HISTOCHEMISTRY

Histochemical Demonstration of Non-specific Esterase in Mast Cells of the Albino Rat with Properties Similar to Mast Cell Enzyme activated in Anaphylaxis

RECENT investigations suggest that release of histamine from isolated mast cells of the albino rat by an antigen-antibody reaction is due to an enzymatic process^{1,2}. Further experiments with inhibitors which are more selective reveal that certain specific chymotrypsin substrates or inhibitors prevent the reaction, whereas substrates and inhibitors of carboxypeptidase, leucine-aminopeptidase or trypsin do not³⁻⁵. Toxic organic phosphorus compounds such as diisopropylfluorophosphate (DFP) or 'Paraaxon' also inhibit in relatively high concentration⁶. By virtue of these findings we have concluded that the enzyme responsible for the antigen-antibody reaction of isolated tissue mast cells of the albino rat is a chymotrypsin-like peptidase having pronounced esterolytic properties⁵.

By means of histochemical techniques a number of enzymes can be detected in mast cells of the albino rat; in particular, one finds strong activities of leucine-aminopeptidase and adenosine-triphosphatase⁷. Benditt and Arase⁸ have also demonstrated a chymotrypsin-like enzyme using naphthol-AS-chloroacetate as substrate.

I have endeavoured to discover which of the histochemically demonstrable enzymes displays properties similar to those of the enzyme responsible for the anaphylactic release of histamine. Normal isolated mast cells of the rat⁹ were spread on slides and fixed by formalin vapour for 20 min. Following Pearse¹⁰ and Löffler's¹¹ methods, the following histochemical tests were carried out on fixed smears: leucine-aminopeptidase according to Nachlas, Crawford and Seligman; acid phosphatase according to Gomori and Pearse; adenosine-triphosphatase according to Wachstein and Meisel; non-specific esterase according to Pearse (α -naphthyl-acetate), to Gomori (naphthol-AS-acetate), to Moloney *et al.* (naphthol-AS-D-chloroacetate) and to Holt (β -bromo-indoxyl-acetate). Comparative control tests were performed after heating slides to 90° C and with incubation mixtures lacking substrate. Several preparations were always examined simultaneously. The experiments with the inhibitors were performed according to the methods of Pearse¹⁰, and Hess and Pearse¹².

The results are set out in Table 1. This shows that the mast cells of the albino rat, in accordance with the findings of Eder and Schauer⁷, display pronounced activity of leucine-aminopeptidase, acid phosphatase and adenosine-triphosphatase. Histochemical detection of these enzymes is not influenced by eserine, DFP or 'Paraaxon' (2×10^{-6} and 10^{-3} M). The

detection of non-specific esterase is unsuccessful or unsatisfactory with the substrates α -naphthylacetate and naphthol-AS-D-chloroacetate. On the other hand, the use of naphthol-AS-acetate or β -bromo-indoxylacetate results in an intensive staining which is clearly localized in the granules of the mast cells. This activity is not influenced by eserine (2×10^{-6} M), DFP (10^{-6} M) or 'Paraaxon' (10^{-6} M); however, incubation with higher concentrations of these substances (10^{-3} M) results in virtually complete loss of enzymatic activity. In addition, copper sulphate (10^{-3} M), lead nitrate (10^{-3} M), silver nitrate (10^{-2} M), and β -phenylpropionic acid (10^{-2} M) are also inhibitory. Histochemically the enzyme thus appears to be a non-specific organophosphate-resistant esterase. In so far as comparative experiments are available, its properties are identical with those of the enzyme responsible for the anaphylactic release of histamine.

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PATHOLOGY

Established Bone Marrow Cell Cultures with High Degree of Iron Incorporation

THE literature contains numerous reports of cell strains derived from normal or pathological human bone marrow or from elements of peripheral blood¹. In the great majority, cell lines of such derivation are known to transform into fibroblastic or epithelial forms after a certain period of time². Their experimental application is extensive, but almost exclusively confined to the field of virology³.

Theoretically, it is possible to develop a permanent population of cells which would retain the basic properties of the primitive elements of normal bone marrow. With this possibility in mind, attempts were made to culture under varied conditions human bone marrow derived aseptically from surgically resected ribs. Of 19 such cultures, 17 were discarded, because of degeneration or fibroblastic transformation. The two remaining cultures derived from two young persons operated on for diaphragmatic hernias showed signs of stability.

The cells (RI) in these two lines were originally grown in T-flasks (60 ml.) with 25 ml. of culture medium. The culture fluid used consisted of Eagle's minimum essential medium, inactivated horse serum 10 per cent, glutamine 1 per cent, and antibiotics; it was supplemented with 0.5 ml. of folic acid ('Leucovorin' Lederle, 3 mg/ml.) and 1 ml. of vitamin B₁₂ ('Rubramin' Squibb, 3 µg/ml.). Since after 25 transfers the folic acid and vitamin B₁₂ were tested in

Table 1. EFFECT OF VARIOUS INHIBITORS ON HISTOCHEMICALLY DEMONSTRABLE ENZYMES IN MAST CELLS OF THE ALBINO RAT

	Untreated normal mast cells	Mast cells pre-incubated with:			
		Eserine (2×10^{-6} M)	DFP or 'Paraaxon' (10^{-3} M)	DFP or 'Paraaxon' (10^{-4} M)	DFP or 'Paraaxon' (10^{-6} M)
Leucine-aminopeptidase	+++	+++	+++	+++	+++
Acid phosphatase (Gomori)	+++	+++	+++	+++	+++
ATPase	+++	+++	+++	+++	+++
Esterase					
Naphthol-AS-acetate	+++	+++	-	(+)	++
β -Bromo-indoxylacetate	+++	++	-	(+)	++