

Fig. 2 Results of the serum immunoglobulin levels and the serum intrinsic factor blocking antibody (IFAB) titre from 16 patients with pernicious anaemia.

#### References

- Ardeaman, S., and Chanarin, I. (1963). A method for the assay of human gastric intrinsic factor and for the detection and titration of antibodies against intrinsic factor. *Lancet*, 2, 1350-1354.
- Chanarin, I. (1969). The Megaloblastic Anaemias, pp. 550-574. Blackwell, Oxford and Edinburgh.
- Geigy, J. R., A.G., Basle (1962). *Scientific Tables* (Documenta Geigy 6th ed., edited by K. Diem, p. 91. Geigy Pharmaceuticals, Division of Geigy (Australasia) Proprietary, Ltd.
- Irvine, W. J. (1966). Immunoassay of gastric intrinsic factor and the titration of antibody to intrinsic factor. *Clin. exp. Immunol.*, 1, 99-118.

## Technical method

### Simultaneous staining of phospholipids, basic proteins, and glycogen on the same slide

B. PRESENTEY AND K. PERK *From the District Laboratory, Kupat Holim, Rehovot, and the Hebrew University of Jerusalem, Department of Animal Anatomy and Physiology, Rehovot Campus, Israel*

At least three different sections or smears are normally required when staining for glycogen, phospholipids, and basic proteins. The following procedure uses a single section or smear for the identification of the three substances, and demonstrates the site of each of the three components in the same cell. It is also useful when the number of sections or smears is limited.

The procedure is based on our observation that Luxol fast blue selectively stains basic proteins in the media used for staining phospholipids according to Lison (1952). This double staining does not interfere with the period acid Schiff (PAS) staining method for polysaccharides.

A combined staining procedure for Luxol fast blue and PAS has already been introduced by Shanklin and Nassar (1959).

#### Materials and Methods

Blood or bone marrow smears, touch preparation (impression smears), and frozen sections.

#### SOLUTIONS

##### *Sudan black B staining solution*

The method is according to Lison (1952).

##### *Luxol fast blue-Sudan black B staining solution*

To 100 ml of Sudan black B solution add 0.1 g Luxol fast blue.

Received for publication 19 January 1972.

#### *Immunoglobulin and intrinsic factor antibody in the sera of patients with pernicious anaemia—concluded.*

Shum, H. Y., O'Neill, B. J., and Streeter, A. M. (1971). A modified method for assaying intrinsic factor blocking antibody in serum. *Pathology*, 3, 311-315.

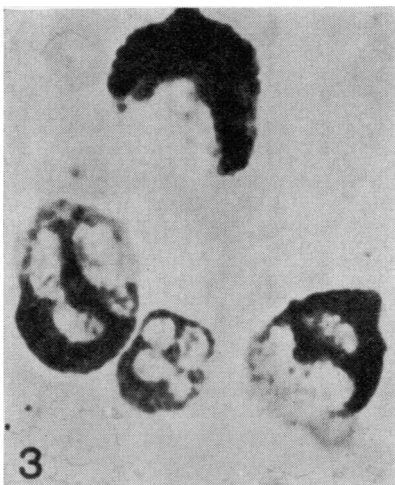
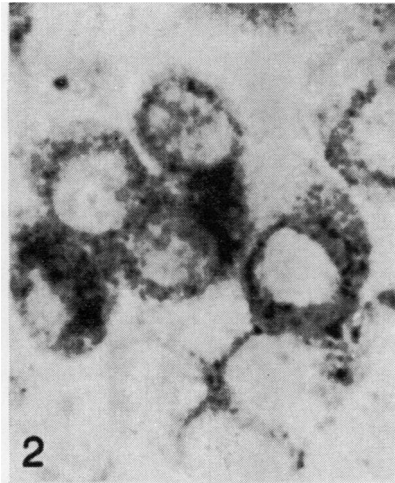
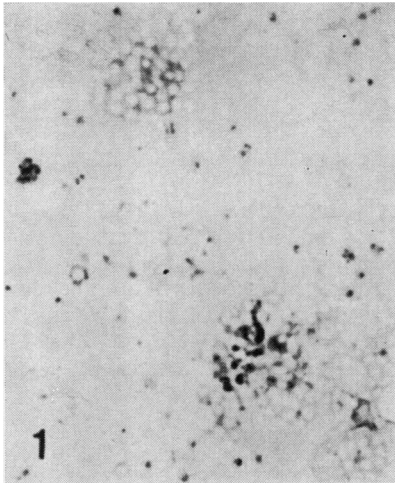
Ungar, B., Whittingham, S., and Francis, C. M. (1967). Pernicious anaemia: incidence and significance of circulating antibodies to intrinsic factor and to parietal cells. *Aust. Ann. Med.*, 16, 226-229.

*One per cent of periodic acid in distilled water Schiff reagent*  
This made up according to Hotchkiss (1948).

*Luxol fast blue*  
Luxol fast blue, 0.1 g in 100 ml of 70% ethanol saturated with urea (Goldstein, 1963).

*Sulphite wash water*  
The method of Hotchkiss (1948) was used.

FIXATION  
Formalin vapour is used.



**Figs. 1 and 2** *Sheep lung carcinoma imprints (impression smears).*

*Neoplastic cells contain either PAS-positive (top left of Fig. 1) or Sudan black B-positive granules (bottom right), or both types of granules in different proportions (Fig. 2). Dark areas in these figures represent phospholipids (appear brown) and lighter areas represent PAS-positive (glycogen) material which appears purple red.*

**Fig. 3** *Normal blood smear. The Sudan black B-positive reaction overlaps the colour reaction for basic proteins. Compare with Fig. 4 ( $\times 1200$ ). Cells show the presence of both phospholipids and glycogen.*

**Fig. 4** *The Presentey eosinophil anomaly-phospholipids are absent (Sudan black B negative) ( $\times 1200$ ).*

*Light granules in the two eosinophils (middle of Fig. 4) represent basic proteins which stain blue. Polynuclear neutrophils (bottom) show the presence of both phospholipids and glycogen.*

## STAINING PROCEDURE

- 1 Stain either with Sudan black B or Sudan black B + Luxol fast blue for 30 to 120 minutes.
- 2 Place in 70% ethanol, change twice for half to one minute.
- 3 Rinse in tap water and dry.
- 4 Put in periodic acid solution for 20 minutes. Rinse with running tap water and dry.
- 5 Stain in Schiff reagent for 25 minutes.
- 6 Wash in sulphite wash water three times for two minutes.
- 7 Rinse with running tap water 15 minutes and dry.
- 8 Stain in Luxol fast blue solution for 30 minutes after Sudan black B staining. If Sudan black B-Luxol fast blue was applied at step 1 omit step 8.
- 9 Differentiate in ethanol for half a minute.
- 10 Wash in running water for five minutes.

For the demonstration of glycogen, treat sections or smears with saliva or diastase for 30 minutes at 37°C.

Staining due to glycogen is selectively eliminated.

## Results

Phospholipids appear brown-black, basic proteins appear blue, glycogen and mucin appear red, and glycogen-PAS positive is removed by diastase.

## Comment

Electron microscopic studies of sheep pulmonary carcinoma (Jaagsiekte) showed that the neoplastic cells were apparently derived from the B type alveolar cell, which is one of the two types of lung alveolar epithelial cells (Perk, Hod, and Nobel, 1971a). Many of the B type and tumour cells contain phospholipid granules. One of the most striking morphological differences between the neoplastic and the B type cells is the appearance of cytoplasmic glycogen granules (Perk, Presentey, and Nobel, 1971b). In normal B type alveolar cells of the adult animal such granules are absent. The procedure

described demonstrates the presence of glycogen and phospholipids in the neoplastic cells and assists in establishing the topographical relationship between the two types of granules. As can be seen in Figs. 1 and 2, some of the neoplastic cells contained either PAS- or Sudan black B-positive granules but many neoplastic cells contained both types of granule in different proportions. The significance of this distribution is now under further study.

In normal blood smears, the Sudan black B-positive reaction masks the colour reaction for basic proteins of eosinophil granules (Fig. 3). In an anomaly of human eosinophils recently reported by Presentey (1968, 1969, 1971) (Fig. 4), neutrophils react normally with Sudan black B and PAS, whereas the anomalous eosinophils lack phospholipids and therefore are Sudan black B negative. In the procedure described here, the granules stain blue with Luxol fast blue whereas the cytoplasm gives the normal positive PAS reaction. From this it may be concluded that the phospholipid component of the eosinophil granules is at its surface.

## References

- Goldstein, D. J. (1963). Selective staining of eosinophil granules in sections by alkaline orcein in contracted urea solution. *Stain Technol.*, **38**, 49-51.
- Hotchkiss, R. D. (1948). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.*, **16**, 131-141.
- Lison, L. (1952). Technique de l'examen du sang et de la moelle osseuse. Coloration des graisses au Sudan noir. In *Planches d'Hématologie*, pp. 30-32. Sandoz, Basle.
- Perk, K., Hod, I., and Nobel, T. A. (1971). Pulmonary adenomatosis of sheep (Jaagsiekte). I. Ultrastructure of the tumor. *J. nat. Cancer Inst.*, **46**, 525-537.
- Perk, K., Hod, I., Presentey, B., and Nobel, T. A. (1971). Lung carcinoma of sheep (Jaagsiekte). II. Histogenesis of the tumor. *J. nat. Cancer Inst.*, **47**, 197-205.
- Presentey, B. (1968). A new anomaly of eosinophilic granulocytes. *Amer. J. clin. Path.*, **49**, 887-890.
- Presentey, B. (1969). Cytochemical characterization of eosinophils with respect to a newly discovered anomaly. *Amer. J. clin. Path.*, **51**, 451-457.
- Presentey, B. (1970). Partial and severe peroxidase and phospholipid deficiency in eosinophils. *Acta haemat. (Basel)*, **44**, 345-354.
- Shanklin, W. M., and Nassar, T. K. (1959). Luxol fast blue combined with the periodic acid-Schiff procedure for cytological staining of kidney. *Stain Technol.*, **34**, 257-260.