IMMUNOCHEMICAL AND ELECTROPHORETIC INVESTIGATION OF MULTIPLE FORMS OF ESTERASES HYDROLYZING CARBOXYLIC ACID ESTERS IN RATS

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Experiments on August rats show that their organs differ in the spectrum and relative activity of iso-forms of various types of esterases. In their composition, iso-forms of the tissue esterases contain organ-specific components and fractions mainly present in subcellular structures of many or some rat organs.

Several types of esterases hydrolyzing carboxylic acid esters have been discovered in the blood serum and tissue extracts of different species of animals. In their biochemical properties, these esterases are subdivided into carboxyl-, aryl-, acetyl-, acetylcholine-, and cholinesterases (3.1.1.1, 3.1.1.2, 3.1.1.6, 3.1.1.7, and 3.1.1.8, respectively). Esterases of each of these types are present in the body as a number of molecular forms, the properties and distribution of which, and their role in the metabolism of various substances in the body have been inadequately studied [5, 8-10].

The results of an immunochemical and electrophoretic investigation of multiple forms of esterases in the blood serum and tissues of rats are described below.

EXPERIMENTAL METHOD

Male August rats weighing 160-170 g were kept on a standard diet. Enzymes from the blood serum and hyaloplasm of cells from the liver, kidneys, spleen, lungs, testes, pancreas, brain, mucous membrane of the stomach and large and small intestine, and the heart of the animals were investigated. The conditions of processing of the organs, isolation of the hyaloplasm fraction of the cells, and the technique of electrophoretic and immunoelectrophoretic investigation of the preparations were identical with those described previously [1]. To detect esterase activity, immediately after electrophoresis the gel was incubated at 37° for 30 min in a substrate mixture consisting of 2 mg α- or β-naphthyl acetate in 2 drops of acetone, 9 ml 0.1 M phosphate buffer (pH 7.8), and 3 mg of the dye fast blue B in 1 ml water. If indoxyl acetate was used as substrate, the gel after electrophoresis was placed for 1 h in a solution containing 2 mg indoxyl acetate in 2 drops of acetone and 10 ml 0.1 M phosphate buffer (pH 7.8). To test the sensitivity of different esterase fractions toward inhibitors, the gel after electrophoresis was incubated for 10 min at 37° in a solution of the corresponding inhibitor in 0.1 M phosphate buffer, and then for 30 min in substrate mixture (β-naphthyl acetate) with the same inhibitor. The inhibitors used were 0,0-diethyl-S [β-(cyclohexylmethylamino)-ethyl]-thiophosphate* ($4 \cdot 10^{-4}$ M), neostigmine ($1 \cdot 10^{-3}$-$1 \cdot 10^{-5}$ M), EDTA ($2 \cdot 10^{-3}$ M), p-chloromercuribenzoate ($5 \cdot 10^{-4}$ M), and $CuSO_4$ ($2 \cdot 10^{-3}$ M) [2, 4, 7]. The gels after electrophoresis were fixed in 3% acetic acid so-

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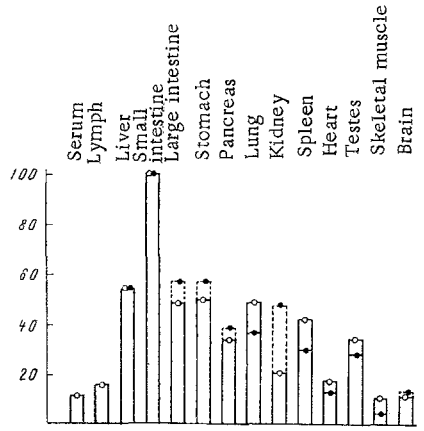

Fig. 1

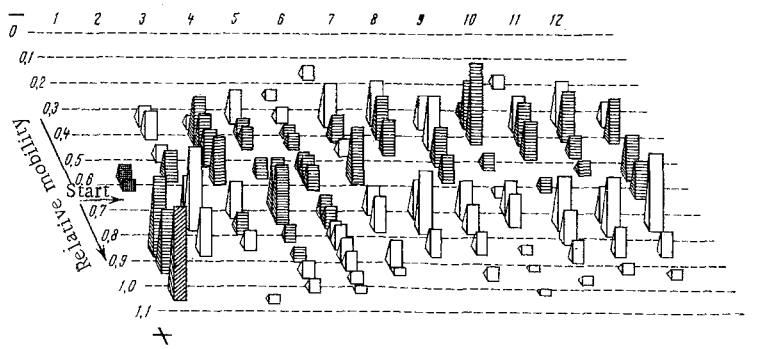

Fig. 2

Fig. 1. Ratio between activities of esterases from different organs of rats. Ordinate, total activity of esterases calculated per mg hyaloplasm protein of different organs (in percent of esterase activity in small intestine). O – activity relative to β-naphtyl acetate; ● – activity relative to indoxyl acetate.

Fig. 2. Isoenzyme spectrum of esterases of blood serum (1) and of cell hyaloplasm from the brain (2), liver (3), gastric mucuous membrane (4), small intestine (5), large intestine (6), pancreas (7), lungs (8), kidneys (9), spleen (10), testes (11), and heart (12) of August rats. Height of prisms is proportional to percentage content, and their position corresponds to relative electrophoretic mobility of fractions of carboxylesterases (horizontal shading), arylesterases (oblique shading), acetylesterases (unshaded), and cholinesterases (cross-hatched). Substrate – β-naphthyl acetate.

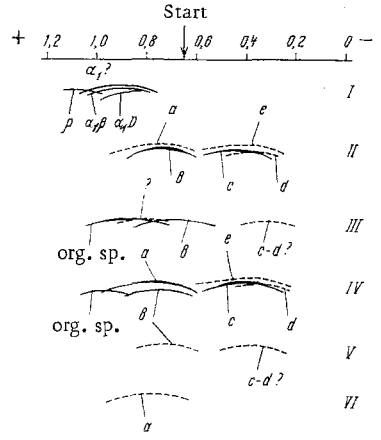

Fig. 3. Immunoelectrophoretic detection of esterases in blood serum (I) and hyaloplasm of cells of the liver (II), mucous membrane of the small intestine (III), kidneys (IV), spleen (V), and brain (VI) of rats. Immunologically identical species-specific esterases are designated in an identical manner, organ-specific esterases are identified as org. sp.

lution, dried under a layer of filter paper, and examined in the ERI-10 integrating densitometer, the width of the light slit in which was reduced to 0.1 mm. Activity of the fractions was expressed in conventional units, proportional to the optical density of each of them on the gel after electrophoresis, and their relative content in the sample was then calculated. The relative electrophoretic mobility of the fractions was calculated by comparison with the mobility of human serum albumin subjected to electrophoresis under the same conditions. In repeated tests, extracts of the organs of 6-8 rats were subjected to electrophoresis.

EXPERIMENTAL RESULTS

Comparison of the specific esterase activity (calculated per mg protein in the sample) of cell hyaloplasm from different organs of August rats showed that relative to β-naphthyl and indoxyl acetate the mucous membrane of the small intestine and other organs developed from the embryonic entoderm possessed the highest esterase activity. The ratio between the activities of esterases in different organs was generally the same, regardless of whether β-naphthyl or indoxyl acetate was used to detect the enzymes. It was only in the kidneys that esterase activity detected by indoxyl acetate was higher than when β-naphthyl acetate was used (Fig. 1).

The most complex spectrum of molecular forms of the esterases was possessed by organs of entodermal origin. For example, the hyaloplasm of cells from the mucous membrane of the small intestine contained up to 15 fractions, compared with 10 and 9 fractions respectively for the stomach and large intestine. Esterases of cells from the liver, pancreas, and lungs were represented by 9, 11, and 8 fractions respectively. The number of esterase fractions in the hyaloplasm of organs of mesodermal

origin varied between 7 and 10 (kidneys – 10, spleen – 9, testes – 8, heart muscle – 7). In extracts of the rats' brain (ectodermal origin) tests with β-naphthyl acetate revealed 7 esterase fractions, while blood serum contained 5 components, 3 of which were located in the electrophoretic zone of albumins and α-globulins, while 2 migrated with serum β-globulins (Fig. 2). The spectrum of esterases from different organs of the rats did not differ whether α- or β-naphthyl acetate was used as substrate for their detection, but it was slightly less active when indoxyl acetate was used to detect esterase activity. However, in extracts of the brain, gastric mucous membrane, and heart muscle of the rats, some esterase fractions were more active and could be detected after electrophoresis only by the use of indoxyl acetate.

On the basis of differences in their sensitivity to the action of inhibitors, it is customary to divide esterases of carboxylic acid esters into cholinesterases (sensitive to organophosphorus compounds and quaternary ammonium bases), carboxylesterases (sensitive to organophosphorus compounds), arylesterases (sensitive to sulfhydryl reagents), and acetylesterases (insensitive to the action of these inhibitors) [2, 4, 7]. The present experiments with a series of inhibitors showed that many of the esterases detected in organs of August rats are isoenzymes of acetyl- and carboxylesterases, while choline- and arylesterases were found only in the blood serum of these animals. Cholinesterases are found mainly in cytoplasmic granules of cells [3], and during fractionation of the organ homogenates under the conditions used in the present experiments they evidently did not enter the hyaloplasm fraction of the organ.

Cells of the liver, gastric mucosa, and small intestine differed in having in their hyaloplasm an electrophoretically fast iso-form of carboxylesterase which was absent from extracts of other organs of the rats. Cathodic iso-forms of carboxylesterases (relative electrophoretic mobility 0.45-0.35) were detected in the hyaloplasm of all tested organs of the rats except in extracts of the brain and lungs. Immunochemical tests showed that these fractions of carboxylesterases are isoenzymes of granular origin and are found mainly in the cytoplasmic granules of several rat organs. Arylesterases of the blood serum were represented by one fraction with the fastest electrophoretic mobility (relative mobility 1.04), while cholinesterases were represented by two labile fractions, not always detectable, in the zone of β-globulins.

The immunoelectrophoretic tests revealed four antigens among the blood serum proteins possessing esterase activity, one of which corresponded to serum prealbumin, and the other three to α_1B-, α_1D-, and α_1?-globulins (nomenclature of the serum proteins proposed by Escribano and Grabar [6]). The use of tissue antisera exhausted with blood serum proteins and erythrocytes revealed 7-8 antigenically different iso-forms of esterases in the extracts from different rat organs (Fig. 3). Organ-specific esterase antigens were found only in the hyaloplasm of cells of the mucous membrane of the small intestine and of the kidneys (relative electrophoretic mobility 0.95-0.85). Anodic esterase tissue antigens (a- and b-antigens) were represented by species-specific acetylesterases, detectable separately or together also during immunoelectrophoresis of the hyaloplasm proteins from various rat organs. Cathodic esterases during immunoelectrophoresis of rat tissue extracts were represented by three carboxylesterases, revealed particularly clearly in specimens from the rat liver and kidneys. Two of these isoenzyme antigens (c- and d-antigens) were antigens of granular origin and could be found among proteins of the cytoplasmic granules of practically all rat organs.

Immunochemical and electrophoretic investigation of tissue and serum esterases of August rats thus showed that, regardless of the embryonic origin of the organs, the spectrum of the hyaloplasmic esterases of cells from different organs differs not only by the presence of organ-specific isoenzymes, but also by the individuality of the assortment of isoenzymes and the fact that the ratio between the activities of different enzymes detectable in extracts from many organs of the animals was characteristic for each organ. At least some of these "universal" esterase fractions are not enzymes of cells of connective-tissue origin (they are absent from extract of a spindle-cell rat sarcoma) and, irrespective of their degree of differentiation they are found in specialized cells of many animal organs.

Esterases of rats are immunologically distinct from those of rabbits, and after immunization of rabbits they cause the formation of antibodies which do not react with rabbit tissue esterases. However, the active centers of the molecules of rabbit and rat esterases are evidently indistinguishable, because rabbit antibodies, while precipitating rat esterases, do not inhibit their enzymic activity, while the precipitation lines of some esterase antigens could be detected only through their enzymic activity.

LITERATURE CITED

1. K. P. Kashkin, A. N. Sharetskii, B. P. Surinov, et al., Vopr. Med. Khimii, No. 3, 235 (1969).
2. V. I. Rozengardt, Trudy Leningrad. Pediat. Med. Inst., No. 32, 140 (1965).
3. W. N. Aldridge and M. K. Johnson, Biochem. J., 73, 270 (1959).
4. K. V. Augustinsson, Ann. N. Y. Acad. Sci., 94, 844 (1961).
5. M. Chmelarova and M. Chmelar, in: Abstracts of the 5th Meeting of the Federation of European Biochemical Societies, Prague (1968), p. 163.
6. M. Escribano and P. Grabar, C.R. Acad. Sci. (Paris), 255, 206 (1962).
7. R. V. Holmes and C. J. Masters, Biochim. Biophys. Acta, 146, 138 (1967).
8. J. H. Lepow, J. B. Naft, E. W. Todd, et al., J. Exp. Med., 117, 983 (1963).
9. W. Pitz and H. Horlein, Hoppe-Seylers Z. Physiol. Chem., 335, 221 (1964).
10. W. Pitz and H. Horlein, Hoppe-Seylers Z. Physiol. Chem., 345, 65 (1966).