Studies on the Immunoglobulin Spectrum of Porcine Serum and Colostrum

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ABSTRACT. An analysis of the immunoglobulin region of porcine blood serum proved the presence of a number of protein fractions which is higher than in other animal species. The presence of antibody activity to tetanus anatoxin examined by radioimmunoelectrophoresis was found in three γ G-globulin fractions, which could be distinguished from each other, and also in two additional fractions. One of the latter was the γ-macroglobulin fraction and the other one might correspond to γ A-globulin even though its mobility was higher than the mobility of γ A-globulin of human serum. A comparison of the immunoglobulin spectrum of porcine serum, colostrum, and of the suckling pig serum after ingestion of the colostrum showed that the main component of porcine colostrum constitutes the γ G-immunoglobulin, even though the colostrum seems to contain other components of the latter which have a higher electrophoretic mobility. The antibody activity of colostrum was found only in fractions γ G and γ M. The fraction of colostrum which might correspond to γ A did not display any activity at all. The activity in suckling pig serum was also concentrated in fractions γ G and γ M.

The importance of immune globulins of the colostrum and milk of mammals for the transfer of immunity from the mother to her descendants has been followed by many authors for a number of years (Timmermann, 1931; Smith & Holen, 1948; Edsall, 1956; Boorman *et al.*, 1958; Greenbaum & Miller, 1960; Dixon *et al.*, 1961; Sussman, 1961; Pierce & Feinstein 1965). The course of the process has been studied most intensively in man and the examination of the relationship between serum and milk proteins (Gugler *et al.*, 1958; Gugler & Muralt, 1959; Rejnek *et al.*, 1960; Hanson, 1960, 1962; Schwick *et al.*, 1959; Silva & Monteiro, 1959; Rejnek, 1964) showed that, in spite of the fact that the immune globulins of colostrum and milk come from the serum, (Askonas *et al.*, 1954; Larson & Gillespie, 1957; Dixon *et al.*, 1961; Feldman, 1961) both colostrum and milk contain above all the immunoglobulins of the γA and γ M type. The γ G-globulin which is preponderant in the serum is present only in small quantities. The ability of the mammary glands to absorb only certain types of the serum immunoglobulins becomes even more interesting when we consider the fact that the colostrum is abundant in types of immunoglobulins not found in the umbilical serum of the newborn and therefore obviously unable to pass through the placenta which is responsible for the passive transfer of immunity in man.

The diaplacental transfer of immunity has not been found in all mammals. Thus, in certain rodents, e.g. rabbits the antibodies are supplied to the fetus, via the vessels of the yolk-sac splanchnopleur (Brambell *et al.*, 1951; Batty *et al.*, 1954; Hemmings, 1956; Hemmings & Oakley, 1957). A certain portion of the antibodies is probably transferred to the newborn organism in the colostrum and milk. The colostrum represents the only source of antibody transfer in animals with an epitheliochorial placenta (pig, horse)

where the transfer of immunoglobulins via the placenta does not exist.

We thought therefore that it might be useful to determine whether in such animal species as the pig, whose six-layer placenta is completely impenetrable for antibodies, the colostrum contains all classes of immunoglobulins present in the mother serum, or whether it is deficient in some type of antibodies, similarly to the human serum.

MATERIALS AND METHODS

Hyperimmune porcine serum to tetanic toxoid was prepared by immunization of an adult animal or a pregnant sow with 2 ml of the tetanic toxoid (Institute of Sera and Vaccines, Prague). The latter was mixed with 3 ml of the complete Freund's adjuvant and applied into the foot pads of the animal 10 and 6 weeks before the withdrawal of blood or before the expected date of parturition. Two weeks before this date 2 ml of the tetanic toxoid was administered intravenously. The serum was then prepared by centrifugation of blood removed from the aural vein and kept frozen until the beginning of the experiment.

Immunoelectrophoretic analyses were carried out as described before (Škvařil & Rejnek, 1958). Rabbit antisera to porcine serum and colostrum proteins prepared as described in one of the previous communications (Rejnek *et al.*, 1965) were used for precipitation.

Radioimmunoelectrophoretic analyses were carried out as described before (Rejnek & Bednařík, 1960), using the arrangement reported by Yagi and co-workers (1963). Preparation of ^{131}I-tetanic toxoid 1 ml of tetanic toxoid*) was added to a solution containing 0.5 of 2×10^{-5} M potassium iodide, 1 ml of radiactive sodium iodide ($Na^{131}I$) of a specific activity 5 mc/ml, and 2 ml of 0.14 % solution of chloramine T in a borate buffer at pH 9.0. The mixture was allowed to stand for 30 minutes at room temperature and then 2 ml of 0.01 M solution of sodium sulfite were added. The mixture was dialyzed against phosphate physiologic solution (pH 7.2) until free ^{131}I was removed completely. After the dialysis the mixture was made up to 1,000 ml and the solution was used for radioimmunoelectrophoresis.

Electrophoretic analysis in agar gel was carried out using the arrangement of Popadiuk (1961).

*) The preparation was a generous gift of Dr. A. Stejskal of the Institute for Sera and Vaccines, Prague.

RESULTS

The agar-gel electrophoretic analysis of human and porcine serum and colostrum is shown in Fig. 1. Contrary to the human serum the porcine serum is more abundant in the cathode region where 3 zones are distinctly separated whereas only 2 zones can be observed in human serum. The zones of the α-globulin and the albumin regions are identical in their characters and mobilities. The protein spectrum of the colostrum shows in both cases only one intensive broad zone in the cathode region. In human colostrum this zone migrates in the β_2-globulin region whereas in porcine colostrum it has the same mobility as serum γ-globulin. Another pecularity of porcine colostrum is a strong zone of prealbumin which has not been found in human colostrum.

The immunoelectrophoretic analysis of porcine blood serum (Fig. 2) indicates the presence of a number of precipitation lines in the cathode region, i.e. where immunoglobulins migrate. Two lines precipitate in the close vicinity of the origin well, one distinct line and one less distinct one. The mobilities of these lines indicate that proteins of the β_1-globulin group are involved. Two other lines which are located closer to the cathode can be seen. One of them is weaker and the other one is a narrow line obviously corresponding to β-macroglobulin. Very close to the cathode end of the latter we find two weaker zones which resemble both in shape and location γ A-globulin as it is

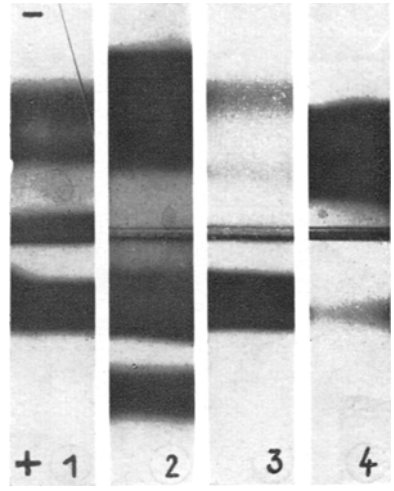

Fig. 1. Electrophoretic analysis in agar gel. (strip 1 — porcine serum; strip 2 — porcine colostrum; strip 3 — human serum; strip 4 — human colostrum).

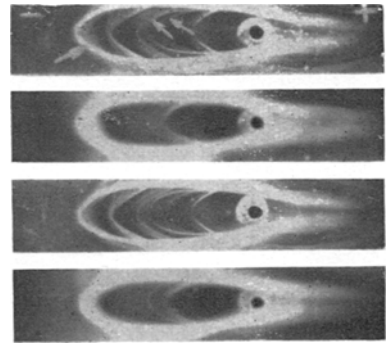

Fig. 2. Immunoelectrophoretic and radioimmunoelectrophoretic analysis of hyperimmune porcine serum. (Strips 1 and 3 — immune porcine serum precipitated by rabbit antiserum to porcine serum proteins; strip 2 and 4 — autoradiography of strips 1 and 3).

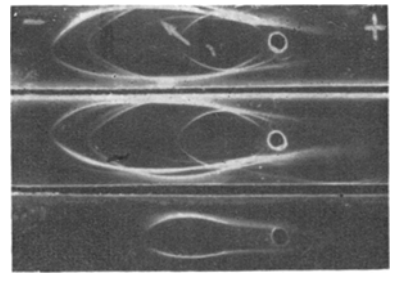

Fig. 3. Immunoelectrophoretic analysis of γ-macroglobulin. (Precipitated by rabbit antiserum to porcine serum proteins. The antiserum was saturated with γ-macroglobulin between strip 1 and 2; Strip 1 and 2 — porcine serum, strip 3 — γ-macroglobulin).

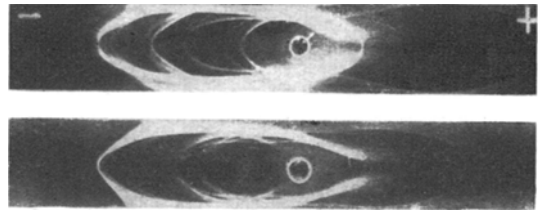

Fig. 4. Immunoelectrophoretic analysis of porcine serum and colostrum. (Strip 1 — porcine serum; strip 2 — porcine colostrum precipitated by rabbit antiserum to porcine serum proteins).

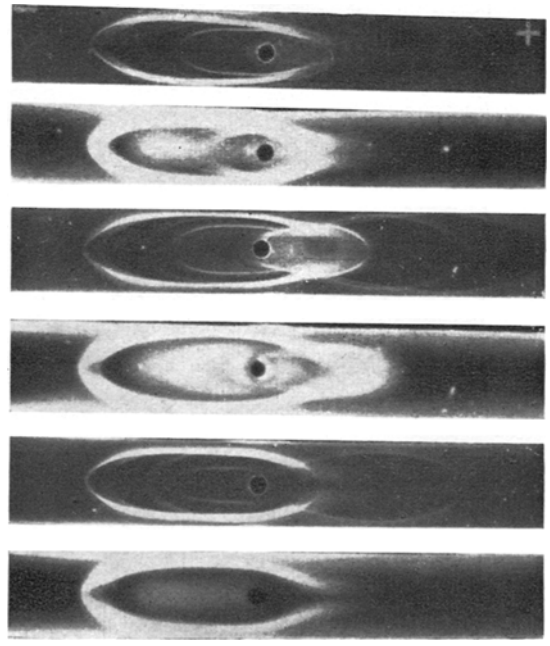

Fig. 5. Immunoelectrophoretic and radioimmunoelectrophoretic analysis of serum of immunized sow of her colostrum, and of suckling's serum. (Strip 1 — serum of the sow; strip 3 — colostrum; strip 5 — serum of the suckling pig. Strip 2, 4, and 6 — autoradiography of strips 1, 3, and 5. Precipitated by rabbit antiserum to proteins of porcine colostrum).

usually encountered when human serum is analyzed. Still further in the direction toward the cathode one intensive and three almost merging lines are found. The anode end of the most intensive one of the latter reaches beyond the origin well and corresponds to γ G-globulin. The remaining two less intensive lines probably represent subfractions of γ G-globulin similar to those detected in human serum with the aid of monkey antisera by Dray (1960), Grey and Kunkel (1964), and Fahey *et al.* (1964).

It is, however, difficult to conclude merely on the basis of the immunoelectrophoretic pattern whether all lines of the γ- and γ_2-globulin region belong to immune globulins, since, as may be seen in Fig. 2, this region is considerably more abundant in fractions than e.g. the same region of the human serum. The strongest line can be identified as the γG- globulin line. A proof of the identity of this line with the immunoglobulin of the γ G-type was obtained in studies on structural subunits of this protein (Rejnek *et al.*, 1965). Similarly, the γ-macroglobulin can be recognized due to the characteristic shape of its precipitation line. The correctness of the identification of the γ-macroglobulin line is evidenced further by the disappearance of this line after the precipitation serum had been saturated with pure γ-macroglobulin isolated by gel filtration using Biogel P-300 (Fig. 3). It is far more difficult to determine whether any of the lines correspond to immunoglobulin of the γA type since nothing is known about its existence in porcine serum. The immunoelectrophoretic analysis of porcine serum shows that any of the four lines of the β_2-globulin region can correspond to this immunoglobulin. An attempt was therefore made by us to solve this problem by radioimmunoelectrophoresis. For this purpose we used the hyperimmune serum prepared by immunization of an adult pig with tetanic toxoid. ^{131}I-labelled tetanic toxoid was used for the detection of antibody activity. The results of this experiment are shown in Fig. 2. It is obvious that in addition to the γ G-globulin fraction the antibody activity was found in two other fractions which lie in the β_2-globulin region. The more intensive one of the latter apparently belongs to the precipitation line localized between the γ-macroglobulin line and a line around the origin well which perhaps corresponds to siderofilin. The second less intensive zone on the autoradiogram corresponds to γ-macroglobulin. The lower intensity of this zone is obviously due to the fact that antibodies of the macroglobulin type are usually not formed when bacterial toxins are used for immunization. These results therefore indicate that the porcine serum, similarly to human blood serum, contains at least three different types of immunoglobulins. The only difference between these two sera is that the protein which should correspond to the γA-type shows a higher mobility than the γ-macroglobulin whereas in human serum the mobilities are reversed.

Since we found three types of immunoglobulins in porcine serum we decided, as in the case of human serum, to determine whether an accumulation of the type corresponding to the γ A-globulin of human colostrum also takes place in porcine colostrum. However, the immunoelectrophoretic analysis of proteins of porcine colostrum with the aid of antisera to serum proteins only confirmed the fact which is obvious in Fig. 1, i.e. that the main component of porcine colostrum is the immunoglobulin of the γ G-type. The only difference which can be seen in Fig. 4 pertains to the line of colostrum γ G-globulin which reaches farther into the anode region than the precipitation line of serum γ-globulin. Hence colostrum contain a higher number of fast migrating components of γ G-globulin. More differences are found when the other lines of the immunoglobulin region of the colostrum are considered. An intensive line located between the lines of γ G- and γ M-globulin, exists only in the sample of serum, while a round precipitation line of a mobility similar to that of γ-macroglobulin can be seen only in the

sample of colostrum. Since, however, these differences can be demonstrated in both samples with the aid of the same precipitation serum, we can assume that the observed differences are of a quantitative character rather than that they represent qualitatively different proteins.

In other experiments the presence of antibody fractions in colostrum of a pregnant sow was followed by the radioimmunoelectrophoretic method using ^{131}I tetanic toxoid. In Fig. 5, which shows the analysis of samples of the mother's serum colostrum, and the suckling's serum prepared 24 hours after parturition, differences in the activity of individual immunoglobulin fractions of colostrum and serum can be seen. Whereas in the mother's serum the activity was found again in fractions γ G, γ M, and in a fraction which might correspond to γA-globulin, the activity in colostrum is pronounced in the γ G-fractions and weak in the γ M fraction. The activity found in the suckling's serum was the same as the activity of colostrum except for fraction γ M where activity was barely detectable. This may be due to the lower content of this fraction in the suckling's serum as obvious from the immunoelectrophoretic analysis.

DISCUSSION

As can be seen from the results presented here, considerably more protein fractions can be detected in the immunoglobulin region of porcine serum than in the same region of human serum. We find 8 precipitation lines in the region where in human serum only fraction γ M, γ A, and the γ G-globulin fraction are detected with the aid of the usual polyvalent antisera. We cannot as yet determine unequivocally whether the great number of fractions in this region is a characteristic feature of this animal species or whether the same number of fractions also exists in the sera of other animals but in different quantitative ratios cannot be detected by conventional immunochemical methods. We have also to take into consideration the fact that the success of the detection of a protein by the immunochemical method depends on the antigenicity of the fraction to the antibody-producing organism. We cannot therefore exclude the possibility that a higher number of fractions could be detected in the immunoglobulin region of porcine serum due to a high antigenicity of porcine immunoglobulins to the rabbit organism. This hypothesis is supported by the fact that we were able to distinguish three fractions corresponding to γ G-globulin, which fractions can be assumed to correspond to the γ Ga, γ Gb, and γ Gc-globulin fractions found in human serum with the aid of monkey antihuman antisera. Another fact which may be adduced in favour of the above hypothesis is that a new class of immunoglobulins designated γD was found in human serum recently (Rowe, 1965).

The problem as to whether all these fractions whose electrophoretic mobilities correspond to the mobilities of immunoglobulins virtually belong among the latter was examined by the radioimmunoelectrophoretic method. The results obtained by this approach show that antibody activity can be proved, in addition to the three distinguishable γ G-globulin fractions, also in the γ-macroglobulin fraction and in another fraction which may perhaps correspond to the γA-globulin of human serum even though its mobility is slightly higher. A fact deserving interest is that this fraction displays a considerably high antibody activity, since its line which is very weak on the immunopherogram gives a distinct picture on autoradiography. It is therefore obvious that at least three types of immunoglobulins exist in porcine serum similarly to human serum. As far as other lines in the immunoglobulin region are concerned whose antibody activity has not as yet proved; we cannot conclude as yet whether they belong to the immunoglobulins or whether they are proteins of another type.

The results of comparison analyses of the protein spectra of serum and colostrum

showed that the main component of porcine colostrum is the immunoglobulin of the γ G-type. It is well known that this type of γ-globulin represents a heterogeneous mixture of very closely related proteins which ratio in serum may be different from colostrum since the latter seems to contain electrophoretically faster components. Similar results were also obtained by Pierce and Feinstein (1965) who examined the immunoglobulins of bovine serum and colostrum and also found colostrum immunoglobulins of the γ G-type to be abundant in faster migrating components.

The fact that γ G-globulin represents the main component of porcine colostrum was further confirmed by radioimmunoelectrophoretic analyses. Unlike in mother's serum the activity was concentrated only in this fraction and in the γ-macroglobulin, whereas the immunoglobulin which should correspond to γ A-globulin was not proved by this method in colostrum at all. This difference is obviously significant since the antibody activity should be more pronounced in colostrum than in serum due to a higher content of proteins in the former. An analysis of the suckling's serum yielded the same results save for the γ-macroglobulin line, which was far less apparent than in colostrum. This finding seems to indicate that the intestinal barrier of the suckling pig is less permeable for this protein than it is for γ G-globulin.

When these results are compared with the data obtained from studies on immunoglobulins of human serum or colostrum (Gugler & Muralt, 1959; Hanson, 1960; Rejnek *et al.* 1960; Hanson & Johanson, 1962) we can see that while the human mammary gland selectively absorbs mostly γ A-globulin and relatively little of γ G-globulin, it was predominantly γ G-globulin which was detected in porcine colostrum. The γ A-globulin (provided we can consider the immunoglobulin found in porcine serum as corresponding to human γ A) was not found in colostrum at all.

Even though we are not as yet able to draw any final conclusions, a fact deserving interest is the relationship between the type of placenta and the immunoglobulin spectrum of colostrum. Human placenta of the hemochorial type is permeable for immunoglobulins of the γ G-type whereas the type γ M and γ A do not pass through this placenta at all (Rejnek *et al.*, 1960). And it is these components which either do not pass through the placenta at all or to a negligible degree which represent the main components of human colostrum. On the other hand the epitheliochorial placenta of the pig is completely impermeable for γ G-globulin and at the same time it is the immunoglobulin of the γ G-type which is the main component of porcine colostrum. It seems therefore that colostrum represents a source which supplies the newborn organism with those types of antibody which were not transmitted during the intrauterine life.

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