CHANGES IN PHOSPHATASE ACTIVITY IN CIRCULATING BLOOD LEUKOCYTES OF MICE WITH EXPERIMENTAL AMYLOIDOSIS

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During development of experimental amyloidosis in albino mice the number of blood leukocytes containing acid phosphatase increases. The number of neutrophils with phosphatase activity increases even before the development of amyloidosis. The number of lymphocytes containing this enzyme increases parallel with the degree of development of amyloidosis.

Acid and alkaline phosphatases play an important role in synthesis and tissue utilization of mucopolysaccharides and protein [8]. Since amyloid is a mucoglucoprotein [1, 3, 4, 6], it is interesting to study these enzymes at various periods of development of experimental amyloidosis.

In the investigation described below, activity of acid and alkaline phosphatases was investigated in circulating blood leukocytes of healthy albino mice and mice developing amyloidosis.

EXPERIMENTAL METHOD

Experiments were carried out on 40 mice. Phosphatases were determined in all mice before the beginning of the experiment, after which 10 mice were sacrificed (to study the initial histological picture of the liver, spleen, and kidneys), while amyloidosis was produced in 30 mice by injection of casein [2].

Alkaline phosphatase was determined by Kaplow's method [7] and acid phosphatase by the method of Goldberg and Barka [5]. No descriptions of the last method, which is the most specific, could be found in the accessible Soviet literature, so that it will be briefly described below. Blood films are made on carefully defatted slides and immediately fixed for 30 sec in 10% formalin solution in 96 or 100° methyl or ethyl alcohol at 0-5°C, well rinsed with distilled water, and dried. Immediately after drying, the films are flooded with a mixture of solutions No. 1 and No. 2. Solution No. 1 consists of 20 mg α-naphthyl monophosphate, 0.5 ml acetone, and 20 ml 0.1 N sodium acetate solution, while solution No. 2 consists of 4 drops 4% pararosaniline and 4 drops 4% sodium nitrate solution. The solutions are mixed immediately before flooding the films and are used only once. Films with the solution are incubated at 37° for not less than 4 h, after which they are well washed with distilled water and counterstained with 1% aqueous solution of methyl green for 5-10 min, well rinsed again with distilled water, and dried at room temperature. On examination under an immersion objective, acid phosphatase is revealed in granulocytes and lymphocytes as bright pink granules in the cytoplasm or as diffuse staining of the cytoplasm.

Staining for phosphatases is usually carried out immediately after blood films are taken. Keeping unstained films, including fixed films, even for as little as 24 h makes them unsuitable for investigation.

The result of determination of acid phosphatase is usually expressed in the literature as the number of cells containing the enzyme from 100 examined. However, the writer's experience suggests that it is necessary to subdivide leukocytes containing phosphatase into the following groups: 1) first degree of activity

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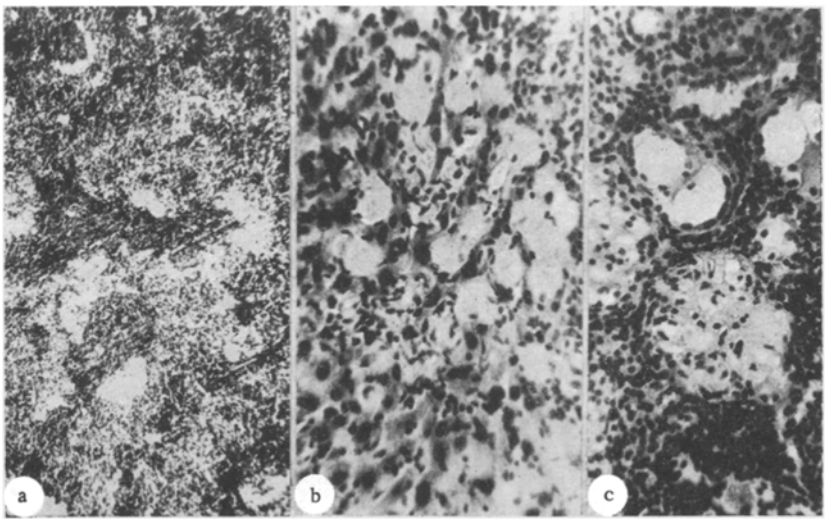

Fig. 1. Amyloidosis of mouse organs: a) spleen (+++), 56x; b) liver (++), 280×; c) kidney (+++), 280×. Hematoxylin-eosin.

TABLE 1. Degree of Visceral Amyloidosis in Mice at Different Times of the Experiment

Mouse No.	Date of investigation											
	Feb. 12 control			April 20			May 20			July 20		
	1	2	3	1	2	3	1	2	3	1	2	3
1	—	—	—	—	—	—	++	—	—	++++	++	+++
2	—	—	—	++	—	—	+++	+++	+++	+++	+	—
3	—	—	—	—	—	—	+	—	—	—	—	—
4	—	—	—	+	—	—	++	—	—	++++	+	++
5	—	—	—	—	—	—				+	+	—
6	—	—	—							+	+	—
7	—	—	—							+++	++	—
8	—	—	—							+++	+	—
9	—	—	—							++++	++	+++
10	—	—	—							+++	++	—
11										++	++	—
12										++++	+	++
13										++	++	—

Legend: 1) spleen; 2) liver; 3) kidney.

(+), 1 or 2 granules of acid phosphatase or slight focal staining of the cytoplasm; 2) second degree (++), 3 or 4 granules or staining of 1/3 of the cytoplasm; 3) third degree (+++), 3-6 granules or staining of 2/3 of the cytoplasm, and finally; 4) fourth degree of activity (++++), more than 5-6 granules or pink staining of the whole cytoplasm.

The greater objectivity of this method of assessing the results is obvious. The percentage of leukocytes containing phosphatase may be the same at the beginning and end of the experiment, but the intensity of staining of the cells will differ.

The experiment continued for 5 months: from February 12 to July 20, 1968. All mice surviving until the end of the experiment received 38 injections of casein; eight mice (Nos. 2, 3, 7, 10, 12, 15, 19, 23) died early, and their data were not included in the experimental results. Besides the initial determination, phosphatases were estimated in the experimental mice on another four occasions (April 20, May 20, June 20, and July 20), and in the healthy mice at the end of the experiment (July 20) as a control of the possible effect of the seasonal factor. The course of development of amyloidosis was checked by periodic postmortem examination of the mice.

RESULTS

Repeated investigations of blood films of mice using human and rat blood films as controls showed conclusively that alkaline phosphatase cannot be detected in circulating blood leukocytes of mice (at least not by Kaplow's method [7]). For this reason, the results of tests for acid phosphatase only will be described below.

TABLE 2. Dynamics of Acid Phosphatase in Circulating Blood Leukocytes of Mice during Development of Amyloidosis (M ± m)

Group, date of experiment, and number of animals (in parentheses)	Percentage of leukocytes containing acid phosphatase							
	neutrophils				lymphocytes			
	+	++	+++	++++	+	++	+++	++++
Control Feb. 12 (40)	0,7±0,12	0,42±0,14	Single cells	Very rarely	1,6±0,16	0,5±0,12	Single cells	Very rarely
Experiment								
April 20 (20)	1,8±0,17	0,4±0,14			1,8±0,17	0,6±0,19		
May 20 (17)	2,0±0,13	0,7±0,17			2,0±0,17	0,9±0,22		
June 20 (13)	2,5±0,17	0,7±0,2			2,5±0,2	0,7±0,19		
July 20 (13)	2,0±0,35	0,8±0,25			3,4±0,5	1,2±0,3		
Control July 20 (10)	1,2±0,11	1,0±0,1			2,0±0,14	0,8±0,09		

The data for development of amyloidosis are given in Table 1 and Fig. 1. By the end of the experiment most of the experimental mice had developed a well-marked degree of visceral amyloidosis.

Results showing changes in acid phosphatase activity in the circulating blood leukocytes of mice during the period of development of amyloidosis are summarized in Table 2. They show that acid phosphatase activity in the neutrophils, after an initial rise in the period before development of amyloidosis or in its early stage, thereafter increased only very slightly. No special changes were observed in the degree of activity, and both in the initial state and at the end of the experiment only solitary cells were seen with the third degree of activity. So far as lymphocytes are concerned, in this case the picture was somewhat different: phosphatase activity in these cells increased gradually, correlating to a definite degree with the development of amyloidosis, and by the end of the experiment it was much higher than both the initial level and the level detected in healthy mice at the same period.

These results are of considerable interest. Miller and Smith [9] showed that acid phosphatase activity increases during an increase in phagocytic activity of cells. From this point of view, it might be postulated that the increase in number of cells with acid phosphatase is associated with the onset of phagocytosis and absorption of amyloid in the mice. However, this can hardly be reconciled with the fact that the increase in number of neutrophils (i.e., of cells participating directly in phagocytosis) with acid-phosphatase activity was slight and was observed before the development of any marked degree of amyloidosis. On the other hand, it is a well-known fact that acid phosphatase plays an important role in the formation of mucopolysaccharides by liberation of essential sugars from hexose phosphates [8]. In addition, acid phosphatase plays a role in protein synthesis [10]. It can therefore be postulated that the increase in number of cells with acid-phosphatase activity, together with the predominant increase in number of lymphocytes, which are concerned with antibody production, is associated not with the absorption, but with the synthesis of amyloid.

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