Br. J. Nutr. (1971), 26, 449

Soluble and insoluble blood serum proteins in fed and fasted newborn pigs

By G. BENGTSSON

Department of Animal Nutrition, Genetics and Hygiene, Royal Veterinary College, S-104 05 Stockholm, Sweden

(*Received* 14 *December* 1970 – *Accepted* 10 *May* 1971)

1. Blood serum concentrations of total nitrogen, soluble N, total protein, precipitable and soluble proteins in suckled and starved piglets during the immediate postnatal period were determined.

2. The major part of the total serum proteins at birth appeared on paper electrophoresis as β-globulin, α-globulin and post-albumin. In addition small amounts of pre-albumin were found.

3. There were no significant changes of serum concentrations of nitrogenous compounds in newborn piglets starved for up to 12 h as compared with those at birth.

4. In suckled piglets there was a significant increase of total N and total protein. This process was confined to the first 12 h from birth. Concentrations of γ- and β-globulin and albumin increased and that of α-globulin decreased during this time.

5. High concentrations of proteins soluble in sodium tungstate were noted in the newborn piglet. At birth, soluble protein N amounted to 105·1 ± 17·6 (SD) mg N/100 ml serum. Two-thirds of these soluble proteins were recovered electrophoretically as two adjacent fast-moving fractions, the slower being the larger. One distinct band migrating at an intermediate rate and one complex slow-moving protein represented the remaining part of the soluble proteins at birth.

6. During the first 4 h after birth there was an abrupt decrease in the concentrations of soluble protein N in suckled piglets, values at 4 h being 29·6 ± 13·6 and 17·2 ± 15·4 mg N/100 ml respectively, and still less at 3 d.

7. It was found that the nursed piglets require more than 150 ml sow's colostrum/kg body-weight during the first 20 h from birth for normal development of the serum N profile.

8. A determination of total protein concentration in blood serum should be a very rapid and simple way to obtain information about the nutritional, developmental, and probably also the immunological state, of the very young suckled piglet.

The piglet possesses at birth a high concentration of non-precipitable serum nitrogen, explained only to a minor extent by an increased level of α-amino-N (Bengtsson, 1970). A part of the soluble N pool was assumed to consist of foetuin, an immature protein with a substituting function when foetal and neonatal concentrations of protein are low. This immature substance disappeared in suckled piglets during the first days after birth. Trichloroacetic acid-soluble proteins are known to occur in large amounts in the blood serum of piglets at birth (see review by Lardinois & Page, 1969). Studies on the neonatal specific serum proteins of the developing pig (Lecce & Matrone, 1960; Dickerson & Southgate, 1967; Lardinois & Page, 1969; Karlsson, 1970) have mostly been conducted in piglets at least 1 d old. For instance, Lardinois & Page (1969) used suckled piglets aged 4–5 d. The rapid development of the blood serum protein profile (Nordbring & Olsson, 1957; Miller, Ullrey, Ackerman, Schmidt, Hoefer & Luecke, 1961; Ramirez, Miller, Ullrey & Hoefer, 1963) that occurs during the first hours after birth in suckled piglets has not been considered by other workers. In the present study the specific neonatal proteins of the piglet have been quantitatively determined. The work has mainly been centred around the first 12 h of life. In addition, the properties of the proteins have been further elucidated, and the effects on the serum N pattern of fasting and of controlled feeding have been investigated.

EXPERIMENTAL

Suckling piglets

Thirty-seven Swedish Landrace piglets of both sexes from four sows aged 1–2 years were used. The sows farrowed after an uneventful normal period of gestation. The mean birth weight of the piglets was 1360 ± 210 (SD) g. Immediately after birth and before suckling, blood samples were obtained from the umbilical vein as reported earlier (Bengtsson, 1970). No differences in the N fractions were found between blood collected from the umbilical vein at birth and blood drawn from the anterior vena cava at the same time. The care of the experimental animals has been described elsewhere (Bengtsson, 1970). Piglets were allowed to suckle at will from birth. Four hours after birth blood was drawn from ten piglets by a blind puncture of the anterior vena cava according to Carle & Dewhirst (1942). A further ten and seventeen piglets were bled at 12 and 72 h after birth, respectively. At least two piglets were bled from each litter at every age. The milk yield of the sows was checked in all experiments by weighing piglets before and after suckling.

Fasted piglets

Blood was obtained from the umbilical vein at birth in sixteen piglets of both sexes. They were given 5 ml isotonic saline/kg body-weight subcutaneously, caged individually at an ambient temperature of 34° and starved from birth. After 4 h blood was drawn from the anterior vena cava in eight piglets and after 12 h blood was sampled from the remaining eight piglets.

Tube-fed piglets

To obtain information about the amount of sow's colostrum required for the development of the serum N profile, a litter of ten piglets was used. At birth and before suckling, blood was obtained from the umbilical vein in all piglets. Five control piglets were permitted to suckle the sow *ad lib.* for 20 h. The mean birth weight of the piglets was 1466 ± 294 (SD) g. The remaining five piglets were caged individually and given five feeds of 30 ml colostrum/kg body-weight every 4th hour during the first 20 h from birth. The mean birth weight of these piglets was 1464 ± 154 g. Both tube-feeding and suckling began immediately after blood sampling at birth. The colostrum used for tube-feeding had been obtained from another sow in the same pig-house. It was collected from the sow at approximately the same time after birth as it was fed to the piglets. The milk had been stored at −20° for several months before being used. Two hours after the last suckling and feeding, blood was obtained from each piglet by a blind puncture of the anterior vena cava.

Quantitative determinations

Serum was removed at 4° and either analysed at once or immediately frozen and stored at −20° until analysed. Storage at −20° and subsequent thawing had no apparent influence on the substances studied. In suckled and fasted piglets total N content of serum was determined by the micro-Kjeldahl method. The error of the method was calculated by the formula $\surd(d^2/2n)$, where d is the difference between duplicate determinations and n is the number of duplicate determinations; calculated on duplicate analyses, it was ±2·9% at a mean value of 436 mg N/100 ml serum. The concentration of the soluble N pool was determined according to the method of Rappaport & Eichhorn (1947) modified by Goa (1951). The concentration of protein N was determined in two ways: by the modified biuret method of Weichselbaum (1946) using the factor 1/6·25, this N being designated biuret protein N, and from the micro-Kjeldahl N after correction for soluble N, this protein N being designated Kjeldahl protein N.

In tube-fed piglets and their controls biuret protein N and total soluble N were determined according to the above-mentioned methods. To get further information about the development of the serum protein profile in tube-fed piglets and their controls, α-amino-N was determined by the method described earlier (Bengtsson, 1970).

Paper electrophoresis of normal and deproteinized serum

Serum from newborn, suckled and fasted piglets was investigated by paper electrophoresis. In addition, portions of the serum samples were deproteinized by the method of Goa (1951), which gives high concentrations of soluble N compounds in serum from the newborn unsuckled piglet (Bengtsson, 1970). The supernatant fraction was evaporated to dryness under reduced pressure at 37° and dissolved in 1 ml tris-buffer (Aronsson & Grönwall, 1957) before being run on paper electrophoresis. This was performed according to Valmet & Svensson (1954) with Schleicher and Schüll paper 2043 b (Schleicher and Schüll, Dassel, W. Germany) at 225 V and 6 mA for 16 h. The tris-buffer had a pH of 8·9. Amido black 10 B (E. Merck, Darmstadt, W. Germany) was used for staining the proteins. The colour intensities of the fractions were determined by a Beckman-Spinco Analytrol 212 (Beckman Instruments Inc., Belmont, Calif., USA).

Differences between group means were analysed by Student's t test.

RESULTS

Mean values and standard deviations for serum total N, soluble N, Kjeldahl protein N and biuret protein N in newborn and suckled piglets of different ages are given in Fig. 1. No significant differences were found between groups before suckling. Concentrations of total N and protein N were low at birth. The concentration of biuret protein N amounted to 441·7 ± 71·3 (SD) mg N/100 ml in the newborn unsuckled pig. The relative concentration of the indirectly determined Kjeldahl protein N, 336·9 ± 58·8 mg N/100 ml, was significantly lower than that of biuret protein N at birth ($P < 0.001$). There was a significant increase ($P < 0.001$) in the content of total N and protein N at 4 h in suckled piglets; at the same time the concentration of soluble N was significantly decreased ($P < 0.001$). The difference between biuret protein N and Kjeldahl protein N was not significant at 4 h or later.

During the next 8 h total N and protein N increased further ($P < 0.001$) and soluble N decreased ($P < 0.05$).

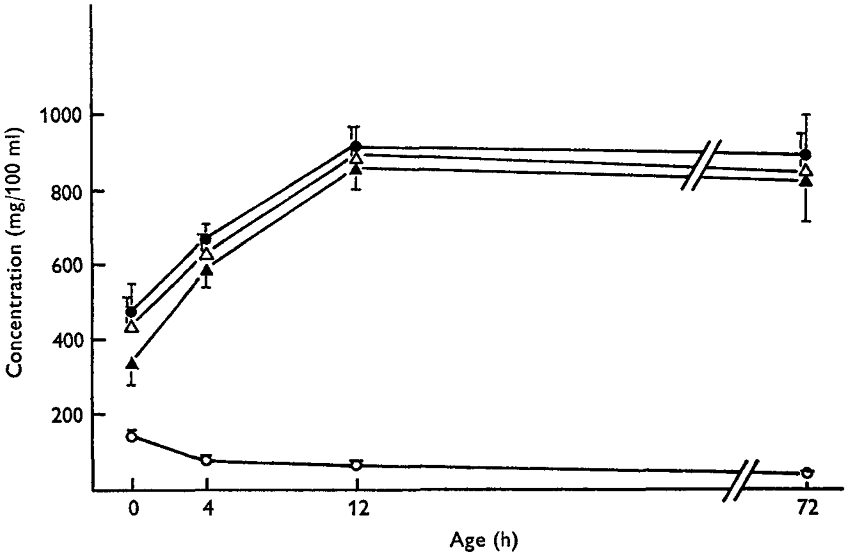


Fig. 1. Concentrations of total nitrogen (●—●), soluble N (○—○), biuret protein N (△—△) and Kjeldahl protein N (▲—▲) in the blood serum of newborn suckled piglets at various ages. Mean values and standard deviations for thirty-seven piglets.

Table 1. *Total nitrogen (mg/100 ml), soluble N (mg/100 ml) and biuret protein N (mg/100 ml) in blood serum of starved piglets*

(Mean values and standard deviations)

	At birth (16)	After 4 h (8)	After 12 h (8)
Total N	450·0 ± 47·6	468·8 ± 68·5	481·4 ± 41·8
Soluble N	146·2 ± 14·9	137·3 ± 13·0	155·6 ± 11·9
Biuret protein N	410·0 ± 69·3	394·0 ± 51·3	428·2 ± 54·6

Figures in parentheses are the numbers of piglets.

In piglets suckled for 72 h soluble N was significantly ($P < 0.001$) lower than at 4 h or 12 h after birth.

In Table 1 values for total N, soluble N and biuret protein N in the serum of piglets starved for up to 12 h from birth are given. There were no significant changes during the starvation period in the substances studied.

Pl. 1 shows representative electrophoretograms of normal and deproteinized serum from newborn piglets at various nutritional stages. The percentages of different protein fractions are given in Table 2.

At birth absolute amounts of albumin and γ-globulin in serum were low. The major part of the serum proteins occurred in three bands (Pl. 1 *a*). One moved somewhat more slowly than albumin and one broad band was located in the α-globulin region. These two bands are treated as one in the analysis and are referred to as α-globulin because of difficulties in effecting their complete separation routinely by paper electrophoresis. One protein fraction moved distinctly as β-globulin and contributed about a fourth of the total serum protein at birth. In addition, a rapidly moving fraction produced a faint coloured band in the pre-albumin area. This part could not be quantitatively determined separately but was included in the albumin fraction in the analysis.

Table 2. *Blood serum proteins in neonatal piglets*

(Mean values and standard deviations)

Age	No. of piglets	Total protein N (mg/100 ml)	Relative percentages of protein fractions: Albumin	α-globulin	β-globulin	γ-globulin
Newborn	53	410·0±69·3	12·4±6·2	58·5±11·5	22·6±7·3	6·5±3·4
4 h, starved	8	394·0±51·3	13·2±5·8	52·4±8·6	26·4±6·8	7·9±4·7
4 h, suckled	10	622·4±56·7	10·4±3·7	20·1±7·1	22·1±5·1	47·4±9·1
12 h, suckled	10	889·6±75·5	7·7±4·9	8·8±5·7	13·6±8·9	72·6±16·5

Table 3. *Total soluble serum protein nitrogen* (*mg*/100 *ml*) *and relative percentage of soluble protein fractions in piglets at birth*

(Mean values and standard deviations)

Total soluble protein N	Relative percentage of fraction: *a*	*b*	*c*	*d*
105·1±17·6	12·0±8·1	20·3±6·2	52·0±14·7	15·5±7·0

a, slow-moving broad band; *b*, distinct band that migrated at an intermediate rate; *c* and *d*, fast-moving fractions that were adjacent to one another.

There were no qualitative or quantitative changes of the protein pattern in piglets fasted for 4 h from birth as compared to the pattern at birth (Table 2, Pl. 1 *c*). In suckled piglets at 4 h and 12 h of age the most striking change was the appearance of large amounts of γ-globulin (Table 2, Pl. 1 *e*, *f*). Moreover, suckling for 4 h was accompanied by an absolute increase of albumin and β-globulin and an absolute decrease of α-globulin.

Electrophoresis of the supernatant fraction of deproteinized serum from newborn and starved piglets yielded one diffuse and three distinct protein bands (Pl. 1 *b*, *d*). In Table 3 the relative percentages of soluble protein fractions are given. Fraction (*a*) consisted of a slow-moving broad band. One distinct band (*b*) migrated at an intermediate rate. Two fast-moving fractions (*c* and *d*) were observed. They were adjacent to one another. Together they represented 67·5±11·6 (SD) % of the soluble protein fractions at birth.

A faint band, located at the leading edge of the fastest-migrating soluble serum protein found at birth, was seen in piglets suckled for 4 h. No proteins could be observed in deproteinized serum of other suckled piglets.

The results of giving controlled amounts of sow's colostrum to the piglets are given in Table 4. No significant differences were found between the groups at birth. As can be seen from the table, the concentration of total protein was significantly higher and those of soluble and α-amino-N were significantly lower in suckled and tube-fed piglets compared with the values at birth. The N fractions studied were significantly higher for piglets that were allowed to suckle *ad lib.* for 20 h from birth than for piglets given 150 ml sow's colostrum/kg body-weight by stomach tube during the same period.

Table 4. *Total protein nitrogen (mg/100 ml), soluble N (mg/100 ml) and α-amino-N (mg/100 ml) in blood serum of control and tube-fed piglets*

	Mean values ± SD in piglets			
	At birth (10)	Suckled *ad lib.* for 20 h (5)	Tube-fed 150 ml colostrum during 20 h period (5)	Significance of difference†
Total protein N	392·0 ± 61·9	976·0 ± 25·3	534·0 ± 78·9	***
Soluble N	184·0 ± 19·4	60·1 ± 6·7	78·2 ± 9·0	**
α-amino-N	25·3 ± 2·5	11·9 ± 2·3	16·2 ± 2·3	*

Figures in parentheses are the numbers of piglets.

† Between suckled and tube-fed piglets: *** $P < 0·001$, ** $P < 0·01$, * $P < 0·02$.

DISCUSSION

Solubility of neonatal proteins

The changes of the plasma volume occurring in the piglet during the early postnatal period (Ramirez *et al.* 1963) did not influence the significance of the results in the present study. The high concentration of soluble N and low concentration of serum proteins in the newborn unsuckled piglet are in accordance with results reported earlier (Nordbring & Olsson, 1957; Bengtsson, 1970). The present study also showed that the concentrations of total protein N determined by the direct colorimetric biuret method was 441·7 ± 71·3 (SD) mg N/100 ml serum, a value that agrees well with that found by Waddill, Ullrey, Miller, Sprague, Alexander & Hoefer (1962) using a direct method. However, when the protein N concentration is calculated from the total N content less the content of soluble N, a mean value of 336·9 ± 58·8 mg N/100 ml serum is obtained. On the assumption that the serum proteins of the newborn piglet contain 16% N, the difference between biuret protein N and precipitable protein N is 105·1 ± 17·6 mg N/100 ml. This difference is thus due to soluble nitrogenous compounds which appear as proteins at direct determination. The high neonatal concentration of unidentified N, soluble in sodium tungstate–sulphuric acid (Bengtsson, 1970), may therefore be attributed to this protein-imitating compound. In this context the finding of a specific, trichloroacetic acid-soluble and immature (Jakobsen & Moustgaard, 1950; Rook, Moustgaard & Jakobsen, 1951; Lecce & Matrone, 1960) neonatal pig protein may be borne in mind.

Though the term 'protein-imitating' is used for the soluble proteins, the blood serum proteins of the newborn piglet as a whole are assumed in this work to contain 16% N. This assumption was also made by Dickerson & Southgate (1967), in contrast to Deutsch (1954) who used a N factor of 7·43 for calf foetuin and of 6·25 for residual protein. Spiro (1960) found a N value of 12·2% for calf foetuin.

It is impossible to say whether soluble proteins are responsible for the high concentrations of α-amino-N found in the piglet at birth (Bengtsson, 1970). Analysis of serum-free amino acids in newborn and suckled piglets (Bengtsson, 1971) did not show simultaneous changes in concentrations of α-amino-N and of the free amino acid pool. Moreover, when precipitation with 2 M instead of 1/3 M sulphuric acid was used, the same quantity of non-protein N, about 5% of the total N, was found in newborn piglets as in older ones (Jakobsen & Moustgaard, 1950). If the biuret protein in the present study is assumed to contain 16% N, then 441·7 ± 71·3 (SD) mg N/100 ml newborn piglet serum, or about 93% (w/v) of the total N amounting to 474·3 ± 73·0 mg/100 ml serum, appeared in biuret protein. That means that 32·6 ± 5·7 mg N/100 ml is true non-protein N in piglet serum at birth. The remaining part of the soluble N is present as soluble proteins.

Disappearance rate of soluble proteins

The content of total soluble N, being 139·9 ± 15·4 mg/100 ml serum at birth, decreased very rapidly in suckled piglets, values at 4, 12 and 72 h being 76·3 ± 10·4, 65·1 ± 8·7 and 36·8 ± 5·2 mg/100 ml serum respectively. On the other hand, the concentration of real non-protein N did not change significantly during the observation period. The decrease thus appears to be related to soluble proteins. The fall occurred mainly during the first 4 h of life. With the methods used in the present study no significant difference was demonstrated between biuret protein and Kjeldahl protein 4 h after birth or later. Based on the differences between soluble N and true non-protein N, using the factor 6·25, the concentration of soluble protein N was 29·6 ± 13·6 (SD), 17·2 ± 5·4 and 5·3 ± 1·3 mg/100 ml blood serum at, respectively, 4 h, 12 h and 72 h of age.

The unchanged concentrations of real non-protein N in the developing piglet do not *per se* exclude decreasing levels of α-amino-N reported earlier (Bengtsson, 1970). The changes are too small to be revealed by the methods used in this study. Moreover the increase of amino acid N during the period (Bengtsson, 1971) may have partly masked the decrease of α-amino-N.

In piglets starved for up to 12 h from birth there are no significant changes in the level of soluble proteins. Suckled piglets show a rapid and continuous fall of these proteins. On the assumption that solubility in conventional deproteinizing agents is a characteristic of the specific, foetal and neonatal blood serum protein of the pig, the finding of continuously decreasing amounts of this protein in the present study is in contrast to the observations of Karlsson (1970), that α-foeto-protein increases during the first days after birth. Lardinois & Page (1969) noted decreasing amounts of trichloroacetic acid-soluble proteins in blood serum during the first 10 d of life in the piglet, but did not investigate suckled piglets younger than 4 d.

The development of the serum N pattern reported here is observed in normal healthy piglets that have absorbed colostrum and milk in sufficient amounts. In piglets starved for up to 12 h from birth no significant changes in the content of total N, soluble N or biuret protein were found in the present study. Indeed fasting for 3 d from birth does not change serum concentrations of Kjeldahl or soluble N in the piglet (Olsson, 1959). In piglets nursed inadequately the maturation of the serum N pattern is thus retarded. This could be the situation in agalactia in the sow, and malabsorption or other dysfunctions in the piglet. Piglets with low concentrations of biuret protein and high concentrations of soluble serum N are less vigorous and more likely to die than those nursed normally (Bengtsson, unpublished). A determination of biuret protein in the serum of very young suckled piglets is a very simple and rapid way to obtain an impression of their nutritional, developmental and probably their immunological state.

Protein fractions of neonatal serum proteins

Paper electrophoresis revealed low concentrations of γ-globulin and albumin in the newborn piglet. This is in accordance with findings of Rook *et al.* (1951), Nordbring & Olsson (1957), Lecce & Matrone (1960), Miller *et al.* (1961) and Lardinois & Page (1969), but is in contrast to high concentrations of albumin reported by Moore, Shen & Alexander (1945), Foster, Friedell, Catron & Dieckmann (1951) and Dickerson & Southgate (1967). Moreover, according to the present study β-globulin, α-globulin and post-albumin are the major components of the serum proteins in the piglet at birth. In addition, small amounts of pre-albumin were found. This is in contrast to findings of Lardinois & Page (1969) that, on polyacrylamide gel electrophoresis, most of the serum protein of newborn piglets appears in two prominent bands moving faster and more slowly than albumin, but agrees entirely with the findings of Nordbring & Olsson (1957) and Miller *et al.* (1961), except that these authors, using paper electrophoresis, did not observe pre-albumin.

The protein fraction moving slightly more slowly than albumin in this study is probably identical with the protein located between albumin and the α-2-globulin first reported by Rook *et al.* (1951) as component X and commonly designated α-1-globulin or, more recently, post-albumin (Lardinois & Page, 1969). α-2-Globulin was then found to be the major component of the serum of newborn unsuckled piglets in the present study. About a quarter of the neonatal protein appeared as β-globulin. The results concerning α-2- and β-globulin in this study confirm those reported by Rook *et al.* (1951), Nordbring & Olsson (1957), Rutqvist (1958), Miller *et al.* (1961) and Waddill *et al.* (1962). Using cellulose acetate electrophoresis, Dickerson & Southgate (1967) found high concentrations of α-2- and β-globulin in the newborn pig but also high levels of albumin.

Soluble protein fractions

Of the protein N at birth, 105·1 ± 17·6 mg/100 ml, or 24% (w/v), was soluble in the sodium tungstate–sulphuric acid used as precipitant. This deproteinization agent is more effective than 5% trichloroacetic acid, which according to Lardinois & Page (1969) yielded 60–85% of the proteins unprecipitated. On paper electrophoresis, the tungstic acid-soluble proteins in this study appeared as one small, complex, slow-moving, and three faster-migrating, distinct, fractions. These proteins are responsible for the unidentified soluble N constituents that occur in large amounts in blood serum of newborn unsuckled piglets (Bengtsson, 1970). The major part of the soluble proteins consisted of two fast-migrating fractions positioned close to one another. The finding of three distinct soluble protein fractions in the newborn unsuckled piglet is in accordance with results obtained by Lardinois & Page (1969) from trichloroacetic acid-deproteinized serum, in which the greater part of the fractions appears to be. The two fastest-moving trichloroacetic acid-soluble protein fractions have been shown by Lardinois & Page (1969) to be pre- and post-albumin. In a very recent paper Karlsson (1970) reports that α- and β-foeto-protein occur in blood serum of newborn piglets, but no further details of the β-protein are given. By contrast, Dickerson & Southgate (1967) found that high concentrations of non-protein N in the serum of the newborn piglet depend on one or more mucoproteins. Work is in progress to further identify the serum proteins of the newborn unsuckled pig that are soluble in sodium tungstic acid.

Probable fate of soluble proteins

Though a high urinary excretion of N during the 1st day of life is observed in suckling piglets (Bengtsson, unpublished), the very rapid fall of the soluble proteins in suckled piglets and the rapid increase of real protein fractions other than γ-globulin suggests that the soluble proteins enter mature proteins. The major part of this process takes place within 4 h after the commencement of suckling. Whether the changes depend on a primary or secondary effect of the colostrum is not yet clear. Recently Page (1969) reported an abruptly increased synthesis of albumin in the liver of newborn suckled piglets. This synthesis was sufficient to account for the rising concentration of albumin in the serum of the postnatal piglets. However, the author gives no specified information about piglets only a few hours old.

Amount of colostrum required for maturation

There are only a few references in the literature to the amount of colostrum obtained by the piglet during the very first days after birth. Barber, Braude & Mitchell (1955), who weighed piglets before and after hourly suckling, found the daily yield of milk obtained by the piglets during the 1st week of life to be about 550 g. As marked changes in the blood serum N pattern occur very rapidly after birth in suckled piglets, an attempt was made to estimate the amount of sow's milk required for this development. Piglets nursed on the sow for 20 h from birth had a more developed serum N profile than piglets tube-fed with sow's colostrum in five feeds of 30 ml/kg body-weight during the same time. On the assumption that the effect of suckling *per se* is negligible, and provided that storage for a long time at −20° does not influence the milk, the results show that the piglet consumes more than 150 ml sow's colostrum/kg body-weight during the 1st day of life. According to the author's experience, storage for several months at −20° does not change the biological properties of sow's colostrum. Another objection could be that the piglets remaining with the sow received disproportionately more milk, since five litter-mates had been removed. The concentrations of blood serum N fractions of the suckled piglets are, however, in agreement with those reported by Nordbring & Olsson (1957) in suckled piglets of corresponding age.

This work was supported by grants from AB Lactamin, Stockholm, Sweden.

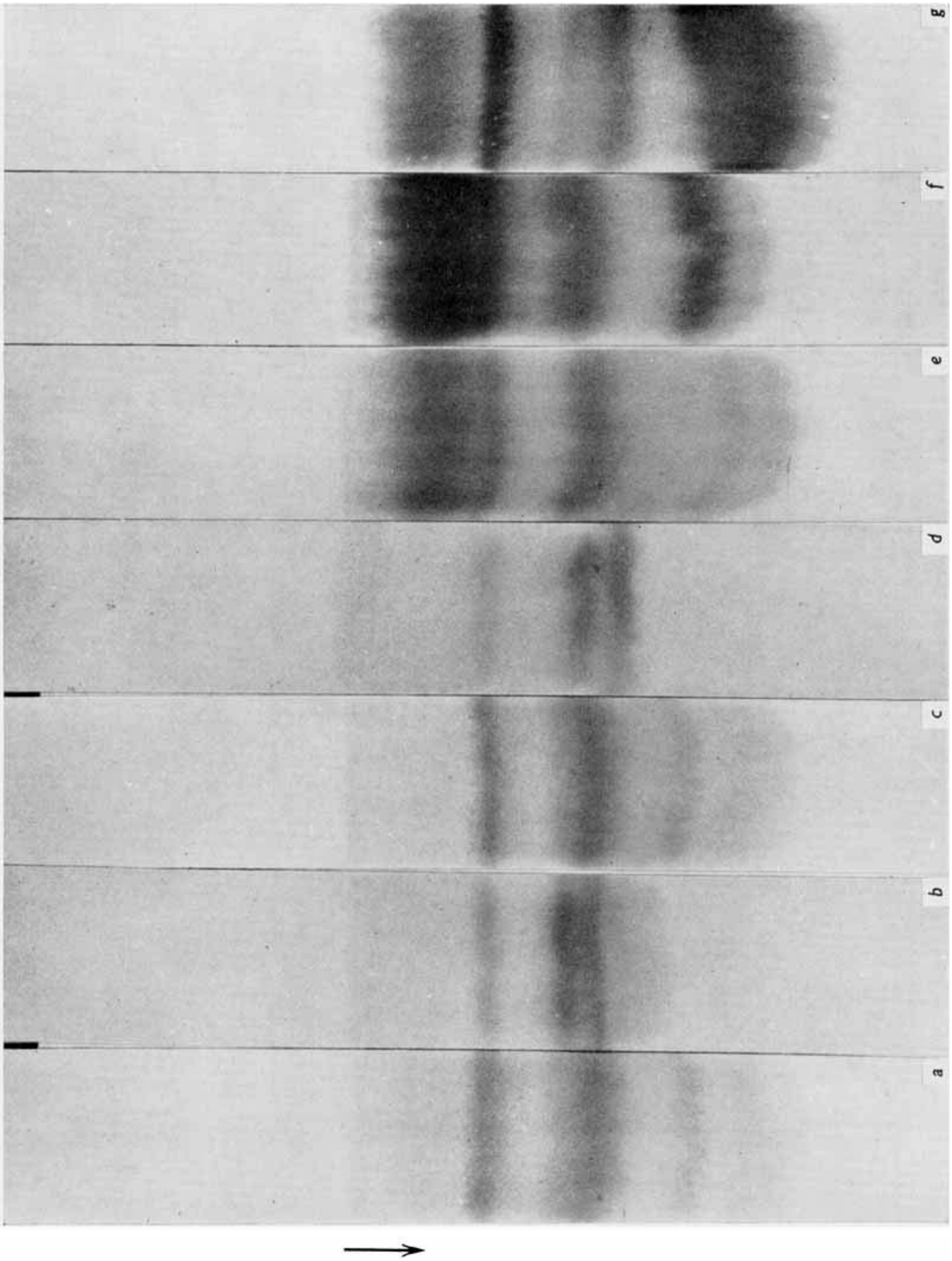
REFERENCES

Aronsson, T. & Grönwall, W. A. (1957). *Scand. J. clin. Lab. Invest.* **9**, 338.
Barber, R. S., Braude, R. & Mitchell, K. G. (1955). *J. agric. Sci., Camb.* **46**, 97.
Bengtsson, S. G. (1970). *J. Anim. Sci.* **31**, 531.
Bengtsson, S. G. (1971). *J. Anim. Sci.* **32**, 879.
Carle, B. N. & Dewhirst, W. H. Jr (1942). *J. Am. vet. Med. Ass.* **101**, 495.
Deutsch, H. F. (1954). *J. biol. Chem.* **208**, 669.
Dickerson, J. W. T. & Southgate, D. A. T. (1967). *Biochem. J.* **103**, 493.
Foster, J. F., Friedell, R. W., Catron, D. & Dieckmann, M. R. (1951). *Archs Biochem. Biophys.* **31**, 104.
Goa, J. (1951). *Scand. J. clin. Lab. Invest.* **3**, 311.
Jakobsen, P. E. & Moustgaard, J. (1950). *Nord. VetMed.* **2**, 812.
Karlsson, B. W. (1970). *Comp. Biochem. Physiol.* **34**, 535.
Lardinois, R. & Page, L. A. (1969). *Devl Biol.* **19**, 261.
Lecce, J. G. & Matrone, G. (1960). *J. Nutr.* **70**, 13.
Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A. & Luecke, R. W. (1961). *J. Anim. Sci.* **20**, 31.
Moore, D. H., Shen, S. C. & Alexander, C. S. (1945). *Proc. Soc. exp. Biol. Med.* **58**, 307.
Nordbring, F. & Olsson, B. (1957). *Acta Soc. Med. upsal.* **62**, 193.
Olsson, B. (1959). *Nord. VetMed.* **11**, 375.
Page, L. A. (1969). *Devl Biol.* **20**, 125.
Ramirez, C. G., Miller, E. R., Ullrey, D. E. & Hoefer, J. A. (1963). *J. Anim. Sci.* **22**, 1068.
Rappaport, F. & Eichhorn, F. (1947). *J. Lab. clin. Med.* **32**, 1034.
Rook, J. A. F., Moustgaard, J. & Jakobsen, P. E. (1951). *Årsskr. K. Vet.-Landbohøjsk.* p. 81.
Rutqvist, L. (1958). *Am. J. vet. Res.* **19**, 25.
Spiro, R. G. (1960). *J. biol. Chem.* **235**, 2860.
Valmet, E. & Svensson, H. (1954). *Sci. Tools* **1**, 3.
Waddill, D. G., Ullrey, D. E., Miller, E. R., Sprague, J. I., Alexander, E. A. & Hoefer, J. A. (1962). *J. Anim. Sci.* **21**, 583.
Weichselbaum, C. T. E. (1946). *Am. J. clin. Path. tech. Suppl.* **10**, 40.

EXPLANATION OF PLATE

Paper electrophoresis of blood serum of the developing piglet. The vertical arrow indicates the direction of migration. In all instances 20 μl of the sample were applied to the paper. *a*, Newborn unsuckled; *b*, supernatant fraction of deproteinized (*a*); *c*, fasted for 4 h from birth; *d*, supernatant fraction of deproteinized (*c*); *e*, suckled for 4 h from birth; *f*, suckled for 12 h from birth; *g*, piglet suckled for 6 d from birth for comparative purposes.

Printed in Great Britain



G. BENGTSSON

(Facing p. 458)