the inability to correct a low two-stage test by the parenteral administration of vitamin K might not necessarily indicate a severe parenchymal liver lesion.

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IMMUNOLOGY

Immunofluorescent Staining of Globule Leucocytes in the Colon of the Sheep

The nature and fate of the globule leucocyte are of particular interest in relation to the immune mechanisms of the gut since it has been shown to increase during helminth infection[1] and appears to have certain affinities to plasma cells and Russell's body cells which have been shown to contain antibody[2].

Four previously worm-free sheep were experimentally infected with 1,500 infective larvae of *Oesophagostomum columbianum* and killed after ten weeks of infection. Tissue samples were taken from the colon and caecum of each animal and fixed in Helly's and Carnoy's fluids and cold acetone, embedded in paraffin wax and sectioned at 4μ according to the method of Culling[3].

Fluorescein isothiocyanate-conjugated rabbit anti-guinea-pig and rabbit anti-sheep globulin were obtained from Difco, Detroit. Rabbit anti-human serum was prepared according to the technique of Kabat and Mayer[4] and conjugated together with normal rabbit and human sera using fluorescein isothiocyanate following the technique of McDevitt *et al.*[5].

Sections of sheep colon were stained using rabbit anti-sheep globulin to detect globulins within cells; the rabbit anti-guinea-pig globulin and anti-human serum were used to test the specificity of this reaction. Further controls were made using conjugated normal rabbit and sheep globulin and unconjugated rabbit and sheep serum to test for non-specific attachment and for fluorescence of the serum proteins themselves. Phosphate buffered saline controls were used to test for fluorescence of the unstained sections. A slide of the aqueous mountant alone was also tested for fluorescence.

All the histological sections were first taken to water in the usual way and rinsed in phosphate-buffered saline, *p*H 7·2, for 15 min. Each slide was then flooded with the test fluorescent stain or control solution and left for 1 h. The slides were all rinsed using phosphate-buffered saline twice for 15 min and once for 1 h; these were then mounted using aqueous mountant and scanned.

All microscopy was done using a Leitz 'Ortholux' microscope with transmitted ultra-violet and white light with a dark ground condenser D1.20 A and an Fl. Oel. 95/1.32 objective with an Oel. 100/Fl. Oel. 95 × D1.20 stop inserted. Photographs of all the sections were taken with a Leitz 'Orthomat' photographic attachment using Ilford Pan *F* 50 *ASA* film.

Only in the sections stained with conjugated rabbit anti-sheep globulin was any fluorescent staining noted. All the globules in the globule leucocytes fluoresced (Fig. 1) and the plasma cells showed a similar fluorescence. In many of the plasma cells large fluorescent globules were shown which were of a similar size to those in the globule leucocytes. No fluorescence above a faint non-specific level was noted on any of the control sections (Fig. 2).

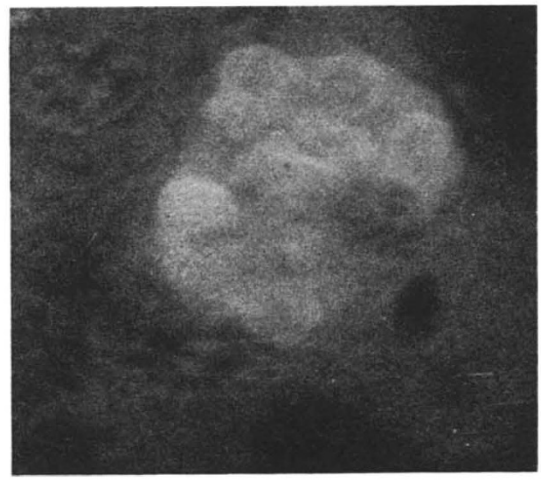

Fig. 1. Globule leucocyte stained with fluorescein isothiocyanate-labelled rabbit anti-sheep serum

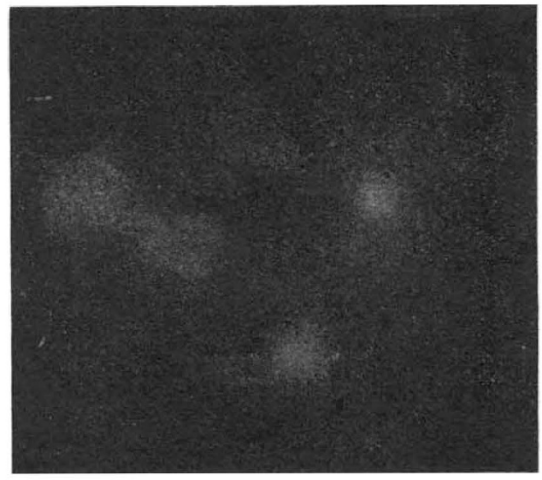

Fig. 2. Globule leucocyte from a control slide

It can thus be concluded that the cytoplasm of the globule leucocyte contains condensations of globulin. The similarities between the staining, the nuclear structure and the presence of globules of similar size and shape in the plasma cells and the globule leucocyte suggest that there is some relationship between these cells.

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