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ON THE TRANSFERRIN CONCENTRATION IN SERUM OF SOWS AND GROWING PIGS AND IN SOW COLOSTRUM

By

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THORÉN-TOLLING, KERSTIN and KJELL MARTINSSON: *On the transferrin concentration in serum of sows and growing pigs and in sow colostrum.* Acta vet. scand. 1974, *15*, 120—134. — Immunologically pure transferrin was isolated from swine serum by means of ammoniumsulphate precipitation and ion-exchange chromatography. Rabbit anti-swine transferrin serum was prepared and used for immunological determinations of transferrin in serum and colostrum. The transferrin concentration in serum from piglets was 180 ± 56 mg/100 ml at birth and rather constant by the first days of life. The levels increase to 610 ± 78 mg/100 ml at 6 weeks after birth. The transferrin level of colostrum was 100 mg/100 ml and decreased rapidly. A negative correlation was established between the concentration of haemoglobin and transferrin. The importance of transferrin is discussed.

immunological determination of transferrin; piglet; transferrin concentration; transferrin preparation.

Transferrin or siderophilin, an iron-binding β_1-globulin was first discovered in human serum by *Holmberg & Laurell* (1945) and *Schade & Caroline* (1946). *Laurell & Ingelman* (1947) isolated transferrin from pig serum with a combination of ammoniumsulphate precipitation and fractionation with cold ethanol. *Inman* (1956) crystallized human transferrin after ethanol fractionation and established actual physical and chemical data for this protein. Human transferrin was prepared by rivanol precipitation and chromatography on carboxymetyl and DEAE cellulose (*Nagler et al.* 1962), and *Jeppsson* (1967) has described a method for transferrin preparation using DEAE Sephadex A 50 chromatography combined with Sephadex gel filtration.

Porcine transferrin has a molecular weight of 88,000 as shown by *Laurell & Ingelman* and every molecule has an ability to bind two free ionic iron (Fe^{3+}). This means that under physiological conditions the concentration of free iron in serum is very low, and in this way the toxic effects of ionic iron are eliminated.

During normal conditions the iron-binding capacity of transferrin in serum only is utilized to one third. The concentration of transferrin is mostly given as the total iron-binding capacity (TIBC) of serum, but the concentration may also be determined by immunological techniques i.e. precipitation in agar gels by a specific antiserum.

Information on the transferrin concentrations in sera of piglets and sows, as well as in colostrum, seems to be important for several reasons. Thus the time of iron administration can be correlated to the amount of serum transferrin present in order to avoid the occurrence of ionic iron in serum. Furthermore, the importance of colostrum for the supply of transferrin in piglets can be determined.

The aim of this investigation was to isolate porcine transferrin in a pure state for the production of a specific antiserum and to determine the concentration in serum of growing pigs and in serum and colostrum from sows. Furthermore, the relationship between concentrations of transferrin and haemoglobin was investigated.

MATERIAL AND METHODS

Animals: Four sows (3, 6, 178 and 182) of Swedish Landrace and their litters including 46 piglets were examined. The pigs were allowed to suckle the sow from farrowing. They were divided into 3 groups and treated orally with 1.5 ml iron-dextran (Vetrifer, Agrivet, Uppsala) (150 mg Fe^{3+}) at 8, 16 and 24 hrs. after birth, respectively.

Blood and colostrum samples. Blood for transferrin and haemoglobin determinations was drawn from anterior vena cava at birth and thereafter at intervals shown in Figs. 1 and 9. Samples for transferrin determinations were drawn from the sows from 2 weeks before partus to 3 weeks after partus at regular intervals, and samples from colostrum and milk were taken up to 3 weeks after partus, as seen in Figs. 7 a—d. Furthermore, 4 newborn colostrum-deprived pigs of Swedish Landrace were examined.

Two pigs were given 20 ml pooled sow colostrum (collected at partus) and 2 were given 30 ml pooled swine serum orally. Blood samples for transferrin determinations were drawn from a catheter in the anterior vena cava at the start of the experiment and at regular intervals during the following 22 hrs. as seen in Figs. 8 a-b.

Preparation of swine transferrin. Pooled blood from newborn colostrum-deprived piglets were stored at 4°C for 24 hrs., and then the serum was removed. Fifty ml serum was precipitated with ammoniumsulphate until 60 % saturation during stirring for 30 min. After centrifugation (6000 r.p.m. for 10 min.) the supernatant was dialyzed against 0.04 M phosphate buffer pH 7.2 for 2 days and concentrated to the original volume by polyvinyl pyrollidone. Twenty ml of the solution was then applied on a column (3×12 cm) with DEAE-Sephadex A 50 equilibrated with the initial buffer (0.04 M phosphate buffer pH 7.2). Eluation with constant flow of the buffer was used (40 ml/hr.) as indicated in Fig. 2. Fractions 7—10 (Fig. 2) were pooled and concentrated to 2 ml.

Immunoelectrophoresis was performed according to the method of *Scheideggar* (1955).

Immunodiffusion was performed in 1 % agar in veronal buffer pH 8.6, ionic strength 0.1.

Radioimmunoelectrophoresis. Five μCi of Fe^{59} ferric chloride was incubated at 37°C for 15 min. with 0.2 ml swine serum at pH 8.2. After immunoelectrophoresis against anti-swine serum the glass slide was put on an x-ray film (Kodak, Kodirex) (Fig. 6).

Antisera. Anti-swine serum was prepared according to *Martinsson* (1970). Anti-swine transferrin was prepared in the following way: Fractions obtained from the chromatographic runs (Fig. 2) and which were shown to be pure by immunoelectrophoretic analyses were used for immunization of 1 rabbit. First 0.5 ml Freund's complete adjuvant was injected into a foot pad to enlarge the popliteal lymph node. Then a transferrin solution containing 1—2 mg TRF precipitated at pH 7 with a few crystals of potassium aluminium sulphate was injected into the enlarged lymph node once a week for 3 weeks. Booster injection was done 2 weeks later, and 1 week later 30 ml of blood was drawn.

Transferrin determinations. The transferrin concentration in serum and colostrum was determined by the single radial immunodiffusion test described by *Mancini et al.* (1965). A standard curve was obtained by the use of serial dilutions of the pure transferrin fraction. A serum from an adult swine was used as a control serum in 4 dilutions of every agar plate.

Protein determination. The protein concentration of the pure transferrin fraction used to obtain a standard curve in the Mancini-technique was done according to the Lowry method.

Analytic errors were calculated from repeated determinations of the control serum in different dilutions on different plates.

Statistical methods were performed according to *Bonnier & Tedin* (1957).

RESULTS

The results of the chromatographic separation of swine serum on DEAE-Sephadex A 50 after precipitation with 60 % saturated ammonium sulphate are seen in Fig. 2. Fractions 7—10 from Fig. 2 were concentrated and contained pure transferrin judging from the immunoelectrophoretic analyses (Fig. 3) and were used

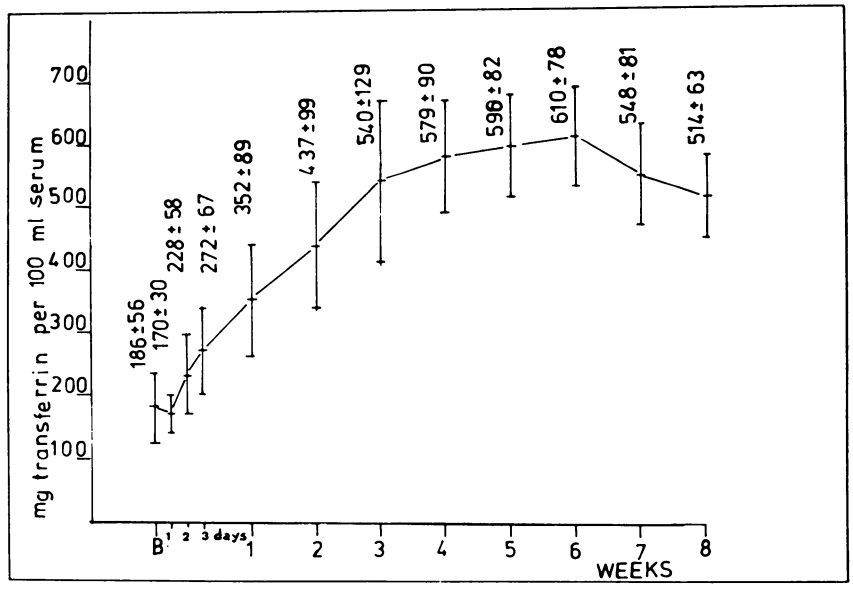

Figure 1. Serum transferrin levels in piglets, litters 3, 6, 178 and 182, from birth to 8 weeks after birth.

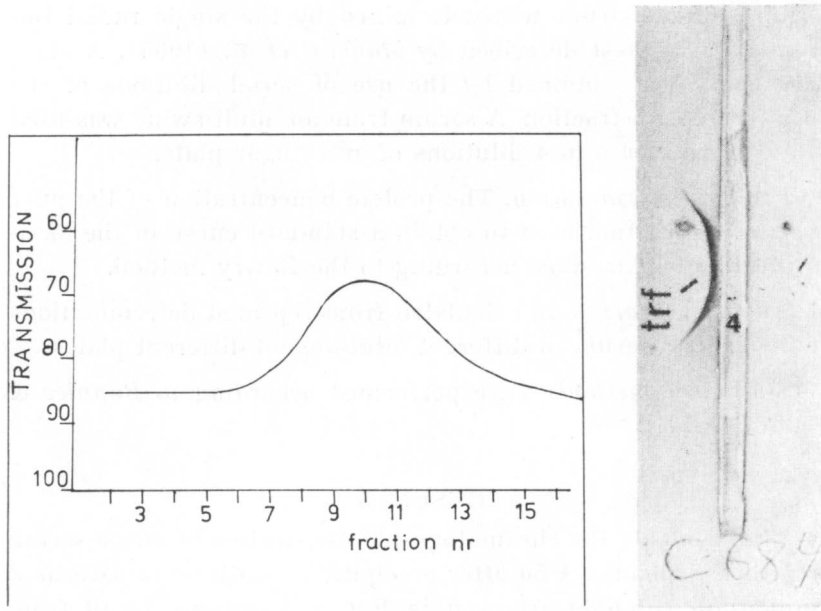

Figure 2. Chromatography of ammoniumsulphate precipitated swine serum on DEAE-Sephadex A 50. Eluation with constant flow (40 ml/hr.) with phosphate buffer 0.04 M, pH 7.2.

Figure 3. Immunoelectrophoretic analysis of the DEAE-Sephadex fractions 7—10 against anti-swine serum (4).

for immunization. The antisera obtained from the rabbit were analyzed by immunoelectrophoresis and immunodiffusion against swine serum and sow colostrum (Figs. 4—5), and only 1 precipitate could be observed. To make sure that the isolated protein was transferrin and thus the antiserum could precipitate transferrin, a radioimmunological test was performed (Fig. 6). It was observed that the antiserum precipitated a corresponding serum protein to which labelled iron was bound.

The results of the transferrin determinations of serum from growing pigs are seen in Figs. 1 and 7 a-d. The transferrin levels in piglet serum at birth varied between 123 ±23 mg/100 ml and 210 ± 16 mg/100 ml between the litters. The transferrin concentration was rather constant during the first days. Then there was an increase, and after about 3 weeks the concentration was rather constant or slightly increased to about 6 weeks of age,

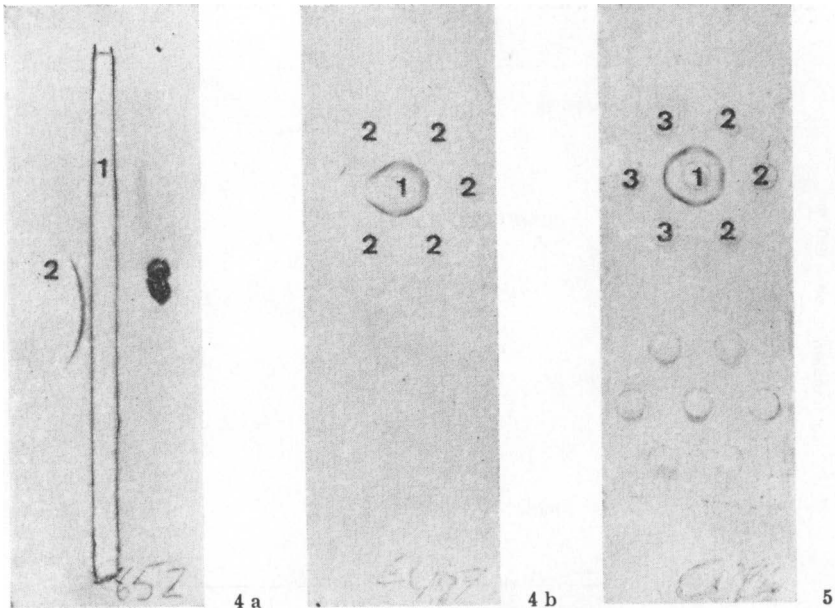

Figure 4. Immunoelectrophoretic (a) and immunodiffusion (b) tests of swine serum (2) against anti-transferrin serum (1).

Figure 5. Immunodiffusion test of sow colostrum (3) and swine serum (2) against anti-transferrin serum (1).

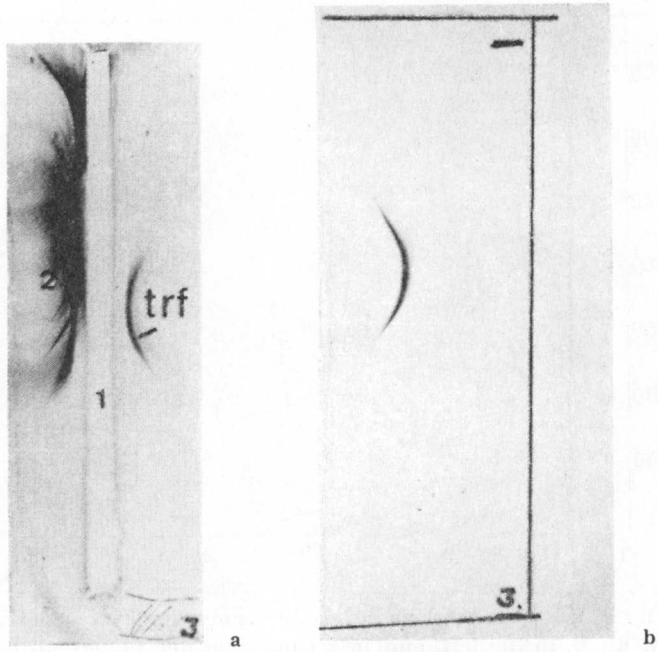

Figure 6 a. Immunoelectrophoretic analysis of swine serum, incubated with ^{59}Fe, to the left, and transferrin (Trf), to the right, against anti-swine serum (1).

b. Radioimmunoelectrophoresis of plate 6 a.

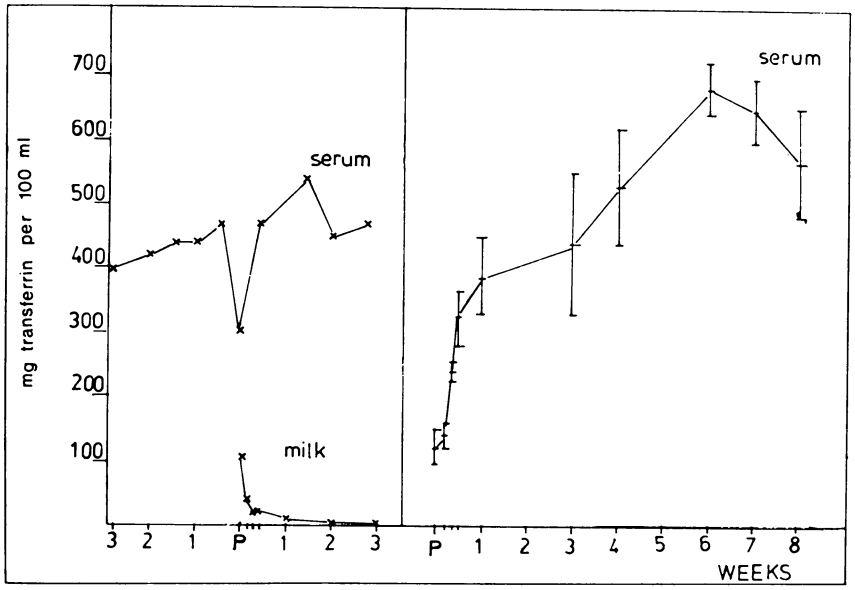

Figure 7a. Transferrin levels in serum and colostrum/milk from sow no. 3, to the left, and in serum from her piglets, to the right.

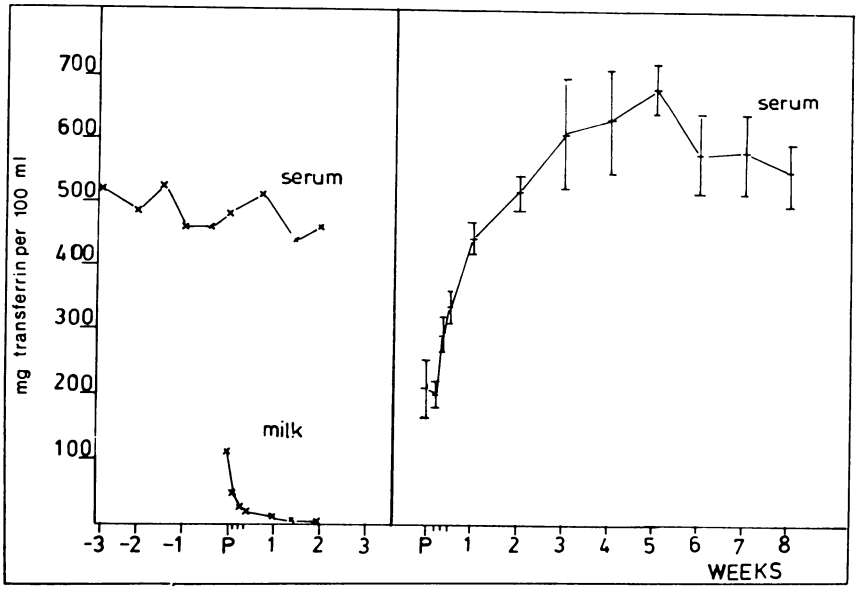

Figure 7b. Transferrin levels in serum and colostrum/milk from sow no. 6, to the left, and in serum from her piglets, to the right.

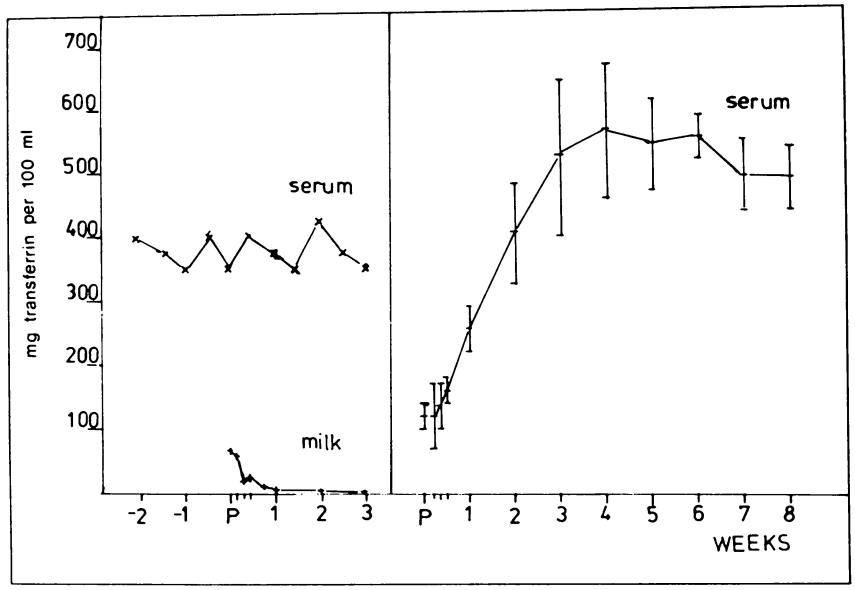

Figure 7c. Transferrin levels in serum and colostrum/milk from sow no. 178, to the left, and in serum from her piglets, to the right.

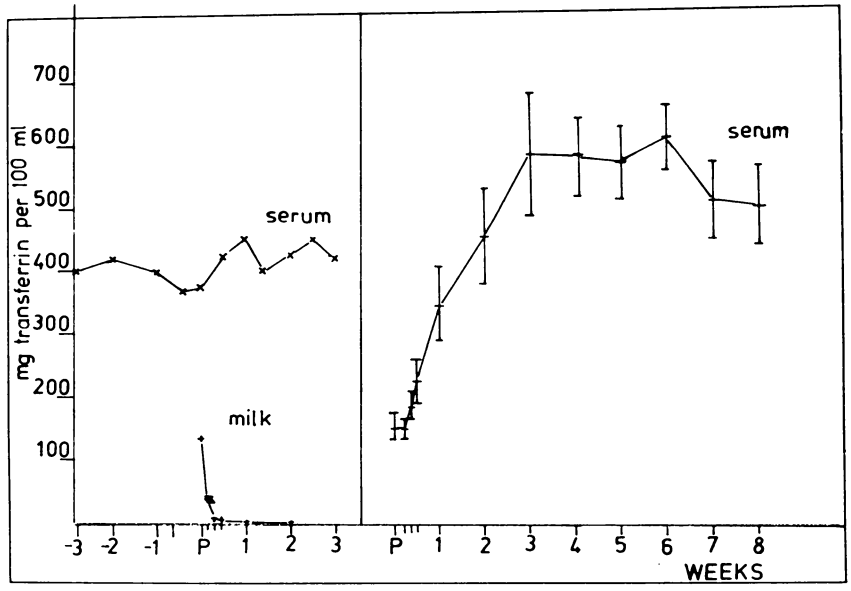

Figure 7d. Transferrin levels in serum and colostrum/milk from sow no. 182, to the left, and in serum from her piglets, to the right.

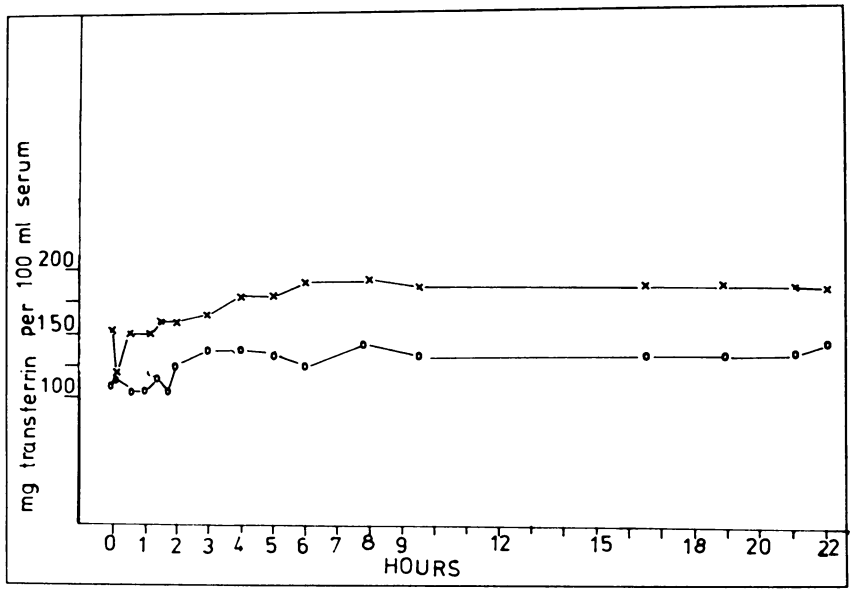

Figure 8a. Transferrin levels in serum from 2 piglets the first 22 hrs. after feeding 20 ml of sow colostrum (43 mg transferrin).

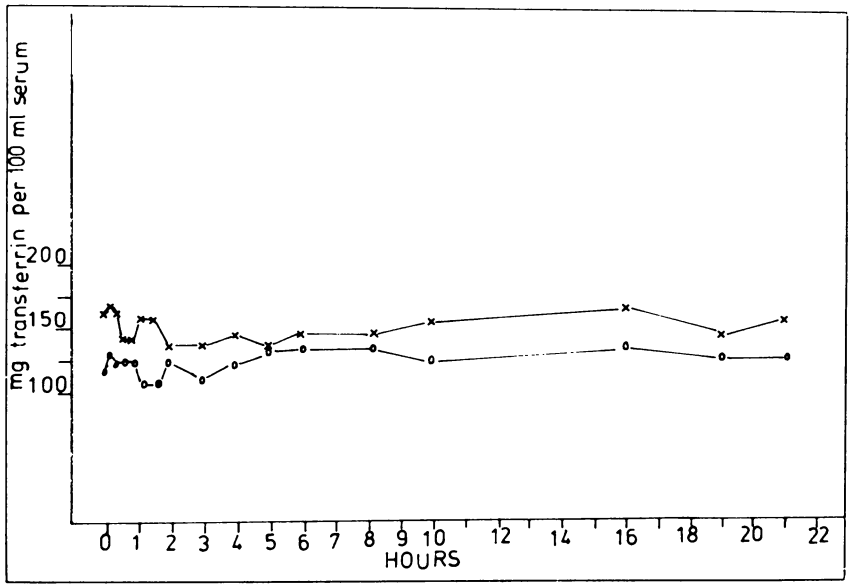

Figure 8b. Transferrin levels in serum from 2 piglets the first 22 hrs. after feeding 30 ml swine serum (113 mg transferrin).

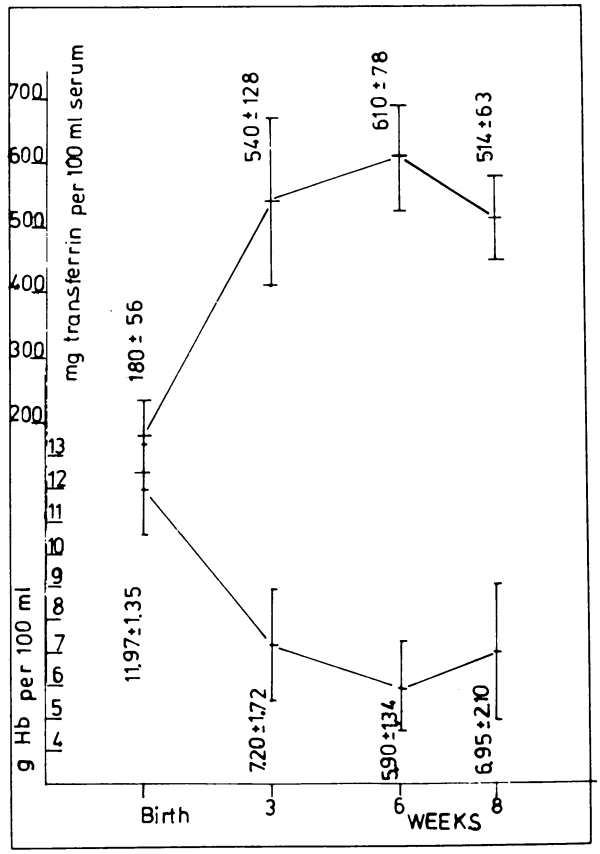

Figure 9. Serum transferrin and haemoglobin levels in piglets from birth to 8 weeks after birth.

when the levels varied between 556 ± 34 mg and 684 ± 35 mg/100 ml. Then the concentration was constant or slightly decreased up to 8 weeks of age.

The transferrin concentration in serum from the sows was rather constant at levels about 400 mg/100 ml (Figs. 7 a-d). Only 1 sow showed a marked decrease in the serum levels at partus. The transferrin concentration in colostrum (Figs. 7 a-d) was about 100 mg/100 ml at partus, but decreased to about 50 mg/100 ml after 1 day and decreased rapidly during the following days.

The transferrin concentration in serum from newborn colostrum-deprived pigs given 20 ml of sow colostrum (containing

43 mg transferrin) showing no or only a very small increase of the serum levels (about 10—15 mg/100 ml) during the following 22 hrs. after the feeding (Fig. 8 a).

The serum levels in pigs given 20 ml serum (containing 113 mg transferrin) showed an increase of 25—35 mg/100 ml with maximum levels about 7 hrs. after the feeding (Fig. 8 b).

The analytical error was calculated from repeated determinations of the control serum in 4 dilutions on every plate. The transferrin content of this serum was found to be 409 ± 41 mg/100 ml ($\bar{x} \pm s$) which means an analytic error of 4.4 %.

The results of details in the haemoglobin determinations of serum after treatment with iron-dextran perorally at different intervals after birth will be published elsewhere, but this parameter was used to elucidate the correlation to the levels of transferrin (Fig. 9). A highly significant negative correlation was observed between the concentrations of haemoglobin and transferrin ($r = -0.69$).

DISCUSSION

In the present investigation immunoelectrophoretic pure transferrin was obtained by a rather simple procedure. It has often been stated that difficulties exist in obtaining transferrin not contaminated with the β-globulins haemopexin and haptoblobin (*Schultze & Heremans* 1966). Serum from newborn colostrum-deprived pigs contains very small amounts of haemopexin and haptoglobin (*Hesselholt* 1969). The precipitation of serum with 60 % saturated ammoniumsulphate removes most of the haptoglobin, and it is obvious that the chromatography of this solution has given some fractions of transferrin free from other β-globulins (Figs. 2 and 3).

The immunization with alun-adsorbed antigen into enlarged lymph nodes seems to be very effective, since only small amounts of antigen are required. The antiserum obtained seemed to be mono-specific, and by radioimmunoelectrophoresis it is obvious that the antiserum precipitates an iron-binding protein, transferrin, in serum.

The mean transferrin levels in newborn presuckled pigs are about 180 mg/100 ml and no greater changes occur during the first day of life. However, the concentration of transferrin in colostrum is about 100 mg/100 ml (one fourth of that in the serum

of the sow). Thus, if the pig has absorbed all the transferrin of the intake of colostrum the first day, about 300 ml, the levels in serum should increase with about 200—300 mg/100 ml. Newborn presuckled pigs given 20 ml colostrum (corresponding to 43 mg transferrin) show no or a very small increase (about 10—15 mg/100 ml) of the serum levels during the first 22 hrs. after the feeding (Fig. 8 a). If all transferrin was absorbed, the serum level should increase with about 50 mg/100 ml. Presuckled pigs given 30 ml serum (corresponding to 113 mg transferrin) showed an increase of the serum levels of about 25—35 mg/100 ml during the first 22 hrs. after the feeding (Fig. 8 b), and if all transferrin was absorbed, the serum level should increase with about 130 mg/100 ml. This indicates that during the first day of life no or only small amounts of the transferrin of colostrum is absorbed, and only about one fourth of the transferrin in swine serum. It should also be noted that the plasma volume increases during the first days of life in pigs (*Ramirez et al.* 1963). Therefore, it is very difficult from the results here to give any definite conclusions on the intestinal transmission of transferrin.

During the second and third days of life a small increase in plasma levels can be seen (20—50 mg/100 ml), and as shown in Fig. 7 there is a rather large increase of the transferrin concentrations during the first 5 weeks after birth, probably due to the decrease of the haemoglobin concentration (Fig. 9). A highly negative significant correlation is found between haemoglobin and transferrin concentrations (Fig. 9). It may therefore be concluded that a decreased concentration of haemoglobin indicating anemia accelerates the synthesis or decreases the catabolism of transferrin, probably due to increased absorption and accelerated transport of iron from the body stores for the erythropoiesis. The occurrence of relatively high concentrations of transferrin at birth in pigs indicates that the risk of iron poisoning after additional supply of iron probably is small, provided a normal status of vitamin E. In this respect another iron-binding protein in sow colostrum also seems to be of importance, namely lactoferrin. The concentration of this protein in sow colostrum during the first days of lactation is relatively high (*Masson & Heremans* 1970). It has not been shown, if lactoferrin is absorbed by the newborn pigs, but it may be concluded that this protein may play a role for the iron-binding capacity in colostrum. According to *Blanc & Isliker* (1963) and *Ezeihiel* (1965) lactoferrin may also

be involved in the transfer of iron from serum transferrin to ferritin, and the latter is probably a form under which iron can also be excreted by the mammary glands.

The iron-binding proteins may protect the organism from toxic effects of free iron. It has also been shown that transferrin and lactoferrin with regard to their metal-chelating and bacteriostatic properties may play a role in the protection against microorganisms by inhibiting their growth (*Schade* 1963, *Masson & Heremans* 1966, *Masson et al.* 1968). Furthermore, it has been shown that iron in excess of the binding capacity of transferrin or lactoferrin allows E. coli to grow in fresh serum. This growth was inhibited by iron-free transferrin (*Fletcher* 1971). It has also been shown that iron-unsaturated lactoferrin has a great growth-inhibitory property of Candida albicans incubated in milk cultures (*Kirkpatrick et al.* 1971). Iron-free transferrin has also been shown to inhibit the oxidation of unsaturated lipids through chelation of inorganic iron. Therefore it has been proposed that the iron-free transferrin has an antioxidant function and may protect from the formation of toxic lipid peroxidases in tissues (*Schade*). It is obvious that the iron-binding proteins of serum and colostrum are very important for the organism in several respects. Especially the effect on bacterial growth seems important and requires further investigations.

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SAMMANFATTNING

Transferrinkoncentrationen i serum hos suggor och smågrisar och kolostrum.

Immunologiskt rent transferrin isolerades ur svinserum genom utfällning med ammoniumsulfat och jonbyteskromatografi. Kanin anti-svintransferrin serum framställdes och användes för en immunologisk

bestämning av transferrin i serum och kolostrum. Transferrinhalten i serum hos smågrisar var tämligen konstant under de första dygnen med 180 ± 56 mg/ml vid födelsen, för att stiga till 610 ± 78 mg/100 ml vid 6 veckors ålder. Transferrinvärdena i kolostrum var ca 100 mg/100 ml och sjönk snabbt. En negativ korrelation mellan koncentrationen av hemoglobin och transferrin i serum förelåg. Betydelsen av transferrin diskuteras.

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