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# STUDIES ON THE TIME OF APPEARANCE OF FETAL BLOOD PROTEINS IN PIGS

By

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Recently a number of studies on electrophoretic investigations of serum from both adult and growing pigs have been published. However, only a very few of the studies deal with electrophoresis of serum from pig fetuses. In 1945 *Moore et al.* published a study on plasma from chicken and pig fetuses examined by electrophoresis and ultracentrifugation. By electrophoresis six electrophoretically different fractions in fetuses were found, although no age group showed more than four of these six fractions. The variations between the single fractions throughout the fetal life were also described. Since then, *Rutqvist* 1958 has worked with 2½ and 3½ months old fetuses in which he could demonstrate albumin, α- and β-globulin and variations between them both in the fetal life and in the first part of the postnatal life. Similar investigations have been made by *Waddill et al.* 1962. The same year immunoelectrophoretic investigations of serum from newborn and growing pigs were published by *Lecce, Morgan* and *Matrone* (1962). These investigators demonstrated the presence of eight precipitin arcs at birth and a gradual increase to nineteen arcs found in the adult pig. The last arc was found when the pig was 63 days old.

As the development of the fetus is an essential part of the life of the pig it was of interest to study the information obtainable by the sensitivity of the immunoelectrophoresis. This paper deals with the first results of such studies.

## MATERIAL AND METHOD

The material comprised nine sows and sixty-five fetuses, the data from this material appear in table 1.

Table 1. Distribution and data of the fetuses.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of fetuses | 8 | 8 | 3 | 9 | 7 | 9 | 2 | 9 | 10 |
| Age in days | 25 | 35 | 40 | 54 | 58 | 63 | 70 | 99 | 112 |
| Av. lenght cm. | 2 | 4 | 6 | 9.3 | 12.8 | 14.9 | 18 | 25.2 | 29.9 |
| Av. weight gm. | 0.5 | 3.2 | 10.4 | 43.9 | 90.1 | 153.5 | 261 | 787 | 1285.5 |

Shortly after the sows had been slaughtered the uteri were taken to the laboratory where the fetuses were removed and when possible blood samples were drawn from both the heart and the umbilical cord. In one case the blood sample was drawn at the slaughter-house and in another case the fetuses were removed by the caesarean operation. It was often rather difficult to obtain blood from the small fetuses, but a thin glass cannula fitted with a rubber tube was found useful for this purpose. By capillary action of the tube and by gentle suction well suited blood samples could be obtained. After drawing, the samples were made to clot by keeping them at 37°C for one to two hours. They were then centrifuged for ten minutes at 3000 r.p.m. Finally the serum was removed. All the serum samples were stored at 4°C until analysis, usually made the following day.

Anti pig serum was used as antibody. This was produced on rabbit No. 146, immunized with pooled serum from adult pigs. The antibody, selected as the best among several antibodies, was used in dilution 1:2.

The immunoelectrophoresis (*Grabar* and *Williams* 1953) in *Scheideggers* modification (1955), was made using 1 % agargel (Special Agar Noble Difco) and veronal buffer pH 8.6 containing calcium lactate as described by *Hirschfeld* 1960. For the electrophoresis Agafor I apparatus with bridges of Whatman No. 3 filter paper was used. The electrophoresis lasted two hours using a gradient of 5 volts per cm. The antibody was then added and the slides stored at 37°C usually for 24 hours.

## RESULTS

To obtain preliminary knowledge about the main fractions from electrophoresis, agargel electrophoresis was used on about 2 $\mu$l. serum, the same amount as used in immunoelectrophoresis.

These investigations show that serum from fetuses as also stated by *Rutqvist* (1958) with paper electrophoresis can be divided into three fractions with the same mobility as albumin, α and β globulin in the adult pigs. There seems to be considerable quantitative variation during the fetal life, but this was not closely investigated. In relation to the three fractions three very pronounced arcs are seen in the immunoelectrophoresis. These three arcs, found in all age groups will, from anode to cathode, be designated 1, 2 and 3. The new arcs appearing during the fetal life may be considered in relation to these three rigid arcs. The different stages are shown by photography in fig. 1. The youngest fetuses, 25 days old, show the three above mentioned arcs, but in several fetuses is observed an arc with slightly slower mobility than arc No. 1 and placed more laterally. This arc is stainable with lipoprotein stains e.g. Sudan black. In some samples an arc with slightly greater mobility than No. 1, is also seen. In the curvature of No. 1 a little arc is seen and the trace of an arc is visible in the curvature of arc No. 2. In all the 25 days old fetuses arc No. 3 is short and blurred. There is considerable development from this first stage to the next stage, ten days later. Arcs 1 and 2 are essentially stronger, and a clear arc, anodic to No. 1 and an arc in the angle between 1 and 2 are also seen. Further a trace of an arc is visible in the curvature of No. 1 and a very strong arc together with some smaller arcs in the curvature of No. 2. Finally, an ach is to be seen in the field between 1 and 2. After thirty-five days the changes observed are only slight. New arcs appear in the curvature of the three great arcs as shown in fig. 1. A new arc may possibly not be distinguished in all the subsequent stages probably due to variations in the concentrations of the small components. In addition possible variations from one litter to another in the same age group cannot be excluded. In the present material fetuses when 40 days old seem then less well developed than at 35 days. The arc which was detectable after 35 days as a trace in the curvature of No. 1 is after 54 days very distinct and can be recognized in all later stages. In serum from the two last stages the concentration of No. 1 has decreased and the arc does not cover the field between the curvature and the antibody trough. In the last stage, after 112 days, about fifteen arcs are seen. With the same antibody immunoelectrophoresis of an adult pig will give the picture seen in fig. 1 below, which shows the mother of litter No. 5. Here we see some twenty arcs. Of these

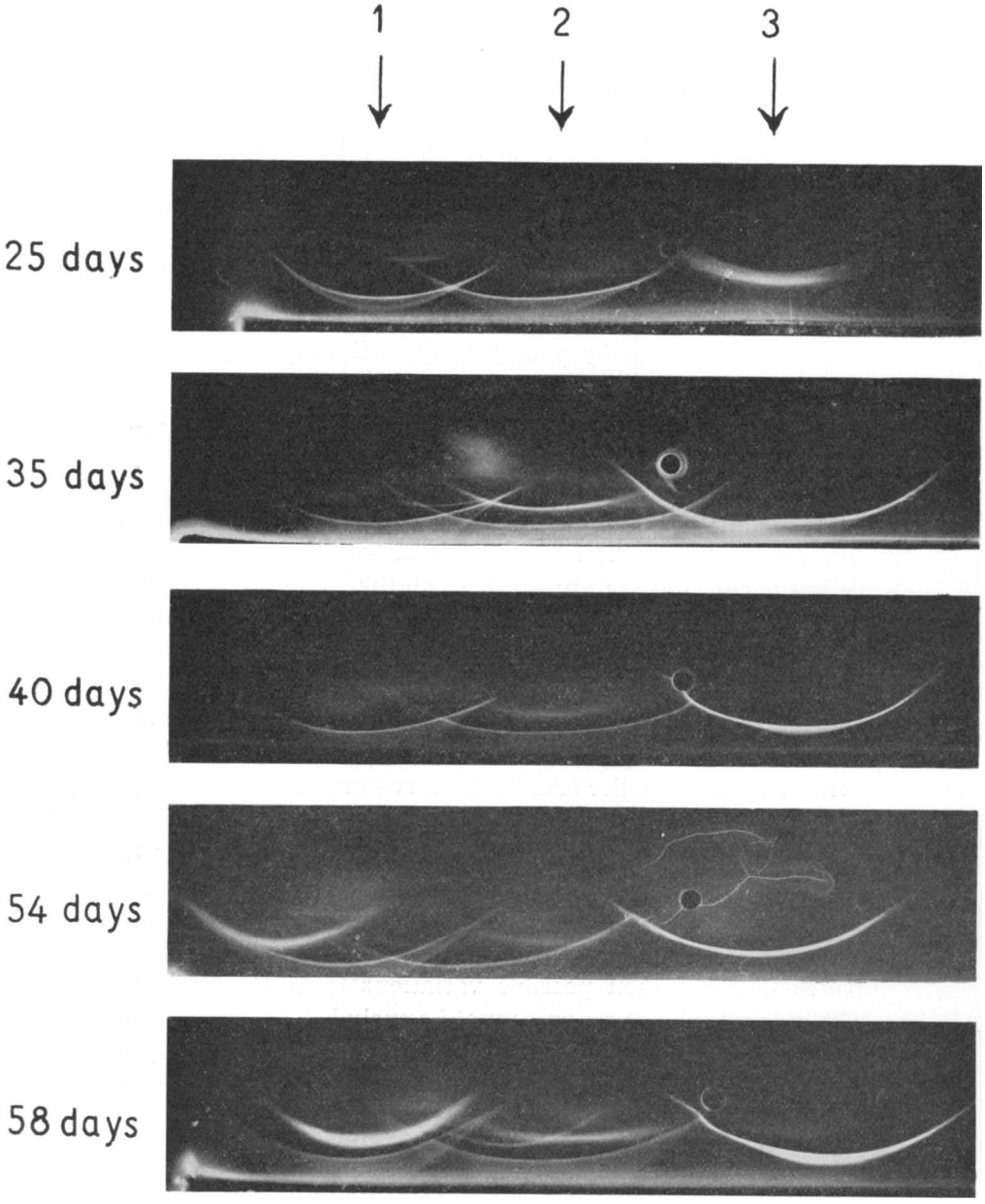
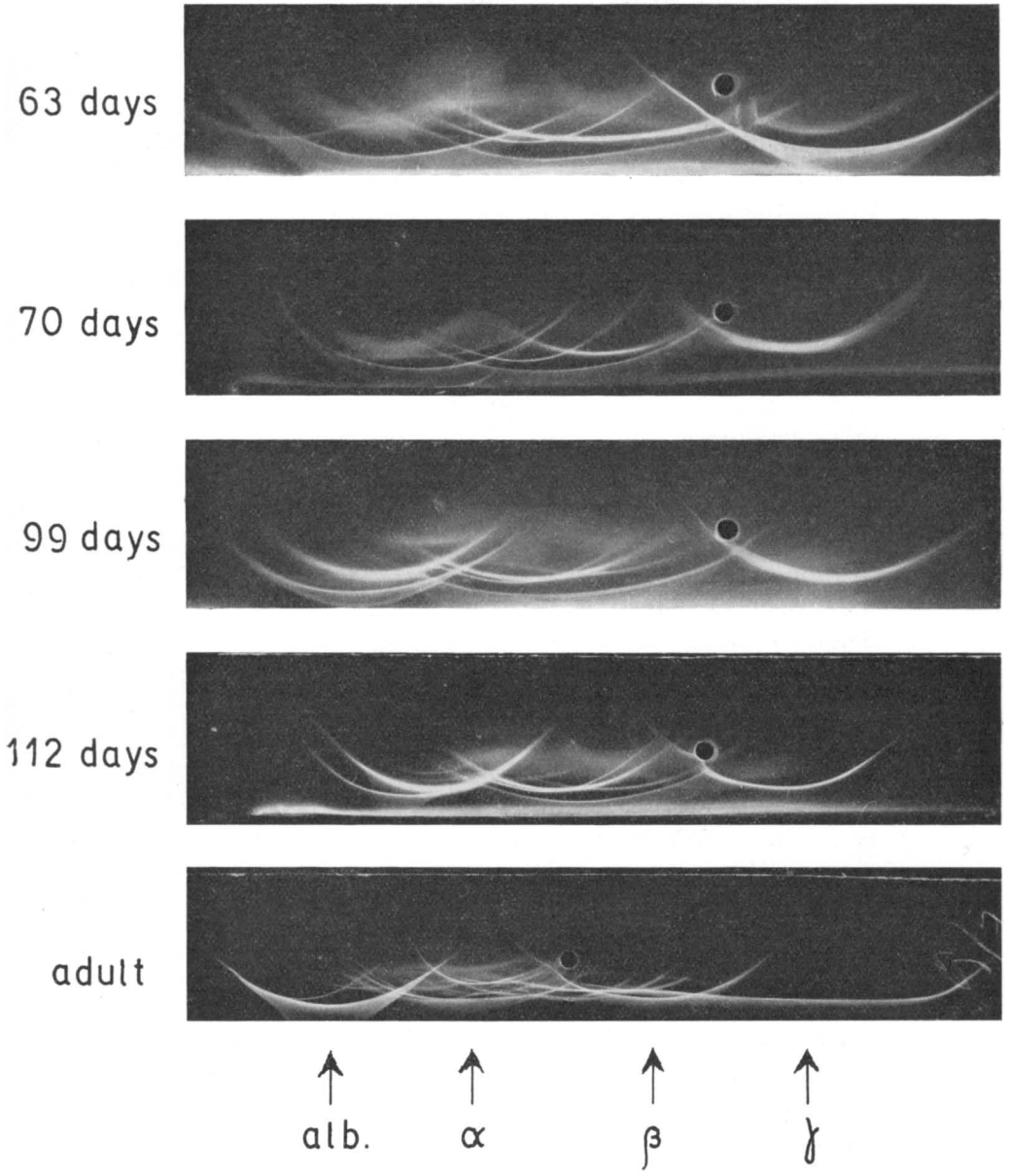



Fig. 1. Photograph showing immunoelectrophoresis of serum from the different fetal age groups and of serum from the adult pig. The arrows Nos. 1, 2 and 3 indicate the position of the three dominating




arcs in the fetal serum. The arrows alb., α, β and γ indicate the different fields in electrophoresis of adult serum. Note that the antigen reservoir in the last picture is moved slightly to the left in relation to the other pictures.

four are dominant viz. the albumin arc, a great α and β arc and the long γ globulin arc stretching from the anode side of the hole to the cathode.

In order to determine to which components in the adult serum the three main arcs in the fetal serum are immunologically related, combined diffusion tests (*Clausen* and *Heremans* 1960) were used. The results are shown in fig. 2. In the combined diffusion test the antibody trough is interrupted by small bridges of agar in which the reaction of immunological identity or non-identity between the fractions of the samples investigated, occurs. The results of this test will be discussed below.

## DISCUSSION

Along the lines of the investigations made by *Rutqvist* (1958), agarelectrophoresis as used in this investigation shows three fractions and immunoelectrophoresis three main arcs with the same mobility as the albumin, α and β arcs in adult serum. As expected no γ globulin arc was found. However, it should be emphasized that *Sterzl et al.* (1960) found γ globulin in serum from newborn non-fed pigs and also in serum from fetuses removed by the caesarian operation one week before the normal birth. Serum was first concentrated 50 to 100 times by alcohol fractionation and on DEAE cellulose.

From the combined diffusion (fig. 2) arc No. 3 seems to be immunologically identical with the great β arc from the adult pig. It was impossible to ascertain to which component arc No. 2 corresponds. However, some of the arcs in the curvature of No. 2 can be identified with components in serum of the adult pig. It proved impossible to make reactions for identity between the albumin arc of the adult pig and the great fetal arc No. 1 which was expected to be albumin. As seen, in fig. 2 and in the schematically drawn fig. 3, the albumin arc in the adult serum is immunologically identical to the arc in the curvature of arc No. 1. This again is related to the arc in the anodic part of the albumin curvature of the adult serum. Provided this is correct, it should be possible to detect all the steps in the development from the fetal arrangement of the two arcs to the adult placement where albumin lies closest to the trough. Furthermore, the fetal arc, as the arc in the curvature of arc No. 1 will now be called, should be greater and arc No. 1 lesser in size. By investigations of serum

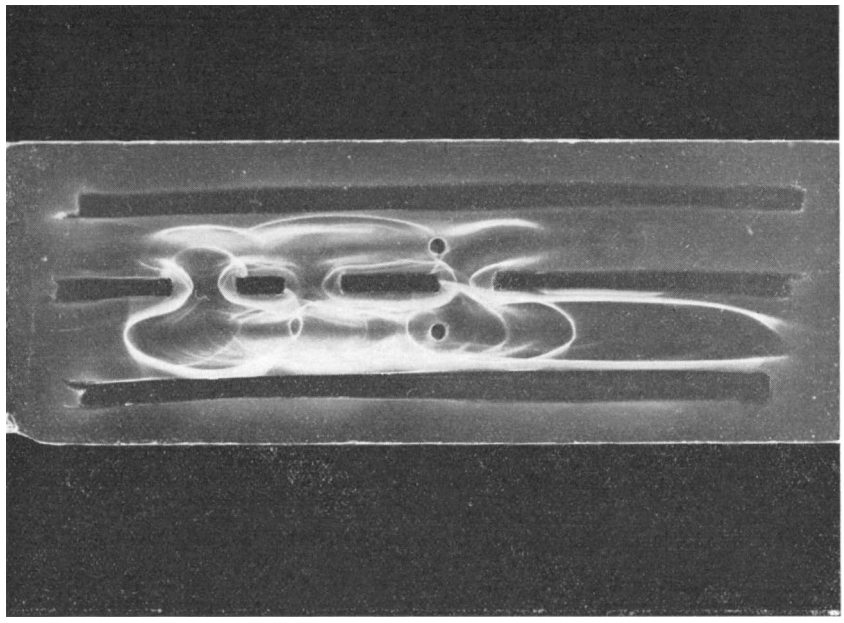


Fig. 2. Combined diffusion test. The fetal serum (112 days) is filled in the upper and the serum from the adult pig in the lower antigen reservoir.

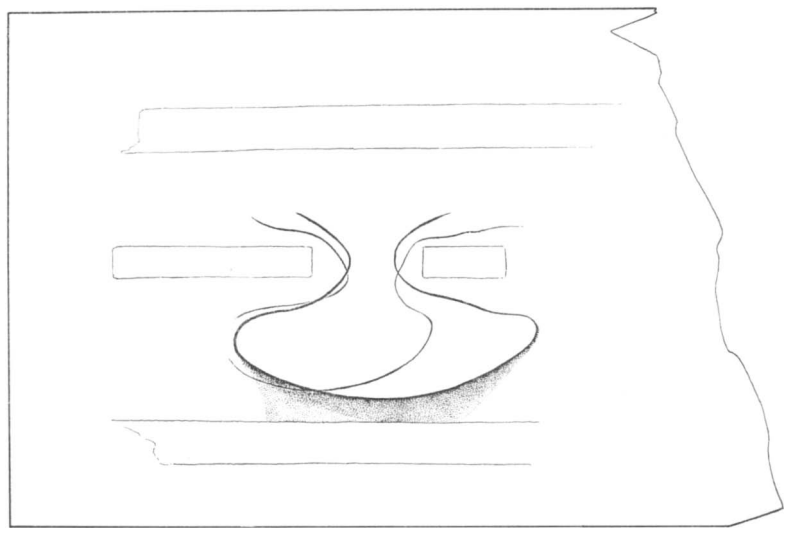


Fig. 3. The anodic section of the slide, fig. 2, is indicated schematically in order to follow the course of the lines. The albumin arc of the adult serum is the large arc. The precipitation of that arc is dotted.

from newborn pigs continued for some time after birth, the result expected was found. In the age group, 112 days, the two arcs are of nearly the same size (fig. 1). Sixteen hours after birth the arcs begin to cross each other. After two days they cross far toward the anode and after one week the picture is the same as for adult pig. It is possible that the division of the most mobile fraction into two fractions, as stated by *Rutqvist* (1958), is related to the problems here presented.

The immunoelectrophoretic pictures from the different age groups are all made with antibody against serum from adult pigs. Therefore, we obtain no information about proteins specific in antigenicity for the prenatal life. Using antibody against serum from newborn pigs *Lecce et al.* (1962) have demonstrated that with a single exception all protein components in serum from newborn pigs are present in adult serum. An arc with a mobility slightly slower than the albumin arc was detected during the first three to four weeks after birth. It should be interesting to study that arc in the prenatal life with a specific antibody.

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## SUMMARY

Serum from pig fetuses in different stages of development, from 25 days to 112 days, were investigated by immunoelectrophoresis. It was found that many of the proteins develop quite early in the fetal life. In fetuses 35 days old about 10 different fractions were observed. These increased to 15 at the end of pregnancy. Adult pigs showed some 20 fractions. However, variations in development between different litters in the same age group cannot be excluded. Using combined diffusion tests it was possible to determine to which fractions in adult serum the two great fetal arcs, Nos. 1 and 3, are related. It was not possible to ascertain to which component arc No. 2 corresponds. However, some of the arcs in the curvature of No. 2 can be identified with components in serum of the adult pig. The albumin arc of the fetus appears from the curvature of arc No. 1. After birth, arc No. 1 diminishes in size and one week later its position is lateral to the arc of the albumin. As only antibodies against adult pigs are used, only those components of the fetus which are immunologically related to the protein components in adult pigs be precipitated. Possibly existing special fetal proteins should be investigated by means of specific antibodies to fetal serum.

## ZUSAMMENFASSUNG

*Untersuchungen über das Erscheinen von Serumproteinen bei Ferkelfötussen.*

Serum von Ferkelfötussen in verschiedenen Entwicklungsstadien von 25 bis 112 Tagen wurden immunoelektrophoretisch untersucht. Wie festgestellt wurde, findet eine bedeutende Entwicklung von Serumproteinen in einem frühen Fötusstadium statt, so dass schon bei 35 Tage alten Fötussen ungefähr 10 verschiedene Proteinfraktionen gegenüber 15 am Ende der Trächtigkeit und gut 20 Fraktionen beim erwachsenen Schwein wahrgenommen wurden. Man kann jedoch nicht Variationen von Wurf zu Wurf innerhalb derselben Altersklasse ausschliessen. Mit kombinierter Diffusion wurde festgestellt, welchen Fraktionen im Serum erwachsener Tiere die beiden grossen Fötusbogen 1 und 3 entsprechen, während es nicht gelang festzustellen, welcher Komponente im Serum Erwachsener der Bogen Nr. 2 entspricht. Mehrere Bogen in der Kurvatur von Nr. 2 lassen sich dagegen mit Komponenten im Serum von erwachsenen Tieren identifizieren. Der Albuminbogen des Fötus wird in der Kurvatur des Bogens Nr. 1 gebildet, der sich nach der Geburt vermindert und lateral zum Albuminbogen verschoben wird. Die Anwendung von Antikörpern gegen Serum erwachsener Tiere bewirkt, dass nur Fötusproteine mit dem

gleichen antigenen Verhalten wie Serumproteine bei erwachsenen Tieren präzipitiert werden konnten. Mögliche eigenartige Fötusproteine sind in bezug auf specifische Antikörper gegenüber Fötusserum zu untersuchen.


## RESUMÉ

*Undersøgelser over tilsynekomsten af serumproteiner hos grisefostre.*

Serum fra grisefostre på forskellige udviklingstrin fra 25 dage til 112 dage gamle er undersøgt immunoelektroforetisk. Det er fundet, at der sker en betydelig udvikling af serumproteiner på et tidligt fosterstadium, således at der allerede hos 35 dage gamle fostre er set omkring 10 forskellige proteinfraktioner mod 15 ved drægtighedens slutning og godt 20 hos den voksne gris. Man kan dog ikke udelukke variationer fra kuld til kuld inden for samme aldersklasse. Med kombineret diffusion er det fundet, hvilke fraktioner i voksent serum de to store fosterbuer 1 og 3 svarer til, medens det ikke er lykkedes at få klarlagt, hvilken komponent i det vokse serum bue nr. 2 svarer til. Derimod kan flere af buerne i kurvaturen af nr. 2 identificeres med komponenter i serum fra voksne dyr. Fostrets albuminbue dannes i kurvaturen af bue nr. 1, der efter fødslen mindskes og rykkes lateralt for albuminbuen. Anvendelse af antistof mod serum fra voksne dyr bevirker, at kun fosterproteiner med samme antigene forhold som serumproteiner hos voksne dyr har kunnet precipitere. Mulige særegne fosterproteiner må undersøges med specifikt antistof mod fosterserum.


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