144, after 7 days - 57, and after 8 days - 8; before injection of pyrogenal the PAN index was 11.

The changes in the PAN after intravenous injection of pyrogenal into the patients are shown in the table.

The following phases could be distinguished in the changes of the PAN under the influence of pyrogenal: a fall and maintenance of the low level of this index during the first 6 h after injection, a rise and maintenance of its high level 1-4 days after injection, and a return to the normal value of the PAN 4-8 days after injection.

A significant ( $P < 0.05$ ) decrease in PAN accompanied the leukopenia (neutropenia), attributable to deposition of the polymorphonuclear neutrophils in the capillaries of the internal organs. The significant decrease in the value of PAN in this time interval indicated that at this stage mostly the polymorphonuclear neutrophils exhibiting alkaline phosphatase activity disappeared from the circulation. The leukocytosis (neutrophilia) observed 3 h after the injection evidently took place as a result of release of the cell reserves located intravascularly in the sinuses of the bone marrow and consisting of mature and not completely mature cells, [16]. The low PAN level 3-6 h after the injection may be explained by the continuing disappearance of the polymorphonuclear neutrophils containing alkaline phosphatase from the circulation and also by the arrival from the bone marrow of cells not exhibiting activity of this enzyme.

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Changes in Phosphatase Activity of Neutrophils after Intravenous Injection of Pyrogenal into 5 Patients with Schizophrenia

Time of investigation	Index	Dose of pyrogenal (in $\mu\text{g}$ )		
		50	25	12
Before injection	$M \pm m$	19,2 $\pm$ 7,7	37,0 $\pm$ 9,4	30,4 $\pm$ 13,8
1 h after injection	$M \pm m$	—	21,0 $\pm$ 9,2	14,6 $\pm$ 4,9
	$P_1$		<0,05*	>0,05
“ 3 h	$M \pm m$	—	6,0 $\pm$ 3,1	7,8 $\pm$ 3,8
	$P_1$		<0,05*	>0,05
	$P_2$		>0,05	
“ 6 h	$M \pm m$	0	7,5 $\pm$ 4,5	5,8 $\pm$ 2,9
	$P_1$	<0,05	<0,05*	>0,05
	$P_2$		>0,05	
“ 1 day	$M \pm m$	76,0 $\pm$ 23,7	87,0 $\pm$ 12,3	64,0 $\pm$ 12,7
	$P_1$	<0,05	<0,01*	>0,05
	$P_2$		<0,01	
“ 2 days	$M \pm m$	189,4 $\pm$ 74,4	172,0 $\pm$ 30,8	67,0 $\pm$ 17,6
	$P_1$	<0,05	<0,01*	>0,05
	$P_2$	>0,05	<0,05	
“ 3 “	$M \pm m$	115,6 $\pm$ 39,9	186,0 $\pm$ 32,6	78,5 $\pm$ 22,6
	$P_1$	<0,05	<0,01*	>0,05
	$P_2$	>0,05	>0,05	
“ 4 “	$M \pm m$	85,2 $\pm$ 19,1	132,0 $\pm$ 17,2	—
	$P_1$	<0,05	<0,01	
	$P_2$	>0,05	>0,05	
“ 5 “	$M \pm m$	40,0 $\pm$ 13,5	32,0 $\pm$ 6,4	26,5 $\pm$ 12
	$P_1$	<0,05	>0,05*	>0,05
	$P_2$	>0,05	<0,01	

Note.  $P_1$ —Significance of difference by comparison with original value.  $P_2$ —Significance of difference by comparison with preceding level.

\* Significance calculated by the method of direct differences.

The results obtained show that an inverse relationship exists between the PAN and the leukocyte (granulocyte) count, although at some moments, for example after 24 h, a high PAN and a high leukocytosis were observed simultaneously. Pyrogenal acted longer on the PAN than on the leukocyte count in the peripheral blood.

The length of stay of the granulocytes in the circulation is known to vary from 8 to 30 h [16, 17, 25], while the duration of medullary hemopoiesis (propagation, maturation, and release) is 96-144 h, and during a natural bacterial infection the duration of hemopoiesis is shortened to 48 h. The question whether the PAN may be increased after the cells have been released from the bone marrow has not been settled. Some authors [21, 27] consider that the PAN continues to rise after the cells have entered the circulation. Others [24, 28] consider that the PAN is determined during maturation of the neutrophils in the bone marrow.

The gradual increase in the PAN of the peripheral blood in the first, second, and third days after injection of pyrogenal shows that the increase in the alkaline phosphatase activity of the neutrophils is associated with the arrival of cells rich in alkaline phosphatase from the bone marrow. If in fact pyrogenal caused an increase in the activity of the neutrophils already in the blood stream, an immediate rise in activity would be observed, followed by a stepwise fall in the PAN, reflecting the disappearance of cells rich in alkaline phosphatase from the circulation.

The authors have suggested earlier [12] that cytochemically detected alkaline phosphatase activity is dependent on the amount of enzyme in the cell, and this in turn is determined by the intensity of its synthesis. The presence of a well developed ergastoplasm in the immature bone-marrow cells of the granulocyte series and its absence in the mature polymorphonuclear neutrophils [22] suggests that the synthesis of alkaline phosphatase takes place during one stage of formation of the neutrophils in the bone marrow. Evidently a single injection of pyrogenal directly or indirectly changes the metabolism of the cells of the neutrophil series being formed at that moment in the bone marrow, and this gives rise to intensified alkaline phosphatase synthesis. In accordance with modern ideas of the regulation of enzyme synthesis [19], it must be assumed that in these circumstances the induction or repression of synthesis of alkaline phosphatase takes place.

#### SUMMARY

A cytochemical method of azocombination was used to study the activity of alkaline phosphatase of human leukocytes after injection of pyrogenal (bacterial lipopolysaccharide). Intravenous injection of pyrogenal at first

causes a decline and then an increase of the phosphatase activity of neutrophils attaining a maximum on the 3rd-4th day, after which the activity returns to normal. No direct relationship has been discovered between the phosphatase activity of neutrophils and the number of these cells in the peripheral blood. It is presumed that the growth of the phosphatase activity of neutrophils of the peripheral blood is connected with supply of cells rich in alkaline phosphatase from the bone marrow.

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