Acta vet. scand. 1975, *16*, 520—524.

From the Research Station of the Veterinary Institute, Skara, Sweden.

AGAROSE GEL ELECTROPHORETIC SEPARATION OF BLOOD SERUM PROTEINS IN CATTLE*

By

Gunnar Carlström and *Per Liberg*

CARLSTRÖM, GUNNAR and PER LIBERG: *Agarose gel electrophoretic separation of blood serum proteins in cattle.* Acta vet. scand. 1975, *16*, 520—524. — The agarose gel electrophoresis described by *Johansson* (1972) was modified so that a buffer of pH 7.9 was used in the gel, whereas the buffer in the electrode vessels had a pH of 8.6.

The cattle blood serum protein picture is described in detail. The β_1-globulin zone shows a very distinct picture of the genetically polymorphic bovine transferrins. The region between the α- and β-globulins shows a number of faint and often very distinct bands. A faint background staining over the whole electrophoretogram may partly be caused by a rather strong lipoprotein in the α_1-region, lipids thus having migrated all over the electrophoretogram.

The modified method described is well suited as a "screen electrophoresis" for cattle serum and is also useful e.g. in studying bovine transferrin polymorphism.

electrophoresis; agarose gel electrophoresis; screen; bovine serum; cattle.

The electrophoretic technique has in recent years been greatly developed within human medicine, the change from separation on paper to separation in other media having provided much improved diagnostic possibilities. With zone electrophoresis on paper the serum proteins in cattle can be separated into only a few groups: albumin, α-, β- and γ-globulins, which not necessarily have any functional relationship. Paper electrophoresis of blood serum from cattle has obvious disadvantages, with considerable zone dispersion and rather bad resolution.

* The investigations were supported by the Swedish Council for Forestry and Agricultural Research.

Using gel as supporting medium in zone electrophoresis there is practically no convection. Proteins are absorbed very weakly by the gel, and the zone dispersion owing to diffusion is strictly limited. Agarose gel is almost free from ionized groups and does not cause electroendosmosis and — unlike polyacrylamide gel, for instance — does not interfere with certain basic proteins. With agar gel and particularly agarose gel electrophoresis there are possibilities of estimating the distribution of several separate proteins within the different fractions. A detailed description of the agarose gel electrophoresis technique and its use on human blood plasma proteins has been presented by *Johansson* (1972).

METHODS

The barbital buffer, pH 8.6, recommended by *Johansson* (1972) has been used in the electrode vessels. Using the same buffer in the supporting gel on the electrophoresis plate, the separation of the β- and γ-globulins in cattle will be poor. As buffer in the agarose gel, use has instead been made of a barbital hydrochloric acid buffer, pH 7.9 (10.3 g barbital sodium + 23.5 ml HCl, 0.1 N, in water to 1000 ml). With this double buffer system a good separation has been obtained over the entire electrophoresis picture, not least in the β-region. During the electrophoresis the sample plate has mostly been cooled with circulating ice-water. Under these circumstances no problems have arisen with gel desiccation or condensation on the gel plate or on the underside of the cover-glass of the electrophoresis apparatus. Circulating cold tapwater has also been tried and the higher temperature has often had a favourable effect on the sharpness in the zones. Drying-out and condensation, however, occur more often in the latter case. With the above-mentioned buffer and cooling system a voltage drop of about 20 v/cm over the gel plate has been used and has given an electrophoresis time (migration 5 cm) of 40—50 min.

The addition of calcium lactate to the buffer has been recommended (*Wieme* 1959, *Laurell* 1966). Calcium ions have been considered necessary for optimal migration of lipoproteins, which otherwise would interfere with the sulphate groups in the gel. No such optimizing effect of calcium lactate has been observed in the present investigations, perhaps because cattle serum normally contains low lipoprotein concentrations. The French aga-

rose Indubiose A 37 (L'Industrie Biologique Française, S.A.) (1 %) gives better resolution than Miles Seravac's agarose. For contact bridges trials have been made with gel as described by *Johansson,* as also with different types of paper. Several layers of filter paper (Munktell's chromatographic paper 302) have been found to give good contact and are considerably easier to handle than agarose gel bridges.

RESULTS AND DISCUSSION

The electrophoresis picture for cattle serum is illustrated in Fig. 1. It is characterized by good resolution with distinct zones. There is a faint background staining over the electrophoresis

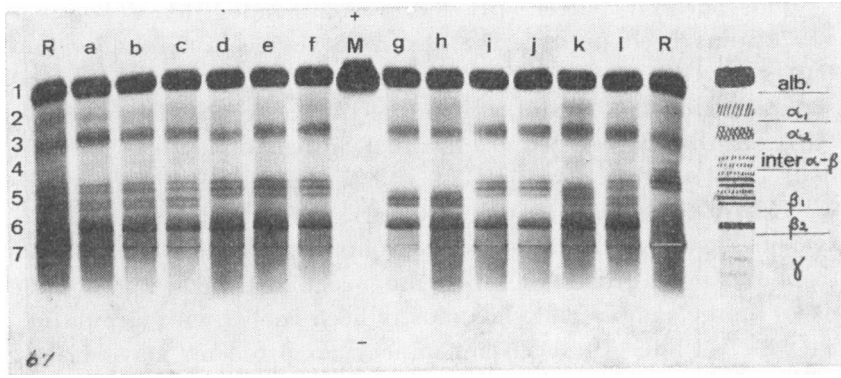

Figure 1. The electrophoresis picture of fresh sera from 12 clinically healthy cows (a-l). R are reference sera. M is a front-marker (albumin).
To the right a general sketch in outline.

picture. Possibly the fairly strong α_1-lipoprotein band which migrates all the way from application slit to just after the albumin front might have some significance for this phenomenon. For the region between β_1-globulin and albumin this is supported by the fact that lipid staining as well as protein staining causes a diffuse background — more so in cattle than in human serum — and in about the same proportion as the α_1-lipoprotein zone is stronger in cattle than in human serum.

The following main components are found, taken in order from anode to cathode:

1. A broad, intensively stained albumin front zone. Lipid staining shows a rather faint band here.

2. A broad, in varying degree diffuse, α_1-globulin band. This area shows the strongest lipoprotein zone. The α_1-globulin band probably appears more diffuse at high α_1-lipoprotein concentrations.

3. A strong, sometimes relatively distinct, sometimes more diffuse, α_2-globuline zone which in the great majority of cases is separated into two or more bands.

4. A broad region between the "classical" α- and β-globulin zones, which contains a series of barely visible but distinct bands. Almost constantly one or two of these bands are distinguishable. Lipid staining shows a lipoprotein band in this area.

5. A broad zone containing several very distinct β_1 bands. The genetically polymorphic transferrin picture seems to appear as clearly as in starch gel electrophoresis, which has usually been used for studies of polymorphism in bovine transferrins. Such studies, performed by agarose gel electrophoresis, are in progress.

6. The β_2-globulin zone, the last zone on the anodic side of the application slit, contains an often strong, distinct band when the sample is fresh, but at least on lenghty frozen storage of serum the zone is often divided into two more or less diffuse bands. In haemolyzed samples haemoglobin appears in this area, as does fibrinogen in plasma samples.

7. The γ-globulin area forms a background to the β_2-globulin area and continues as a very broad diffuse zone on the cathode side of the slit. The major cathodic part consists chiefly of immunoglobulin G. In this area very distinct, sometimes faint, sometimes strong or very strong extra bands are often seen. This is evident in sera from animals with an early and often intensive immunological response (oligoclonal hypergammaglobulinaemia).

The electrophoresis pattern in cattle largely resembles that in man. A number of identified proteins on the human electrophoretogram may be presumed to be a direct counterpart of the bovine. For bovine immunoglobulins, for example, there is a well documented antigen cross-reaction with homologous human immunoglobulins (among others *Murphy et al.* 1965; see review by *Aalund* 1972). The transferrin polymorphism in cattle on the other hand is an example of a species-specific picture. Prealbu-

min, which is sometimes seen in human electrophoretograms, has not been observed in the present investigations on cattle.

The agarose gel electrophoresis has a high capacity, especially compared with conventional paper electrophoresis. The latter is slow, so that the result of the analysis cannot be presented until the next day. The preparation time with agarose gel electrophoresis may be estimated at 45 min. The electrophoresis takes about 45 min. and fixat'on, drying and staining of the electrophoresis plate take roughly 1 hr. This amounts to 2½ hrs. in all. Each plate accommodates 12 serum samples (excluding marker and references).

REFERENCES

Aalund, O.: Immune response of sheep, goats, cattle and swine. *In* Immunity to Animal Parasites. Acad. Press, Inc., New York and London 1972.

Johansson, B. G.: Agarose gel electrophoresis. *In* C.-B. Laurell: Electrophoretic and Electro-Immuno-Chemical Analysis of Proteins. Scand. J. clin. Lab. Invest. 1972, *29*, Suppl. 124.

Laurell, C.-B.: Quantitative estimations by electrophoresis in agarose gel containing antibodies. Analyt. Biochem. 1966, *15*, 45—52.

Murphy, F. A., J. W. Osebold & O. Aalund: Physical heterogeneity of bovine γ-globulins: Characterization of γM and γG globulins. Arch. Biochem. 1965, *112*, 126—136.

Wieme, R. J.: Studies on agar gel electrophoresis. (Arcia Uitgaven N. V. Brussels; and Elsevier, Amsterdam 1965). 1959.

SAMMANFATTNING

Agarosgelelektrofores av blodserumproteiner hos nötkreatur.

Den agarosgelelektrofores som beskrivits av *Johansson* (1972) har modifierats så att buffert med pH 7,9 användes i gelet, medan bufferten i elektrodkärlen är av pH 8,6. Med denna och några andra, smärre modifikationer har metoden blivit lämplig för "screen"-elektroforetiska undersökningar av blod från nötkreatur.

Den serumproteinbild som erhålles hos nötkreatur med denna metod beskrivs i detalj. β_1-globulin-zonen uppvisar en mycket distinkt bild av transferrinpolymorfismen hos nötkreatur. Området mellan α- och β-globulin visar ett antal svaga men ofta mycket distinkta proteinband. En svag bakgrundsfärgning över hela elektroforetogrammet kan möjligen till viss del orsakas av ett ganska starkt α_1-lipoprotein, där sålunda lipoider migrerat hela vägen över elektroforetogrammet.

(Received October 27, 1975).

Reprints may be requested from: P. Liberg, the Research Station of the Veterinary Institute, Box 180, S-532 00 Skara, Sweden.