Erythrophilic Proteins of Bovine Plasma

B. V. GOODGER

CSIRO, Division of Animal Health, Long Pocket Laboratories, Private Bag No. 3, P.O., Indooroopilly (Queensland, Australia 4068), 18 August 1975.

Summary. A saline eluate of sucrose washed bovine erythrocytes contains a few specific plasma proteins. The relationship between the proteins and erythrocyte morphology and physiology is discussed.

Erythrophilic γ-globulin (IgG E-C) is a plasma protein which coats human erythrocytes in vivo and which may be eluted with saline from sucrose washed erythrocytes[1,2]. Recently it has been demonstrated that IgG E-C levels increase in pathological conditions accompanied by acute haemolysis[3]. The present study was made to determine if a similar protein occurred in cattle and, if so, whether changes would be induced by the haemolysis associated with the intraerythrocytic protozoan parasite *Babesia argentina*. It was found that the saline eluate of sucrose washed bovine erythrocytes contained not only a protein similar to IgGE-C but also other plasma proteins.

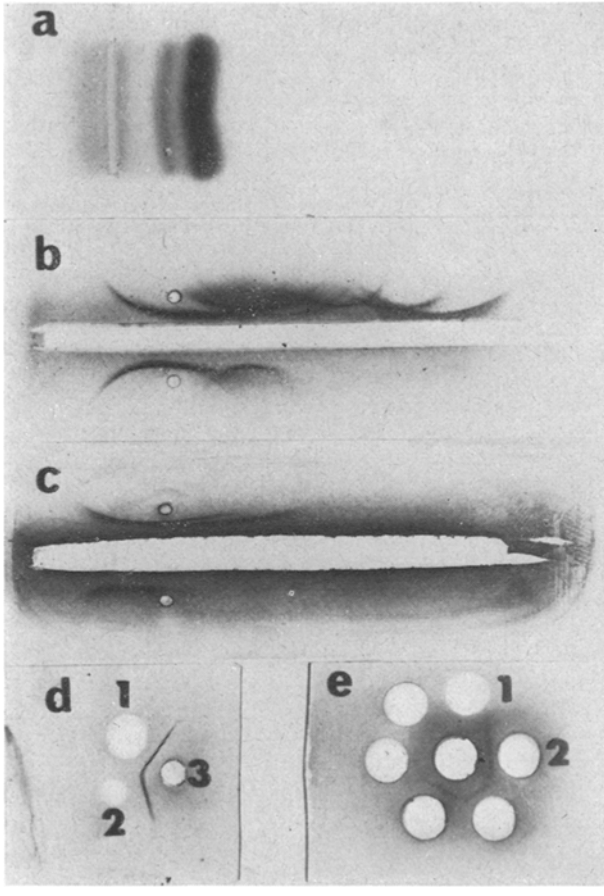

Fig. 1. a) Agar gel electrophoresis of the saline eluate of sucrose washed normal bovine erythrocytes. b) Immunoelectrophoresis of normal bovine plasma (upper well) and the saline eluate of sucrose washed normal bovine erythrocytes (lower well) reacted against rabbit antinormal bovine plasma (central trough). c) Immunoelectrophoresis of normal bovine plasma (upper well) and the saline eluate of sucrose washed normal bovine erythrocytes (lower well) reacted against rabbit antibovine IgG_2 (central trough). The long spur in the normal bovine plasma sample is due to the crossreaction with IgG_1. d) Immunodiffusion showing reaction of identity between the saline eluate of sucrose washed normal bovine erythrocytes (1) and normal bovine fibrinogen (2) when reacted against rabbit antinormal bovine fibrinogen (3). e) Immunodiffusion reaction with rabbit antibovine haptoglobin in the central well, saline eluate from an animal prior to infection with *B. argentina* in well 1 and saline eluate from the same animal on the day of death from *B. argentina* in well 2. The 4 remaining wells contained daily saline eluates for the 4 days prior to death.

Blood was collected into sterile trisodium citrate from healthy cattle and from cattle experimentally infected with *B. argentina* by blood passage from carrier animals[4]. Blood samples were centrifuged at 250 *g* for 10 min and the platelet rich plasma was removed. The blood was washed 3 times in 10 volumes of 0.27 *M* sucrose in 0.0004 *M* phosphate buffer pH 7.2 at 1500 *g* for 10 min at 4 °C. After each wash the top layers of leucocytes and platelets were also removed. The washed blood was suspended in 25 volumes of saline, incubated at 22 °C for 10 min and then centrifuged at 1500 *g* for 10 min at 4 °C. The supernatant was removed and the saline wash was repeated. The 2 saline supernatants were pooled and concentrated by ultrafiltration to the original volume of washed packed blood. Control eluates were obtained from both normal and infected blood by washing the blood 3 times in saline and using the 4th and 5th saline washes as eluate.

Pure bovine IgG_2, pure bovine haptoglobin and their respective rabbit antisera and rabbit antisera to normal bovine plasma were prepared as described elsewhere[5,6]. Rabbit antiserum was also prepared against bovine fibrinogen which had been obtained commercially[7] and further purified by gel filtration and preparative agar gel electrophoresis.

Agar gel electrophoresis (Figure 1a) of the saline eluate of sucrose-washed normal bovine erythrocytes showed a dense haemoglobin band, a lighter β_2-globulin band and a faint slow γ-globulin band. Immunoelectrophoresis and immunodiffusion always demonstrated fibrinogen and IgG_2 (Figure 1, b, c and d) while occasionally a faint band due to an unidentified β_2-globulin was also seen. The saline eluate of sucrose washed erythrocytes infected with *B. argentina* also contained haemoglobin, fibrinogen and IgG_2 and in addition bovine haptoglobin was demonstrated by immunodiffusion with specific antiserum (Figure 1e) and by the series of peroxidase positive molecular polymers separated by disc electrophoresis (Figure 2). By contrast the control saline eluate of saline washed erythrocytes contained only haemoglobin.

Haemagglutination was performed in microplates using 0.025 ml Takatsky loops and dropping pipettes. One drop (0.025 ml) of 0.5% sucrose washed erythrocytes suspended in sucrose-phosphate buffer pH 7.2 was added to each serial 2-fold dilution of heat inactivated antiserum and observed for agglutination after 30 min. Fibrinogen antiserum from a 1/1 to 1/4 dilution and IgG_2 antiserum

[1] B. V. FIDALGO, Y. Y. KATAYAMA and V. A. NAJJAR, Biochemistry *6*, 3378 (1967).
[2] T. S. THOMAIDIS, B. V. FIDALGO, S. HARSHMAN and V. A. NAJJAR, Biochemistry *6*, 3369 (1967).
[3] T. S. THOMAIDIS, H. VALASSI-ADAM and N. MATSANIOTIS, Clin. chim. Acta *57*, 109 (1974).
[4] D. F. MAHONEY, Aust. vet. J. *38*, 48 (1962).
[5] B. V. GOODGER, Res. vet. Sci. *12*, 465 (1971).
[6] B. V. GOODGER, Aust. J. exp. Biol. med. Sci. *50*, 11 (1972).
[7] BDH Chemicals Ltd, Poole, England.

from a 1/1 to 1/6 dilution agglutinated both sucrose washed normal erythrocytes and sucrose washed infected erythocytes whereas haptoglobin antiserum did not agglutinate normal erythrocytes but did agglutinate infected erythrocytes from a 1/1 to 1/4 dilution.

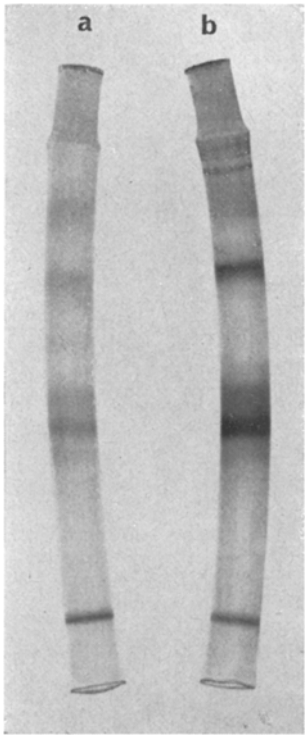

Fig. 2. Disc electrophoresis in 5% acrylamide gel of the saline eluate of sucrose washed normal bovine erythrocyte (a) and the saline eluate of sucrose washed bovine erythrocytes infected with *B. argentina* (b). The series of high molecular weight polymers in (b) was due to haptoglobin. The fast migrating band in both samples was due to a bovine albumin-amido black 10B marker.

These results are in accord with those of FIDALGO et al.[1] and indicate that sucrose washed erythrocytes appear to be coated with immunoglobulin. Whereas the human immunoglobulin is a distinct physiochemical fraction of IgG, the bovine counterpart appears to be solely IgG_2 as judged by its slow mobility and IgG reactivity. Like its human counterpart it probably aids in maintaining erythrocyte shape[8].

The presence of fibrinogen in the eluate of sucrose washed bovine erythrocytes requires some comment. It did not originate in platelets[9] which were removed during preparation of the erythrocytes. In addition, the haemagglutination of sucrose washed erythrocytes with fibrinogen antiserum indicated that the fibrinogen was complexed with erythrocytes. This finding agrees with the hypothesis that there is a basic relationship between erythrocytes and fibrinogen[10] and may explain why repeatedly washed stroma always contains this protein[11,12].

The presence of bovine haptoglobin in the eluate from sucrose washed erythrocytes infected with *B. argentina* is also of interest. Bovine haptoglobin differs markedly in polymerization sequence and chemical structure from its human counterparts and is not normally present in serum, but appears in times of stress[6]. Its presence in the eluate is difficult to explain but because of its specific and avid binding capacity for haemoglobin, it may act as a biological plug to prevent haemoglobin leakage from erythrocytes whose membranes have been damaged by the growth of the intracellular parasite.

[8] A. K. LAHIRI, W. M. MITCHELL and V. A. NAJJAR, J. biol. Chem. *245*, 3906 (1970).
[9] M. BETTEX-GALLAND and E. F. LÜSCHER, Adv. Protein Chem. *20*, 1 (1965).
[10] M. MURRAY and D. E. REARICK, Thromb. Res. *4*, 261 (1974).
[11] H. FURTHMAYR and R. TIMPL, Eur. J. Biochem. *15*, 301 (1970).
[12] B. V. GOODGER, Int. J. Parasit. *3*, 387 (1973).

Autoantibody Formation Against Spleen in Rats 'Chemically Splenectomized' by Ethyl Palmitate

V. ŠEBESTÍK and Z. JEŽKOVÁ

Institute of Haematology and Blood Transfusion, Unemocnice 1, CSSR-128 20 Praha 2 (Czechoslovakia),
3 November 1975.

Summary. The follow-up experiments with rats revealed auto-antibodies against spleens damaged by an i.v. ethyl palmitate injection. The antibodies could be shown in some animals only.

The work of STUART[1] and PROSNITZ et al.[2] report on a single i.v. injection of ethyl palmitate (EP) causing acute spleen destruction in some laboratory animals, a so-called 'chemical splenectomy'. We found a similar pharmaceutical effect of EP in a model experiment with Wistar rats (JIRÁSEK and ŠEBESTIK[3], ŠEBESTÍK et al.[4]). The administration of EP caused an initial storing of this drug chiefly in cells of spleen sinuses; after 24–48 h most animals developed a segmental or even total necrosis of the spleen. On day 5 to 7, following application of EP, some spleens revealed signs of recovery proportional to the extent of damage. The histological effect of EP, especially at the initial stage following injection of EP, was followed by a pronounced metabolic alteration of proteins and nucleic acids in the spleen (KUŽELA and ŠEBESTÍK[5]). Thus the damaged organ, as known from human pathology, may act in some diseases as an alien antigen and induce antibody formation. Since some research workers experiment with the possible application of EP which may abolish non-surgically, partially or completely, the function of the spleen, this finding is expected to become of actual importance.

[1] A. E. STUART, Lancet *2*, 896 (1960).
[2] L. PROSNITZ, SHO. KOWASAKI, G. S. COHEN, J. L. DINEEN, P. E. PERILLE and S. C. FINCH, J. reticuloendot. Soc. *6*, 487 (1969).
[3] A. JIRÁSEK and V. ŠEBESTÍK, Čs. Pat., *11*, 147 (1975).
[4] V. ŠEBESTÍK, I. POTMĚŠILOVÁ, A. JIRÁSEK, J. JELÍNEK, Physiol. bohemos., in press.
[5] L. KUŽELA and V. ŠEBESTÍK, in preparation.