

Fig. 1. Macrophage showing localization of carbon-14 endotoxin label in the region of its cytoplasm. Note the unlabelled transferrin-coated lymphocyte in the right corner of the picture, and unlabelled small lymphocytes below the macrophage (magnification  $\times c. 410$ )

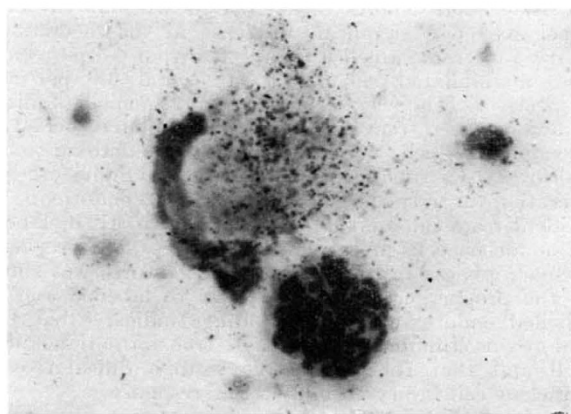


Fig. 2. A macrophage manifesting localization of carbon-14 endotoxin label flanked by an unlabelled early prophase mitotic figure (magnification  $\times c. 410$ )

organisms. Most of these organisms are phagocytized by macrophages, and none is detectable within the transformed lymphocytes<sup>12</sup>. Furthermore, lymphocytes purified in nylon or glass bead columns fail to transform *in vitro* with antigens, despite the fact that these antigens are successful in transforming lymphocytes in cultures of the same leucocytes before separation<sup>13</sup>. These findings suggest that macrophages may play an intermediate part in the secondary *in vitro* lymphocyte transformation response to antigens.

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## HAEMATOLOGY

### N Blood Group System of Pigs

THE N blood group system of pigs has been identified by Hála and Hojný<sup>1</sup> by means of an antiserum called anti-Na. The corresponding antigen, Na, was considered to be the product of a gene,  $N^a$ . They describe a system of two alleles,  $N^a$  and  $N^{(a)}$ —the latter symbol denotes the absence of  $N^a$ . Two additional factors of the N system, Nb and Nc, defined by the antisera anti-Nb and anti-Nc, have been identified in this laboratory. Inheritance studies show that there are three genes:  $N^a$ , producing factor Na,  $N^b$ , producing factor Nb, and  $N^{bc}$ , producing factors Nb and Nc.  $N^a$  and  $N^b$  are a pair of contrasting alleles, forming a closed system; Nc is a sub-group of Nb, and has not been found in its absence. It was not possible to verify Hála and Hojný's claim that Na is a soluble antigenic substance.

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### Large-scale Crystallization of Purified Human Foetal Oxyhaemoglobin from Cord Blood

THE crystal shape of pure human adult haemoglobin (HbA) has unanimously been described as that of well-developed, symmetrical bipyramids<sup>1,2</sup>. Investigations of the crystal forms of human foetal haemoglobin (HbF), however, have revealed divergent results: hexagonal rhombic plates<sup>3</sup>, probably monoclinic forms<sup>4</sup> and a mixture of dodecahedra and square prisms<sup>5</sup> have been reported. All these investigations were carried out on cord blood. Our methods allow the investigation of pure crystalline foetal haemoglobin.

Red cells from fresh heparinized cord blood of full-term and premature infants were washed five times with 0.9 per cent sodium chloride solution and haemolysed by the addition of 1.4 parts of twice distilled water to 1 part erythrocytes. All preparations were carried out at a temperature of 4° C to exclude an increase in methaemoglobin concentration. The relative proportion of HbF was estimated as described by Betke<sup>6</sup>; haemoglobin and methaemoglobin were repeatedly determined as cyanmethaemoglobin. Alkali denaturation of HbA was carried out according to Chernoff<sup>7</sup>. 100 ml. of an approximately 15 per cent haemoglobin solution were denaturated by the addition of 800 ml. 1/12 N potassium hydroxide for 2 min to destroy HbA completely. The reaction was interrupted by the addition of a 450 ml. saturated solution of ammonium sulphate containing 0.95 ml. concentrated hydrochloric acid per 100 ml. of the salt solution. The reaction mixture was adjusted to pH 7.0. After centrifugation (4,000g for 30 min) and after decanting carefully the deep red and clear supernatant, denaturated HbA, stromata and the more insoluble proteins in the sediment were discarded. 100 ml. of 0.5 M phosphate buffer, pH 7.35, were added to 1 l. supernatant. The salt saturation was brought up to 75–80 per cent by gradual addition with thorough stirring of solid ammonium sulphate, while the pH was maintained at at least 6.5 (if necessary more phosphate buffer (pH 7.35) was added). The solution was then centrifuged again (4,000g for 30 min) and a deep red sediment containing the foetal haemoglobin appeared at the bottom. HbF was then dissolved in 0.25 M phosphate buffer (pH 7.4), placed in dialysis bags and dialysed