A DIFFERENTIAL STAIN FOR CELL TYPES IN THE PANCREATIC ISLETS *

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Most, if not all, of the accepted staining methods for the differentiation of cell types in the islets of Langerhans are more or less capricious and unreliable. The neutral gentian violet stain gives beautiful results in guinea pig material, but it is much less dependable in other animal species. The Mallory-Heidenhain azan method can be applied to almost all animal species, but gives a clear definition of the *alpha* granules only, while the *beta* granules are poorly demonstrated. The other methods (copper and iron hematoxylin, acid fuchsin-methyl green) are even less satisfactory than the stains mentioned.

The routine hematoxylin-eosin stain shows in well fixed tissues a definite difference between *alpha* and *beta* cells owing to the relative oxyphilia of the former and the basophilia of the latter. As a result of my attempts to increase this contrast I have found a fairly simple modification of the hematoxylin-eosin stain which brings out the two cell types more distinctly than any of the other stains used for the same purpose. It has the additional advantage of giving uniform results in all animal species examined (guinea pig, rat, mouse, cat, dog, rabbit, *Macacus rhesus* monkey, beef) as well as on human material. Its results were excellent in certain human cases in which all other stains failed. The two cell types were clearly demonstrated in both the normal and the diabetic pancreas sometimes taken as late as 4 hours after death. This method is also suitable for study of the degranulation processes in the pancreas in experimental animals. It can also be recommended for the pituitary. In the pancreas the *beta* granules stain a deep slate blue color, while the *alpha* granules stain red. The D cells of Bloom are not demonstrated. In the pituitary the basophils are blue, the oxyphils red, and the chromophobes are almost unstained.

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METHOD

Fixation: The thickness of pieces of tissue should not exceed 2 mm. in order to ensure rapid and complete penetration by the fixative. Aqueous fixatives, such as formalin, Bouin's, Zenker's, and Stieve's solution, or Zenker-formalin, may be used. The best fixative proved to be a modified Bouin's solution in which half the amount of acetic acid is replaced by sulphosalicylic acid (formalin 1 part, saturated picric acid solution 4 to 5 parts, acetic and sulphosalicylic acid 2.5 per cent each). For use dilute this fixative with equal parts of distilled water. The staining of tissues fixed in any fluid can sometimes be greatly improved by refixing the sections before staining in the undiluted solution for 12–24 hours. The removal of mercury salts from tissues is done by the routine iodine method.

Embedding: Embed tissues in paraffin.

Oxidation: Deparaffinized (and refixed, if necessary) sections are treated for 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and of sulphuric acid. The sections are rinsed in water and then decolorized with a 1 to 5 per cent solution of potassium metabisulphite. After decolorization the sections are thoroughly washed in water. Without oxidation the *beta* granules will not take the stain. For sections of pituitary, oxidation, though it will enhance the color contrast, is not strictly necessary.

Staining: Stain in a well ripened solution of chromium hematoxylin for from 15 minutes to 1 hour under microscopic control. The *beta* granules should be a deep blue and the cytoplasm of the *alpha* cells should be unstained. In the pituitary the basophils should be a deep blue and the oxyphils unstained. Any mucoid material is stained a deep blue. The staining solution is made up as follows:

Mix equal parts of a 1 per cent aqueous solution of hematoxylin and of a 5 per cent solution of chromium alum (potassium chromium sulphate). The brownish mixture is ripened by the addition of about 3.5 cc. of a 5 per cent potassium dichromate solution plus a few drops of sulphuric acid to each 100 cc. of the mixture. The process of ripening will take 1 or 2 days. The solution is ready for use as soon as it is a deep blue-black with a

purplish tinge. It should be filtered before use as a precipitate will form repeatedly on its surface. For some time the staining power and selectivity of the solution increase, but as the solution becomes older the staining time has to be prolonged. Solutions that do not stain deeply enough in 35 to 40 minutes are better discarded as they often do not stain with sufficient precision.

After staining in this chromium hematoxylin solution rinse the sections in water and transfer to alcohol containing 1 per cent of hydrochloric acid. In this acid alcohol solution the color of the section becomes a clearer blue. Rinse again and counterstain rather heavily with any of the routine red or orange acid dyes. Phloxine and ponceau de xylidine have been found to be especially suitable. The latter is used in a 0.5 per cent solution with 1 per cent of acetic acid. Rinse in water and differentiate in a 5 per cent solution of phosphotungstic acid until the acid dye is completely removed from the connective tissue. Only the strongly oxyphilic structures, such as erythrocytes, muscle fibers, oxyphils and *alpha* cells, remain stained. Carry sections through graded alcohols, clear in xylol and mount in balsam.

DESCRIPTION OF PLATE

PLATE 82

Tissues were fixed in modified Bouin's solution and stained according to the method recommended. Phloxine was used as a counterstain.

An orange-yellow filter was used for photographing the sections. The blue stained tissue elements are dark and the pink stained elements pale. All microphotographs were taken at a magnification of $\times 380$.

FIG. 1. Pancreas from a normal guinea pig. A = pale *alpha* cells; B = dark *beta* cells.

FIG. 2. An islet in the pancreas of a guinea pig. The blood sugar level was kept at 200 mg. per 100 cc. for 12 hours preceding death. Profound degeneration of the *beta* cells, of which only a few show very deep blue granules in a cap-shaped area of the cytoplasm, is to be seen.

FIG. 3. Normal human islet. A = pale *alpha* cells; B = dark *beta* cells.

FIG. 4. Human pancreas from a case of severe diabetes. A = pale *alpha* cells; B = dark *beta* cells; C = masses of hyalin.

FIG. 5. Anterior lobe of a human pituitary gland. A = chromophobes with unstained cytoplasm; B = dark basophils; C = pale oxyphils.

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Stain for Cell Types in Pancreatic Islets