Technical methods

Simultaneous demonstration of mast cells and plasma cells

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In order to obtain a compound stain to differentiate between mast and plasma cells, it was necessary to find dyes which provided a distinct colour contrast between the two types of cell.

The aqueous acetic Alcian blue method (McManus and Mowry, 1960) stained mast cells, but also other tissue elements. Using Alcian blue at a pH less than 1.0 (Johnson and Helwig, 1963) the mast cells stained brilliant turquoise-blue, with pale turquoise-blue background staining of connective tissue mucin. An alcoholic solution of Alcian blue at pH 0.5 was found to give the same results, and was stable for many months. White's methyl green-pyronin technique (Dacie, 1950) was modified to allow its application to paraffin sections, and the resultant bright pink staining of the RNA in the cytoplasm of the plasma cells gave a good contrast to the turquoise-blue mast cells.

TECHNIQUE

MATERIALS Sections, each of 5 μ , of paraffin-wax-embedded 10% formal-saline-fixed tissues (10% formalin in 0.85% aqueous sodium chloride solution).

SOLUTIONS All solutions may be stored at room temperature.

A Acid alcohol solution

70% alcohol 90 ml.
Concentrated hydrochloric acid 10 ml.

B Alcian blue solution

Alcian blue 1 g.
70% alcohol 90 ml.
Concentrated HCl 10 ml.

C Methyl green-pyronin solution Pyronin Y, 2.6 g., and 1.4 g. methyl green (washed in chloroform) are stirred into 40 ml. glycerin. To this 10 ml. absolute alcohol and 400 ml. 0.5% aqueous phenol are added, and the whole mixed well.

It is advisable to ripen this solution for one week before use. Specificity is lost after approximately seven weeks.

METHOD The section is treated as follows:

- 1 Bring through xylol, absolute alcohol to 70% alcohol.
- 2 Immerse in three changes of acid alcohol solution for two to five minutes each change.

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- 3 Stain in Alcian blue solution for 10 minutes.
- 4 Rinse in acid alcohol solution.
- 5 Wash in running tap water for at least 10 minutes to remove acid.
- 6 Stain with methyl green-pyronin solution for 10 minutes.
- 7 Rinse in distilled water.
- 8 Differentiate and dehydrate rapidly in three changes of absolute alcohol.
- 9 Clear in xylol.
- 10 Mount in Xam.

RESULTS

Cytoplasm and granules of mast cells stain deep turquoise blue; connective tissue mucin stains pale turquoise-blue; cytoplasm of plasma cells, nucleoli, and other structures containing RNA a deep pink; and nuclei stain purplish green.

COMMENT

It has been found that freshly fixed tissues, both human and animal, give reliable results with this method. The fixative employed is 10% formal/saline. Routine processing through alcohols, xylol, or chloroform is followed by embedding in paraffin wax. Some blocks over three years old gave inconsistent results and failed to stain with the methyl green-pyronin solution.

Only certain batches of dye are completely satisfactory. Pyronin Y and methyl green obtainable from G. T. Gur and Alcian blue from Hopkin and Williams are recommended for use in this method. The methyl green must be washed in chloroform to remove methyl violet if accordance with the usual methods.

Using this modification (adapted from White's technique), the pyronin remains in R.N.A. after quite long periods in alcohol. This appears to be an improvement on other methods in which most of the pyronin staining is removed during treatment with alcohol.

Note on preserving a standard suspension of live vibrios

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It is difficult to prevent changes in biological characters of vibrio strains during storage (Martin, 1964). Even freeze-drying may be unsatisfactory for two reasons. First, only about 5% of viable cells survive the process (Fry and Greaves, 1951) allowing chance for selection of variants. Secondly, the viable cell count of each suspension made from regenerated cultures has to be ascertained. This may lead to large deviations from the intended dose because the number of vibrios in suspensions of equal optical density varies unusually widely (Maaløe,

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The pH of the Alcian blue solution in this method is low, approximately 0.5, and it is important that the section is allowed the full time in the acid alcohol solution (stage 2), otherwise other tissue elements will take up the Alcian blue stain; this cuts down the contrast in the final picture. As the staining of the methyl green-pyronin solution is impaired in very acid conditions, it is essential that the sections are well washed after treatment with Alcian blue.

Solutions B and C can be used separately. For the demonstration of mast cells, steps 1-5 are carried out, followed by a nuclear counterstain, e.g., 1% neutral red. For plasma cells, the section is brought to distilled water, and steps 6-10 are carried out.

Generally the mast cells stain a deep turquoise-blue, but it has been observed that some cells, cytologically resembling mast cells, stain purple.

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1955), and the viable count is not available till the next day. Thus McIntyre and Feeley (1964) noted fifteenfold variations in their challenge doses during a passive protection test. We have, therefore, investigated the possibility of storing a vibrio suspension of known viable count at a very low temperature, i.e., of preparing a vibrio stablate. (A stablate has been defined by Lumsden and Hardy (1965) as 'a population of an organism preserved in viable condition on a unique occasion'.)

Martin (1964) has reviewed the principles of storing microbes at temperatures below -55°C . The problem is to prevent cell death during freezing and thawing. Addition of glycerol or dimethylsulphoxide reduces cell death with some organisms. Once successfully frozen, microbes seem to keep indefinitely without noticeable change.

MATERIALS AND METHODS

Vibrio cholerae 12R (Ogawa) was received from the National Institutes of Health, U.S.A. (McIntyre and Feeley, 1964). *V. eltor* H2 (Ogawa) was sent by Dr. M. Robertson, Queen Elizabeth Hospital, Hong Kong; it had been isolated from a cholera patient in 1963. Both strains were received freeze-dried. The contents of each ampoule were suspended in 0.2 ml. of nutrient broth (Oxoid no. 2) and streaked on a nutrient agar plate (pH 8.0 to 8.4). After incubating for 18 to 20 hours at 37°C . the growth was freeze-dried in a large number of ampoules.

PREPARATION OF A STABULATE The contents of an ampoule were cultured as above. Portions of five to six colonies were streaked on a nutrient agar slope and incubated overnight. The growth was suspended in 2 ml. of broth, and divided in two portions. To one part was added sterile glycerol (7.5% v/v), the second portion received no preservative. In early experiments sterile Analar grade dimethylsulphoxide was added to a third portion to a final concentration of 10% (v/v). The suspensions were filled into capillary lymph tubes and then frozen and stored in solid carbon dioxide (-79°C .) as described by Cunningham, Lumsden, and Webber (1963).

DETERMINATION OF VIABLE COUNT A tube was opened at both ends and weighed. The contents were expelled in 1 ml. of diluent (0.1% peptone in 0.15M saline, pH 7.0 to 7.2), and the tube was weighed again. The volume of diluent was adjusted to contain 10 mg. of stablate in each millilitre. A series of tenfold dilutions was made. With a 0.1 ml. pipette, 0.1 ml. of each dilution was delivered on each of two agar plates and spread with the bent tip of Pasteur pipettes. After overnight incubation the colonies on both plates from an appropriate dilution (giving 100 to 300 colonies per plate) were counted. The number of colony-forming units in each milligram of undiluted stablate was computed from the average.

At least two tubes at a time were examined immediately before freezing and after storage for 48 hours.

INFECTING SUCKLING RABBITS A litter of 11-day-old rabbits was infected as described by McIntyre and