

# ENZYME PROFILE OF SPLEEN CELLS IN MICE WITH AMYLOIDOSIS

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During induction of amyloidosis in mice, the number of histiocytes with a high content of some hydrolytic and oxidoreductive enzymes in the spleen follicles was increased against the background of a general increase in activity of specific phosphatases in the lymphoid cells. The amyloid masses possessed weak aminopeptidase activity.

A crucial question in the problem of amyloidosis is that of the origin of the amyloid masses. Despite the fact that no convincing evidence has yet been brought in support of the local cellular formation of amyloid [16], the role of the reticulo-endothelial system in this process cannot be denied [7, 11]. It was therefore natural to assume that the metabolism of cells participating in amyloidogenesis has certain characteristics which can be detected by histochemical tests for enzymes.\*

## EXPERIMENTAL METHOD

Amyloidosis was induced in 30 male BALB mice weighing 18-20 g by subcutaneous injections of 0.5 ml 5% casein solution in 0.25% NaOH solution 6 times a week for 5 weeks. Eighteen mice served as controls. The animals were killed in small batches every 3-4 days. Only the spleen was investigated because in the model used this organ was affected by amyloidosis before all the others. Sections were cut on a cryostat, and some material was embedded in paraffin wax. Staining with hematoxylin-eosin, methyl green-pyronine, methylviolet, thioflavine T, and Congo red was used. Activity of the following enzymes were studied histochemically: hydrolytic enzymes (nonspecific acid and alkaline phosphatases, ATPase, glucose-6-phosphatase, 5-nucleotidase, esterase, and aminopeptidase) and oxidoreductive enzymes associated with the Krebs' cycle (succinate, malate, and isocitrate dehydrogenases) with the pentose cycle (6-phosphogluconate and glucose-6-phosphate dehydrogenases), with glycolysis (lactate and  $\alpha$ -glycerophosphate dehydrogenases), with electron transport (TPN- and DPN-diaphorases), and with amino-acid synthesis (glutamate dehydrogenase). The enzymes were determined by the usual methods with slight modifications [2, 14].

## EXPERIMENTAL RESULTS

During induction of amyloidosis the spleen became considerably enlarged on account of hyperplasia of cells of the red and white pulp. The number of plasma cells, of histiocytes containing hemosiderin granules, and of megakaryocytes in the red pulp increased. Proliferation of cells of the germinal centers

\* In the course of the investigation described in this paper two reports [9, 12] were published by workers who have examined the same problem.

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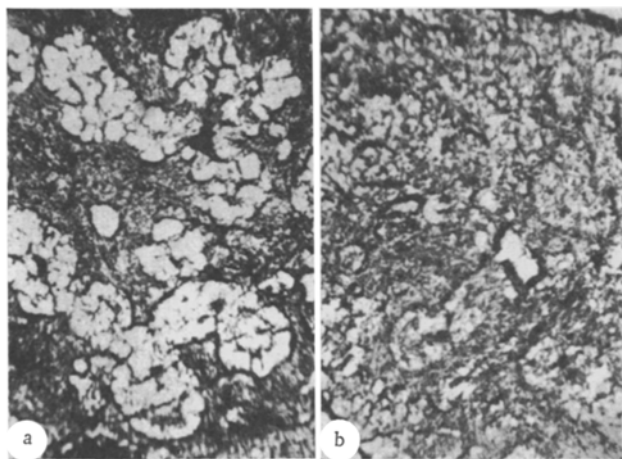


Fig. 1. Adenosine triphosphatase a) control, low enzyme activity in follicles (15  $\times$ ); b) experiment, 17 injections of casein; high enzyme activity in follicles and pulp (15  $\times$ ).

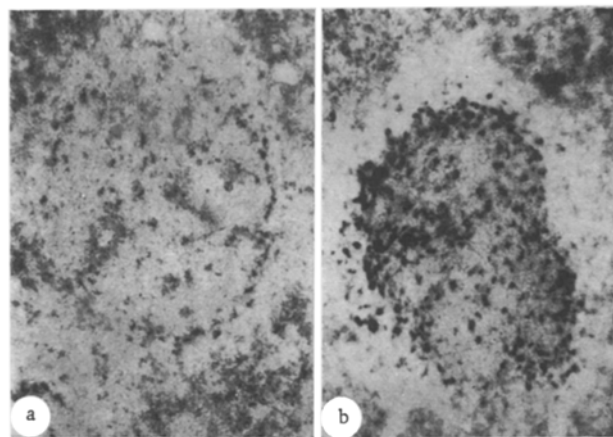


Fig. 2. Esterase: a) control, high enzyme activity in cells at periphery of follicle (100  $\times$ ); b) experiment, 23 injections of casein: deposition of amyloid in perifollicular zone, considerable increase in number of cells with high enzyme activity in follicle (15  $\times$ ).

was observed in the follicles, and groups of histocytes appeared among the lymphocytes, mainly at the periphery.

Repeated injections of casein were followed by changes in activity of nearly all the enzymes studied. There was a marked increase in the number of large histocyte-like cells containing acid phosphatase only in the follicles, whereas in the red pulp of even normal spleens, numerous polymorphs with phosphatase activity were scattered. Whereas in the control spleens the follicles, except for the fibrous structures, remained optically empty after the reaction for ATPase against the background of a generally intensive staining of the red pulp, administration of casein led to activation of the enzyme in all cells of the follicles (Fig. 1). The dynamics was similar after testing for 5-nucleotidase and glucose-6-phosphatase, the only difference being that the total activity, especially of the latter enzyme, was lower. High esterase activity was observed in the spleens of the control animals, in cells located at the periphery of the follicles. Under experimental conditions the zone of activity of the enzyme was widened, and individual histocytes migrated toward the center of the follicles (Fig. 2). In the intact mice, regardless of the level of the general reaction, the activity of all oxido-reductive enzymes were considerably increased at the periphery of the follicle, whereas in the experimental animals histocytes in the central portions of the follicles also had high activity. Deposition of amyloid was not significantly reflected in the level of activity and distribution of the enzymes. The reaction for aminopeptidase was weak in the amyloid masses, and in some places dehydrogenase activity was detected as a local accumulation of blue granules.

Unlike Western investigators [9, 12], the writers found no marked increase in the number of cells containing acid phosphatase in the red pulp of the spleen of immunized mice. High enzyme activity of the histocytes in the follicles was probably due to phagocytosis of the casein [5, 8, 18], like any other antigen [10], or especially because casein is a protein rich in phosphorus compound [13]. Casein, of course, is phagocytosed by cells of the red pulp also [9, 12], but it is difficult to identify them there without the use of special tests. Activity of acid phosphatase in the blood lymphocytes in experimental amyloidosis is also associated with phagocytosis [4].\* However, the fact cannot be ignored that the character of the activity of the lysosomal hydrolyses, even after injection of casein only, is largely explained by the method of investigation, the special features of the tissues and organs [17], and also, possibly, by metabolic features peculiar to inbred animals.

\* Macrophages rich in acid phosphatase have recently been found at the periphery of senile (amyloid?) plaques in the brain [19].

Nothing which was said above rules out the possible synthetic function of the macrophages or, more especially of the lymphocytes of the follicles with respect both to the amyloid fibrils and to the "special factor" recently found in human patients with amyloidosis and in intensively immunized animals [3, 11]. On the other hand, the presence of diformazan granules in the amyloid, together with its aminopeptidase activity, are in agreement with the results of ultrastructural investigations confirming the role of intracellular organelles and their fragments in the formation of amyloid masses [15].

In conclusion, it must be recognized that the results of the present investigation are too inadequate in number to allow exhaustive interpretation. The situation is also aggravated by the absence of histochemical characteristics clearly distinguishing the cells of the reticulo-endothelial system [6, 18].

The changes in the enzyme profile of the spleen cells which were discovered probably reflect the standard response of the organism to massive doses of protein antigen. Any search for specific features of metabolism in amyloidogenesis ought probably to be directed along the lines of studying SH-dependent enzymes, because sulhydryl groups are apparently essential for the aggregation of protein molecules into amyloid fibrils [1].

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