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Brief Communication

THE MIGRATION TEST ON CIRCULATING BOVINE LEUKOCYTES AND ITS POSSIBLE APPLICATION IN THE DIAGNOSIS OF JOHNE'S DISEASE*)

The leukocyte migration test for in vitro studies of delayed type hypersensitivity has recently been reviewed (*Søborg & Bendixen* 1967; *Bendixen & Søborg* 1969). *Søborg & Bendixen* applied the test to circulating leukocytes in man and thereby widely increased the potentialities of this test. They obtained high leukocyte yields with only moderate erythrocyte admixture by harvesting the supernatant plasma after sedimentation of the erythrocytes for 60 min. at 37°C in the normal gravitational field (1 × g). Their procedure was unsuitable for the present investigation because bovine erythrocytes sediment so slowly. Sedimentation after clumping at the interphase of aqueous solutions of polymers, dextran and methylcellulose, in combination with metrizoic acid (*Böyum* 1968) was tried without success because the vast majority of the leukocytes sedimented together with the erythrocytes. Separation of leukocytes from erythrocytes could not be achieved by differential centrifugation.

Leukocytes were removed from heparinized bovine blood in the present experiments by centrifugation of 10 ml blood in 13 mm diameter plastic tubes at 1000 × g for 10 min. to produce a loose buffy coat which was harvested to include 5 mm of the adjacent red cell layer. Excessive centrifugation at this stage trapped the leukocytes in a coagulated buffy coat probably due to high platelet concentration. The buffy coat harvest was resuspended in 10 ml of Hanks solution and centrifuged for 6 min. at 110 × g, followed by 250 × g for 2 min. This 2-speed centrifugation allowed removal of the platelets in the supernatant so that coagulation would not occur at later stages. The sedimented leukocyte-erythrocyte suspension in Hanks solution was thoroughly mixed with 4 volumes of 1:45 Hanks solution for 1 min. to produce lysis of the red cells. Isotonicity was then

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restored with an equal volume of 0.25 M-NaCl plus 0.04 M-Na_2HPO_4, pH 7.0. This protocol has proved equally applicable to blood from swine, rabbit and guinea-pig. The leukocyte yield from bovine blood averaged 39 % ± 12 % (n = 56) and only an insignificant change occurred in the differential leukocyte count. Except for a moderate change in the appearance of the nuclei of the heterophils the cell morphology was unaltered.

The cells were washed in Hanks solution prior to suspension in tissue culture medium "199" (Difco) supplemented with 10 %

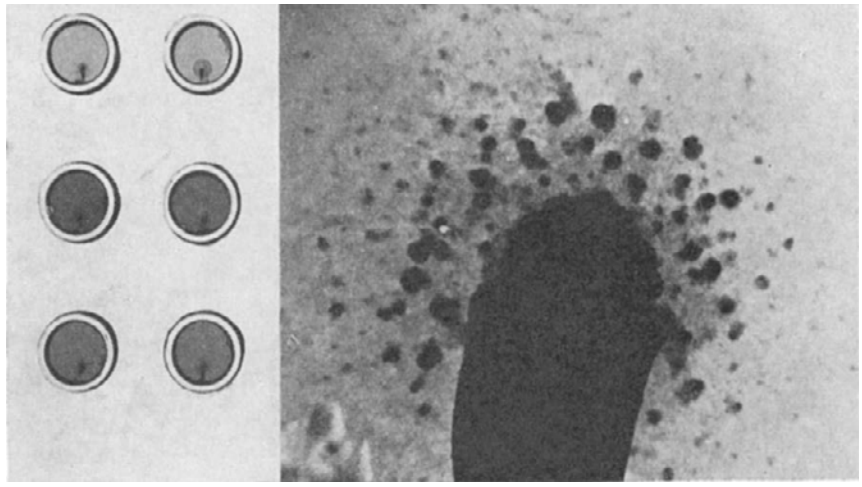

Figure 1. Capillaries and migrating leukocytes in 1 ml tissue culture chambers, scale 5:9 (left). Leukocyte migration colony with dark spots of accumulated cells, scale 16:1 (right).

normal horse serum. Transfer of the cells to the capillaries and the application in tissue culture chambers (Fig. 1) were done according to *Bendixen & Søborg*. After incubation at 37°C for 22 hrs. the area of the migrated cells was traced after projection through a microscope.

Comparison was made between planimetry based on weighing the traced figures and geometry with and without the capillary area included. The geometric value was obtained as the product of the major and minor axes of the elipsoid shaped cell colony. The differences between weighing and geometry were insignificant at all index levels whether or not the area of the capillary was included ($P > 0.30$—0.80). The differences obtained with

geometry with and without the capillary area included were also insignificant ($P > 0.70$—0.80). However, since the capillary area made up a significantly higher proportion ($P < 0.05$) of the geometric value in the group with lower migration indices ($\leqslant 0.59$) as compared to the group with higher indices ($\geqslant 0.90$), the capillary area was subtracted in the routine employment of the geometric planimetry.

Heparin, 25 i.u./ml of blood, was preferred as the anticoagulant since experiments with EDTA/calcium depletion of the blood had demonstrated that the specific reactivity of the leukocytes was reduced to a period of 6 hrs.

In a herd with a low, but persistent incidence of cattle infected with Mycobacterium paratuberculosis 72 animals more than 2 years of age have been examined. Animals were scored positive when the specific antigen, i.e. the Johnin, caused the migration index to fall below the point of the mean index for the whole group minus 1 s. Nineteen animals were positive to the migration inhibition test and 10 animals had complement fixing antibodies to the Johnin and/or avian antigen, but only 4 animals in each group were positive to both tests. It may be possible to titrate the test antigens to differentiate between infections with M. avium and M. paratuberculosis. Thus, the preliminary experiments with routine-testing of the migrating cells against the avian tuberculin and the Johnin may be interpreted to demonstrate that the titration point where a given increment in the dosage of antigen causes a substantial drop in the migration index is considerably more sharp with the homologous than with the heterologous antigen. Stimulation was seen when cells from reactors were exposed to sub-inhibition levels of the antigen.

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