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APPLICATION OF RADIOIMMUNOLOGICAL METHODS FOR CHECKING THE QUALITY OF CLASS-SPECIFIC ANTIBODIES AGAINST BOVINE AND PORCINE IMMUNOGLOBULINS

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ABSTRACT

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Class-specific antibodies against bovine IgG1, IgG2, IgM and IgA and porcine IgG, IgM and IgA immunoglobulins were prepared. Their class specificity was assessed by two radioimmunological methods, namely, radioimmunoelectrophoresis and double antibody sandwich radioimmunoassay. The methods are highly specific and sensitive and do not require the use of purified immunoglobulins, but can be performed with normal serum or colostrum. It was confirmed that antibodies found satisfactory in these tests were suitable for a wide range of use including radioimmunoassay and enzyme linked immunosorbent assay.

INTRODUCTION

A number of immunological methods make current use of anti-immunoglobulin antibodies for the needs of both research and practical diagnosis. Labelled with radioactive isotope or conjugated with enzyme, these antibodies are also used in a number of radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISA). However for more detailed immunological studies on the dynamics and character of the immune response, it is necessary to use specific antibodies to individual immunoglobulin classes. Since suitable class-specific antibodies to bovine and porcine immunoglobulin classes are not currently available, we decided to prepare our own monospecific antisera and to isolate antibodies suitable for labelling with ^{125}I or conjugation with enzyme.

The class specificity of the antibodies was assessed by radioimmunoelectrophoresis and double antibody sandwich RIA. These methods are highly specific, sensitive, do not require the use of purified immunoglobulins, but can be performed using normal serum or colostrum. The results obtained in our study confirmed good

quality of the antibodies and their suitability for a wide range of use including RIA and ELISA.

METHODS

Preparation of antisera and isolation of immunoglobulins

The animals employed were Chinchilla rabbits weighing 2 to 3 kg. Antisera against various immunoglobulin classes were prepared by repeated immunization of the rabbits with purified immunoglobulins or antigen-antibody complexes in Freund's adjuvant (Duncan et al., 1972; Nansen et al., 1971).

Isolation of bovine and porcine immunoglobulins was carried out according to standard procedures (Masseyeff et al., 1965; Bourne 1969; Butler and Maxwell, 1972; Cambier and Butler, 1974).

Isolation of specific antibodies

The antisera prepared were freed from non-specific antibodies by affinity chromatography on Sepharose 4B (Pharmacia) with covalently bound bovine or porcine immunoglobulins of the corresponding classes (March et al., 1974). The same method was used to isolate specific antibodies against individual immunoglobulin classes from the monospecific antisera. The yields depended on the quality of the antisera, ranging from 1 to 3.5 mg antibody/ml antiserum. After elution with 0.1 M glycine-HCl buffer, pH 2.8, and dialysis, the antibodies were lyophilized and stored in sealed ampoules until use.

Labelling of antibodies with ^{125}I

Specific antibodies were labelled by means of chloramine T iodination (Mc Conahey and Dixon, 1966). More than 98 % of non-dialysable activity was precipitated with 10 % trichloracetic acid. Specific activity of the labelled proteins ranged between 18.5 and 185 MBq/mg. The labelled antibodies were kept in phosphate buffered saline (PBS) supplemented with 10 mg/ml rabbit serum albumin (RSA) or with 10 % rabbit serum obtained from non-immunized animals.

RESULTS

Radioimmunoelectrophoresis

Electrophoretic separation of pig and bovine sera and bovine colostral whey was carried out in 1.5 % agar (Noble agar, Difco) containing 0.05 M veronal buffer, pH 8.6, on glass plates (Rejnek and Bednařík, 1960). After the separation was completed, rabbit precipitating antisera against porcine (RASw) or bovine (RAB) serum and colostrum proteins were placed into troughs. Antiserum in individual troughs contained supplements of ^{125}I-labelled anti--immunoglobulin antibodies of different class specificity with an activity of 30 to 70 KBq/ml antiserum. After precipitation lines had been formed, the plates were thoroughly washed in PBS for 2 days, air-dried and covered with X-ray film (Medix Rapid, Foma). The film was developed after exposure for 2 to 3 days, the plates were stained with amido black 10B and the precipitation lines in agar were compared with the lines exposed on the film. With this method, serum and colostral proteins are precipitated with rabbit antisera (RASw or RAB) which form precipitation lines with individual proteins. ^{125}I-labelled antibodies added to these antisera have only a share in the formation of precipitation lines.

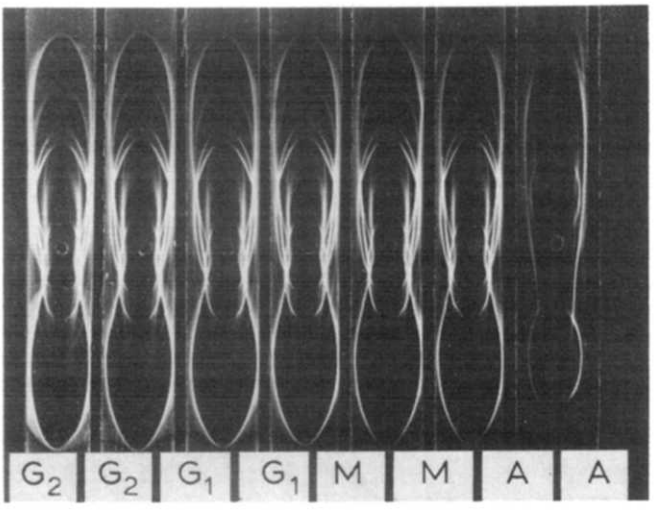

Fig. 1A. A radioimmunoelectrophoretic check on the class specificity of antibodies against bovine immunoglobulins. Wells left to right: Nos. 1-6, bovine serum; No. 7, colostral whey. RABS was placed in all troughs. ^{125}I-labelled antibodies against bovine IgG2, IgG1, IgA and IgM were added to antiserum in troughs designated as G2, G1, A and M, respectively.

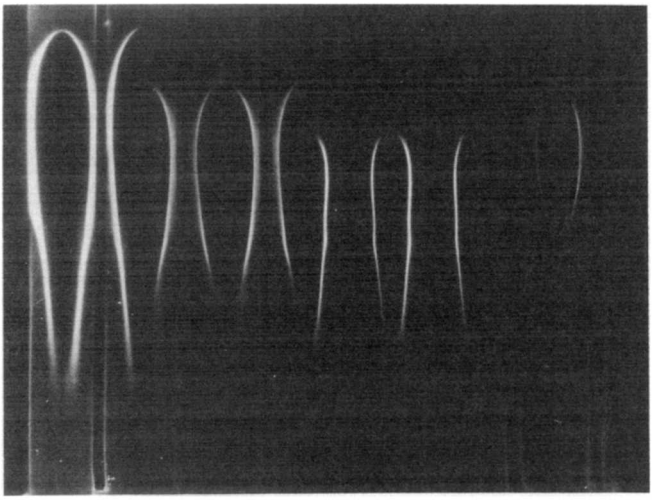

Fig. 1B. X-ray film after exposure with electropherogram 1A. The binding of the labelled antibodies was demonstrated always in a single precipitation line. The location and character of the lines confirm the monospecificity of the labelled antibodies.

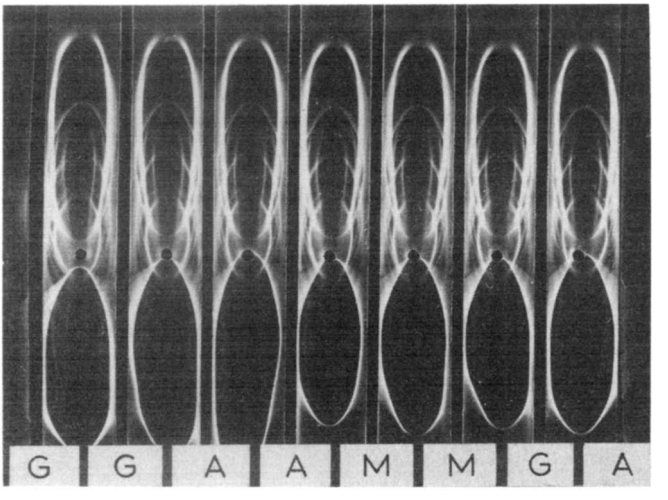

Fig. 2A. A radioimmunoelectrophoretic check on the class specificity of antibodies against porcine immunoglobulins. Pig serum was placed in wells of the electropherogram and RASw was placed in the troughs. ^{125}I-labelled antibodies against porcine immunoglobulins IgG, IgA and IgM were added to antiserum in troughs designated as G, A and M, respectively.

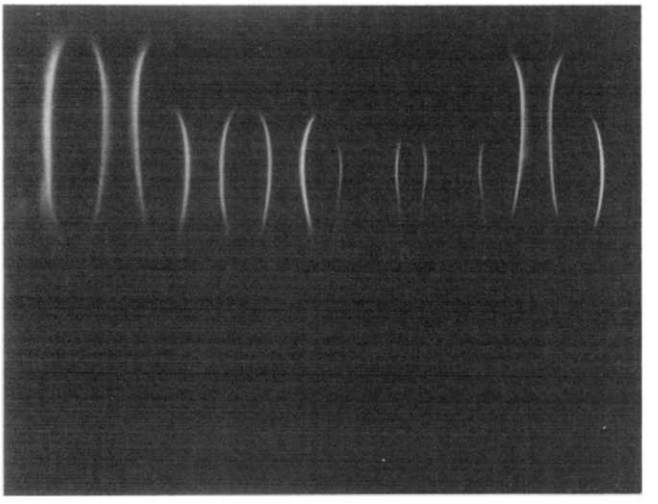

Fig. 2B. X-ray film after exposure with electropherogram 2A. The binding of the labelled antibodies was demonstrated always in a single precipitation line. The location and character of the lines confirm the monospecificity of the labelled antibodies.

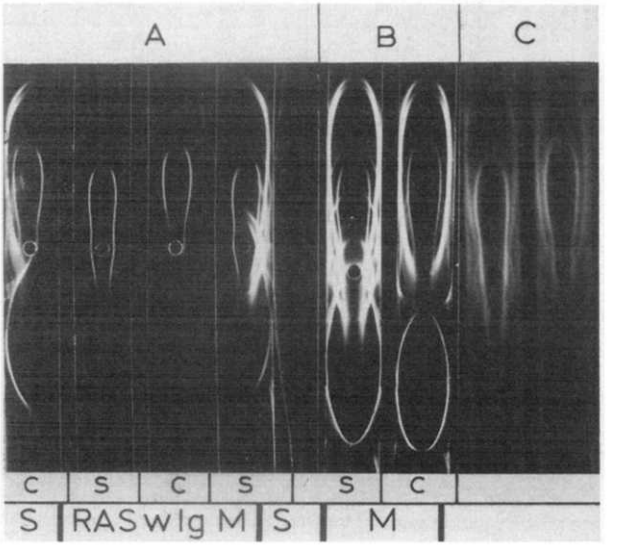

Fig. 3. Comparison of the sensitivity of immunoelectrophoresis and radioimmunoelectrophoresis in checks on the specificity of rabbit antiserum against porcine IgM (RASwIgM). A, immunoelectrophoresis; B, radioimmunoelectrophoresis; C, exposed X-ray film. Wells: pig serum (s) or colostrum (c). Troughs: rabbit antiserum against pig serum and colostrum (RASw designated here as S); RASwIgM; RASw supplemented with ^{125}I-labelled Ig fraction of RASwIgM (M).

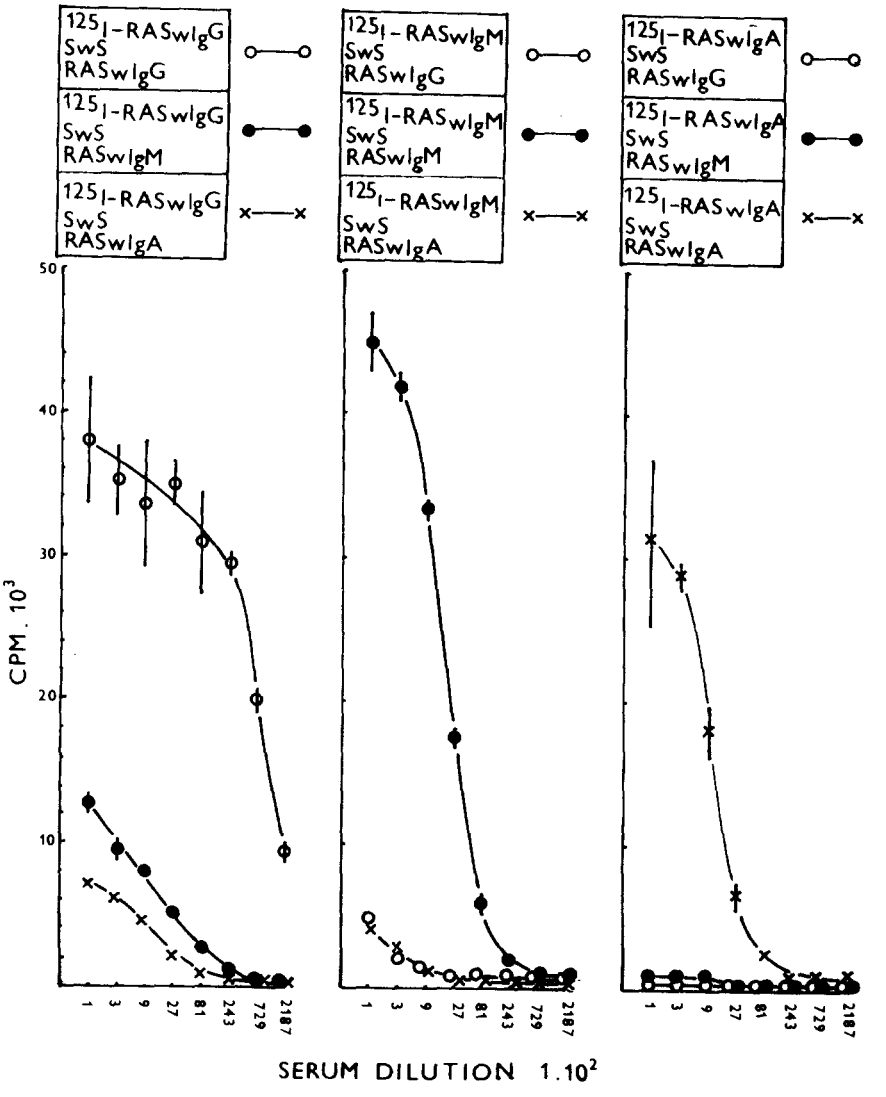

Fig. 4. Graphical presentation of the results of a check on the specificity of antibodies against porcine immunoglobulins by double antibody sandwich RIA. Mean radioactivities ± SD of quadruplicate wells coated with rabbit antibodies against porcine immunoglobulins RASwIgG (o——o), RASwIgM (●——●) and RASwIgA (x——x). After incubation with pig serum diluted 1:100 to 218 700 the specificity of reactions was demonstrated by means of the same antibodies labelled with ^{125}I.

Labelled monospecific antibodies are required to incorporate only into a single, precisely determined precipitation line and not to react with other protein antigens. Presence of non-specific antibodies is manifested by radioactivity binding also in other precipitation lines. The results obtained (Fig. 1 and 2) show that ^{125}I-labelled antibodies against bovine and porcine immunoglobulins are strictly class-specific because they react only with the required immunoglobulin classes and their precipitation lines are the only ones exposed in X-ray film.

The higher sensitivity of radioimmunoelectrophoresis as compared with electrophoresis was demonstrated by checks on the quality of rabbit antiserum against porcine IgM (RASwIgM). On immunoelectrophoretic examination this antiserum was monospecific and formed precipitation lines only with serum and colostral IgM (Fig. 3A). The crude immunoglobulin fraction of this antiserum obtained by precipitation with ammonium sulphate (twice at 45 % saturation) and labelled with ^{125}I was used for radioimmunoelectrophoretic examination (Fig. 3B). The lines exposed on X-ray film (Fig. 3C) show that the labelled antibodies reacted not only with porcine IgM but also with IgG and serum alpha 2-macroglobulin.

Checks on the specificity of antibodies by double antibody sandwich RIA

The quality of the antibodies obtained was also checked by double antibody sandwich RIA. However, only the results of evaluation of antibodies against porcine immunoglobulin classes are presented here in detail.

Disposable polystyrene flat-bottomed microtubes in plastic plate holders were employed (the size and arrangement of the wells were the same as in the plates for the microtitre ELISA). In each of three plates employed, four of the 12 vertical rows of wells were filled with one of the solutions of the tested rabbit antibodies against porcine immunoglobulin classes (RASwIgG, RASwIgM, RASwIgA). A 100 µl amount of antibody solution in 0.1 mol carbonate-bicarbonate buffer, pH 9.6, having the antibody concentration of 10 µg/ml, was placed in each well. After adsorption of the antibodies overnight at 20 °C and subsequent washing, all the wells were filled with 100 µl amounts of normal pig serum diluted threefold in the range of 1:100 to 1:218 700 with PBS containing 0.5 % bovine serum albumin (BSA) and 0.02 % Tween 20.

Each horizontal row of 12 wells contained one serum dilution. After incubation for 2 hours at 20 °C and subsequent washing the plates were incubated with the solution of the tested ^{125}I--labelled class-specific antibodies under the same conditions. All the wells of one plate were filled with labelled antibodies of one class specificity. To each well, 100 µl of the antibody solution in PBS supplemented with 0.5 % RSA and 0.02 % Tween, having the activity of 15 KBq/ml, was added. After final washing (each washing three times with PBS) the activities bound in individual wells were measured with an NE 1600 counter for ^{125}I (Nuclear Enterprises, Ltd, Edinburgh) and mean activities and standard deviations were calculated from groups of four equally incubated wells (Fig. 4). On the third incubation with ^{125}I--labelled specific antibodies during this procedure, the binding of antibodies and therefore also of radioactivity occurred only in those wells that contained the specifically bound pig serum immunoglobulin. In other words, the radioactivity of the wells treated on the first and third incubations with antibodies of the same class specificity must have been significantly higher than the radioactivity in the wells incubated with labelled antibodies of different class specificity. The mode of treatment of the wells and the results are presented graphically in Fig. 4. The curves showing the mean radioactivity of quadruplicate wells incubated with various serum dilutions confirmed high specificity of the antibodies. The gradual decrease of the activities bound in the wells was directly proportional to the degree of dilution and to the initial concentration of immunoglobulin classes in the pig serum. Another measure of the quality of antibodies is the highest ratios of the radioactivities bound in wells incubated with various antibody combinations on the first and third incubations. If in both cases antibodies against porcine IgG were employed, i.e. in the combination IgG-SwS-^{125}I IgG (thereafter referred to as G-G, etc.), the activity in the wells was as much as 59.9 and 35.6 times as high as in the combinations A-G and M-G, respectively. The highest ratios were obtained at the serum dilution of 1:72 900. Analogously, in the combination A-A the activities in the wells were as much as 93.9 and 37.3 times as high as in the combinations G-A and M-A, respectively. The highest ratios were obtained at the serum dilution of 1:300. In the combination M-M the mean activity in the wells was as much as 24.9 and 23.1 times as high as in combinations G-M and A-M, respectively, and the highest ratios were obtained at the serum dilution of 1:900.

DISCUSSION

Antibodies combine with the corresponding antigen to produce a complex which is sufficiently stable over a wide range of pH. An important criterion in addition to the avidity and activity of antibodies is their specificity. Antibodies having insufficient specificity react with a number of other similar antigenic structures and their applicability for immunological studies is thus limited. Class-specific anti-immunoglobulin antibodies are required to react only with a certain immunoglobulin class, i.e. with specific antigenic structures of the heavy chains of immunoglobulin molecules. There are a number of methods for checks on class-specific anti-immunoglobulin antibodies. The most widely used are those based on agar-gel precipitation, namely, immunoelectrophoresis, double radial immunodiffusion (Ouchterlony) etc. (Butler and Maxwell, 1972; Duncan et al., 1972). Antibodies or antisera that prove satisfactory by these methods are generally of sufficient quality also for other immunological studies. The presence of non-specific antibodies, however, may not be demonstrated, particularly if the non-specific precipitation activity was removed by dilution or insufficient immunosorption of the antiserum. Therefore agglutination method is used for more exact purposes (Hirota et al., 1980). In this method monospecific antiserum agglutinates only those erythrocytes on the surface of which immunoglobulin of the corresponding class has been adsorbed. The disadvantage of this test is that accurate results can be obtained only with highly purified immunoglobulins and that it is not possible to reveal the presence of antibodies against proteins of non-immunoglobulin nature. On the analogous principle of the direct reaction of antibody with the corresponding antigen (immunoglobulin), a check on the class specificity of antibodies was developed using an enzyme linked immunosorbent assay (Franz and Corthier, 1981).

The antibodies prepared by us were therefore evaluated by means of the radioimmunoelectrophoretic method. This method is highly specific and has the advantage over the latter test in that it uses normal serum or colostrum as the source of antigens. In this method, ^{125}I-labelled specific antibodies participate in the formation of a certain precipitation line. The presence of

non-specific antibodies is manifested in that the radioactivity is demonstrated also in other precipitation lines. The fact that ^{125}I-labelled antibodies have only a share in the formation of the precipitation lines makes it possible to demonstrate even low non-specific activity which is not revealed by common precipitation methods (e.g. immunoelectrophoresis). The results presented here (Fig. 1, 2 and 3) confirm that the antibodies tested are strictly class-specific and that low concentrations of non-specific antibodies in insufficiently saturated monospecific sera cannot be detected by immunoelectrophoresis where considerable disproportion between the antigen and antibody concentrations makes formation of the respective precipitation lines impossible.

The quality of the antibodies prepared was also assessed by means of double antibody sandwich RIA. This method, too, uses normal serum or colostrum as the source of antigen. We presumed that class-specific antibodies bound to the microtube walls would react only with immunoglobulins of one class on incubation with the serum. The binding of these immunoglobulins would be demonstrated by ^{125}I-labelled antibodies. The activity demonstrated in wells incubated repeatedly with solutions of antibodies of the same class specificity would be significantly higher than that found in wells incubated with antibodies of different class specificity. In our experiments this presumption was confirmed (Fig. 4) and the activities for same-class were as much as 23.1 to 93.9 times higher than for different-class specificity. In RIA demonstration of anti-virus antibodies using triplicate determinations, even a double increase in activity as compared with control samples was regarded as highly significant (Rodák et al., 1981, 1982). Antibodies that proved satisfactory by this method can therefore also be regarded as class-specific. After labelling with radioactive isotopes or conjugation with enzyme such antibodies can be used even for radio- and enzymoimmunological studies. Their usefulness was confirmed in a study by RIA of the dynamics of the production of class-specific antiviral antibodies in cattle (Rodák et al., 1983).

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REFERENCES

Bourne, F.J., 1969. IgA immunoglobulin from porcine serum. Biochem. giophys. Res. Commun., 36: 138-145.

Butler, J.E. and Maxwell, C.F., 1972. Preparation of bovine immunoglobulins and free secretory component and their specific antisera. J. Dairy Sci., 55: 151-164.

Cambier, J.C. and Butler, J.E., 1974. A rapid method for the purification of immunoglobulin M (IgM) from the sera of certain mammalian species. Prep. Biochem., 4: 31-46.

Duncan, J.R., Wilkie, B.N., Hestand, F. and Winter, A.J., 1972. The serum and secretory immunoglobulins of cattle: characterization and quantitation. J. Immunol., 108: 965-976.

Franz, J. and Corthier, G., 1981. Measurement of porcine faecal IgA, IgG and IgM levels by a competitive enzyme-linked immunosorbent assay. Clin. exp. Immunol., 44: 645-649.

Hirota, Y., Vainio, O., Sawada, T. and Bito, Y., 1980. A simple method for detecting cells producing antibodies of specific immunoglobulin classes in the chicken. J. immun. Meth., 33: 379-386.

March, S.C., Parikh, I. and Cuatrecasas, P., 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. Anal. Biochem., 60: 149-155.

Masseyeff, R., Gombert, J. and Josselin, J., 1965. Methode de préparation de la beta$_2$-macroglobuline du sérum humain. Immunochemistry, 2: 177-186.

McConahey, P.J. and Dixon, F.J., 1966. A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy, 29: 185-189.

Nansen, P., Flagstad, T. and Pedersen, K.B., 1971. Preparation of antisera to bovine immunoglobulin classes by immunization with agar-gel precipitates. Acta path. microbiol. scand. B, 79: 459-465.

Rejnek, J. and Bednařík, T., 1960. Radioimmunoelectrophoresis. Clin. Chim. Acta, 5: 250-258.

Rodák, L., Šmíd, B., Holub, A. and Sedláček, M., 1981. Application of solid-phase radioimmunoassay in the diagnosis of Aujeszky's disease in naturally infected pigs. Acta vet. Brno, 50: 213-220.

Rodák, L., Babiuk, L.A. and Acres, S., 1982. Detection by radioimmunoassay and enzyme-linked immunosorbent assay of coronavirus antibodies in bovine serum and lacteal secretions. J.clin. Microbiol., 16: 34-40.

Rodák, L., Pospíšil, Z. and Hampl, J., 1983. A study of the dynamics of the production of class-specific antibodies to Infectious bovine rhinotracheitis (IBR) virus in calves using a solid-phase radioimmunoassay. Zbl. Vet. Med. B., in press.