

PRO EXPERIMENTIS

Staining and Fluorescent Microscopy of Mast Cells with Pseudoisocyanine

There are numerous histological methods for the staining of mast cells. Most of them make use of metachrome dyes: methylene blue¹, alcian blue², azure A³, toluidine blue³, thionine⁴, etc.

It was shown⁵ that the reaction with pseudoisocyanine applied on pancreatic cells and hypothalamus shows a polychromatic effect: the B-cells and the neurosecretional cells appear polychromatic and are stained deep violet. It was also shown that the metachromatic phenomenon of the neurosecretional cells⁷ and the B-cells^{6,8,9} of pancreas is accompanied by a fluorescent effect.

Since mast cells are stained with paraldehyde fuchsin¹⁰ in the same way as the granules of the B-cells in pancreas and the neurosecretional cells of the hypothalamus, we applied the reaction with pseudoisocyanine for the demonstration of the granules in the mast cells.

Material and method. Omentum and pancreas of white rats, gold hamsters and guinea-pigs were studied. The histological technique based upon SCHIEBLER was as follows: (1) fixation in Bouin's solution 12–20 h. (2) After deparaffinization the preparations are immersed in a freshly made solution of 2.5% potassium permanganate, 5% sulphuric acid and distilled water in 1:1:6 ratio. (Better results can be obtained if the organ is embedded for a long time and the deparaffinized preparations are preliminary treated for 24 h in Bouin's solution.) (3) Washing in tap water, 5 min. (4) Decolourization with 1% potassium metabisulphide, 5–10 sec. (5) Washing in tap water, 5 min. (6) Staining with 7 mg% pseudoisocyanine, 15–30 min. (7) Washing in tap water, 5 min. (8) Fixation and inclusion in 1% potassium bisulphide. (9) Observation with light and fluorescent microscope.

Results. With the light microscope, the cytoplasmic granulations of the mast cells are coloured blue, blue-pink. The nucleus and the cell's structures are pale pink, or remain uncoloured. (In the pancreas the mast cells were seen in the interlobular connective tissue and around the larger excretory duct.)

The same stained preparations observed with the fluorescent microscope reveal a very intense secondary gold-orange luminescence of the granules of the mast cells. The nucleus A-cells and the other structures appear dark.

Discussion. It must be pointed out that all structures which are paraldehyde-positive stain equally well when metachromatic staining is applied: the B-cell-granules of the pancreatic gland, the neurosecretory cells of the hypothalamus and the mast cells' granules. What structures in the mast cells are stained? Chemical studies established¹¹ that chondroitin sulphuric acid, heparin and hyaluronic acid can be linked to pseudoisocyanine. A reversible polymere compound is formed which shows a very specific short absorption band. The linking groups are $\text{SO}_3^{(-)}$ and $\text{COO}^{(-)}$. In our case it must be pointed out that the mast cells contain both hyaluronic acid and heparin¹².

The proposed method for the study of mast cells by polychrome staining with pseudoisocyanine is rapid and very sensitive. Only the mast cells are coloured, while the other cell structures remain uncoloured or are poorly coloured. The method has an advantage over other polychrome methods, since the preparation can be observed also with the fluorescent microscope. The demonstration of heparine or hyaluronic acid points to a histochemical value of the method.

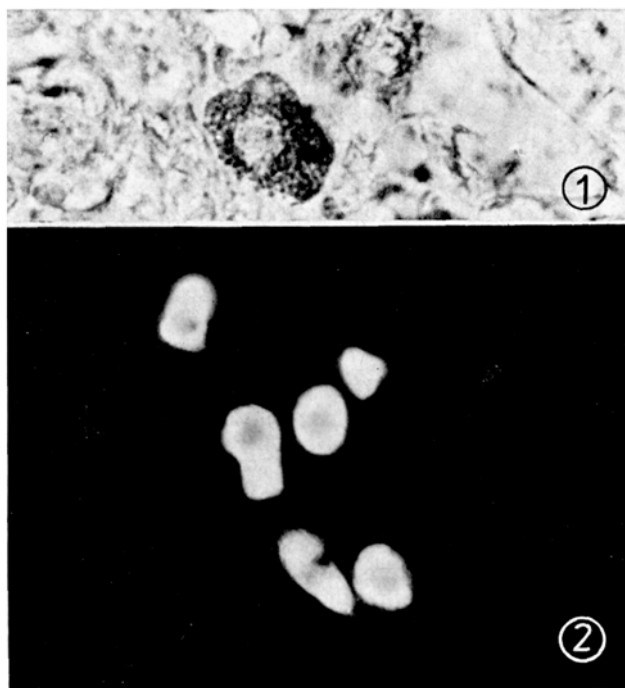


Fig. 1. Mast cell stained with pseudoisocyanine, observed with the light microscope. $\times 504$.

Fig. 2. Mast cells stained with pseudoisocyanine, observed with the fluorescent microscope. $\times 180$.

Résumé. Les mastocytes peuvent être colorés par la réaction polychromatique de pseudo-isocyanine. L'avantage de ce procédé consiste dans le fait qu'on peut obtenir un effet fluorescent qui rend cette réaction très sensible. Une certaine valeur histochimique est envisagée.

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