Acta vet. scand. 1983, 24, 84—98.

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ACID-BASE AND ELECTROLYTE CHANGES IN 1-3 DAYS OLD PIGLETS INFECTED WITH ENTEROPATHOGENIC ESCHERICHIA COLI AND IN SPONTANEOUS CASES OF PIGLET DIARRHOEA*

By

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ANDRÉN, BIRGITTA and SUNE PERSSON: *Acid-base and electrolyte changes in 1—3 days old piglets infected with enteropathogenic Escherichia coli and in spontaneous cases of piglet diarrhoea.* Acta vet. scand. 1983, 24, 84—98. — Infection perorally with enteropathogenic E. coli (ST +LT) bacteria in 57 newborn piglets gave rise to watery diarrhoea in 50 (88 %) piglets and was lethal in 17 (34 %) cases. The diarrhoea was associated with a progressing partially compensated metabolic acidosis indicated by significantly decreased pH, pCO_2 and BE values. The acidosis (BE-values) was significantly correlated with increased blood LA and serum K^+ values. The dehydration during the disease was confirmed by increased Hb, Hct, serum protein and urea values as well as loss of weight. The changes were most pronounced in piglets that died and a BE value of —10 mmol/l seemed to be a critical limit at which the prognosis could be considered poor.

The changes in acid-base status and water balance was confirmed in 64 piglets with spontaneous cases of E. coli diarrhoea.

E. coli; diarrhoea; acidosis; piglet.

The pathogenesis of piglet diarrhoea caused by enteropathogenic Escherichia coli (E. coli) bacteria is similar to that of human cholera. The bacteria proliferate in the intestinal lumen, adhere to the epithelium and produce enterotoxins (LT = heat labile, ST = heat stabile) which induce fluid secretion mediated by cyclic AMP and cyclic GMP, respectively, through the mucosa of the small intestines (*Sack* 1980). The great fluid and electrolyte losses during diarrhoea may in severe cases cause a rapid death.

*This investigation was supported by grants from the Swedish Council for Forestry and Agricultural Research.

Moon et al. (1970) found that experimental infection with E. coli bacteria produced diarrhoea in 84 % of infected piglets and that the diarrhoea was associated with increased plasma potassium (K^+), decreased plasma bicarbonate (HCO_3^-) and decreased plasma sodium (Na^+). Experimental E. coli diarrhoea in piglets was accompanied by low pH and base excess (BE) values (*Kutas & Szabó* 1971).

The purpose of this investigation was to study the acid-base status, water and electrolyte changes after the experimental induction of E. coli diarrhoea in piglets and to compare the results obtained with those found in spontaneous cases of the disease.

A further purpose was to use these data to assess prognosis and optimal replacement therapy for affected piglets.

MATERIAL AND METHODS

Animals

Experimentally induced diarrhoea. In this study 68 healthy piglets from 8 litters were used. They were of Swedish Landrace or mixed breed. Weak or undersized piglets (< 900 g) were excluded from the study. In all, 57 piglets from 7 litters were infected experimentally with enteropathogenic E. coli bacteria. Eleven piglets of 1 litter were used as uninfected controls.

The piglets were divided into 3 groups. Group N consisted of noninfected normal piglets. These were also used in a previous study (*Andrén* 1982). The infected piglets that developed diarrhoea (n = 50) formed group D if they died and DR if they survived. To insure that the diarrhoea was caused by the strain of E. coli used for infection only those piglets in which the strain could be reisolated were included. Subsequently 8 piglets of 1 litter were excluded. Due to failing in the experimental procedure as e.g. kinking of or blood coagulation in the catheter 15 more piglets were excluded. Thus the total number of piglets used in the 3 groups were: N = 8; DR = 13 and D = 14, respectively.

Spontaneous diarrhoea. The spontaneous cases of diarrhoea were 64 piglets from 15 litters and they were used for percutaneous arterial and venous blood sampling. The piglets were reared at 11 different conventional Swedish pig farms and Swedish Landrace, Yorkshire and mixed breed were represented. No piglet was younger than 12 h at the time of sampling and the

age distribution of the piglets were 23 piglets of 1 day, 11 piglets of 2 days and 30 piglets of 3 days. The reference material of healthy piglets used for percutaneous sampling has been described elsewhere (*Andrén* 1982).

Experimental infection

Immediately after birth the piglets were taken from the sow. They were catheterized in a carotid artery (*Andrén* 1982) and infected orally with approximately 7.0×10^9 bacteria. The bacteria used for infection was a E. coli strain (BD 3027/76, O-149, $K88^+$, LT^+, ST^+) isolated from a natural outbreak of watery piglet diarrhoea in a pigherd in Sweden (*Söderlind & Olsson* pers. comm.). The bacteria were cultivated at 37°C for about 10 h in a tryptone yeast extract medium containing per litre: tryptone (Difco), 10 g; yeast extract (Difco), 5 g; NH_4Cl, 2.5 g; $Na_2HPO_4 \cdot 12\ H_2O$, 15 g; KH_2PO_4, 6 g; $Na_2SO_4 \cdot 10\ H_2O$, 0.5 g; $MgSO_4$, 2 mg; $MnSO_4$, 1 mg; $FeSO_4$, 0.6 mg; citric acid 0.6 mg. About 30 min after infection the piglets were returned to their dam and were then allowed to suckle freely throughout the whole study.

Sampling procedure

Experimentally induced diarrhoea. After experimental infection the piglets were watched until diarrhoea first appeared. Then arterial blood sampling was performed successively 2 to 5 times during the course of the disease depending upon the the time of survival of the piglets and the patency of the catheter. About 1 ml of arterial blood was drawn from the catheter at each sampling with a heparinized disposable syringe for acid-base, haemoglobin (Hb), haematocrit (Hct) and lactic acid (LA) determinations. Also 3 ml of blood was collected without anticoagulant and the sera from these samples were stored at —20°C until analysed for Na^+, K^+, Cl^-, protein and urea. The body temperature was measured and rectal swabs for bacteriological isolation and serotyping were taken from 1—2 piglets in each litter.

Spontaneous diarrhoea. Arterial blood samples were taken percutaneously with a heparinized syringe (B 109 Arterial Blood Sampler, Radiometer, Copenhagen) (*Andrén* 1982) and analyzed for acid-base parameters, Hb, Hct and LA. Serum levels of Na^+, K^+, Cl^-, protein and urea were determined from

blood drawn from the jugular vein. After sampling the rectal temperature was taken and rectal swabs for bacteriological isolation and serotyping was taken from at least 1 piglet of each litter and pig farm with diarrhoea. In 2 farms only 1 of 2 litters with diarrhoea was sampled.

Analyzing procedure

The arterial blood samples were analysed for pH, pCO_2 and and pO_2 at 37°C in an automatic acid-base analyser (IL 613, Instrumentation Laboratory S.p.A., Milan) and the BE and HCO_3^- values were calculated on the basis of the Siggaard-Andersen nomogram. The acid-base values were also corrected to the actual body temperature. The BE values were calculated at the actual haemoglobin concentration which was determined spectrophotometrically as cyanmethaemoglobin.

Hct was determined after centrifugation for 3 min at 12,000 rpm in an Adams Autocrite centrifuge (Clay Adams Autocrite, Becton, Dickinson and Company). Blood LA was determined enzymatically (Boehringer Mannheim GmB UV test). Serum Na^+ and K^+ were measured with an emission flame photometer (Corning 450, Halstead, Essex) and serum Cl^- with a CMT 10 Chloride titrator (Radiometer, Copenhagen). Serum urea was determined with an urease/glutamate dehydrogenase procedure using an IL 919 glucose/urea/creatinine analyser (Instrumentation Laboratory, Milan) and total serum protein with a biuret reaction according to *Weichelbaum* (1946).

The bacteriological isolation and identification of the E. coli strains were made at the National Veterinary Institute, Uppsala, Sweden, by the biochemical and serological methods of *Söderlind* (1971 a, 1971 b).

Statistical analyses were made by standard methods (*Colton* 1974). Differences between mean values were tested by the Student's t-test and analyses of regression were made by the method of least squares.

RESULTS

Experimentally induced diarrhoea

Within 8—24 h after infection 50 (88 %) of the piglets developed a watery diarrhoea and 17 (34 %) of these died 9—54 h after diarrhoea first occurred. The duration of the disease in

surviving piglets was 4—34 h. The E. coli strain used for infection was isolated from every litter but 1 of those that developed diarrhoea.

The mean loss of weight from onset of diarrhoea until death was 175 g for piglets of group D while piglets of group DR lost only a mean of 50 g during the course of the disease.

Fig. 1 shows the changes of the individual acid-base values of group D piglets in the Siggaard-Andersen nomogram. These values indicated a progressive non-respiratory acidosis during the course of the disease as manifested by increasing deviations from the hexagon representing normal values. In group DR (Fig. 2) these deviations although less pronounced indicated a metabolic acidosis also during the diarrhoeic period. The pCO_2 values

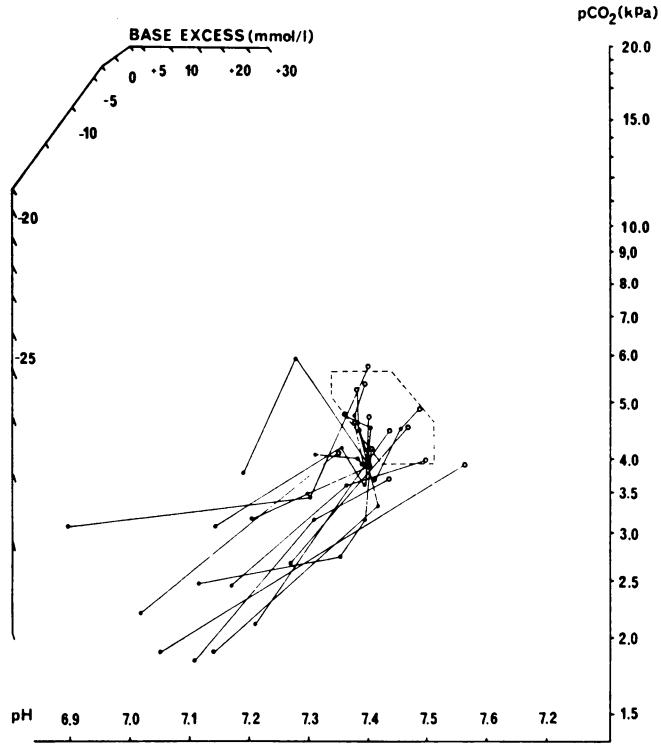

Figure 1. The individual change of the acid-base status of piglets of group D after lethal experimental infection with enteropathogenic E. coli bacteria. Open symbols (o) indicate the initial value observed at the onset of diarrhoea. The dotted hexagon represents the reference values (*Andrén* 1982).

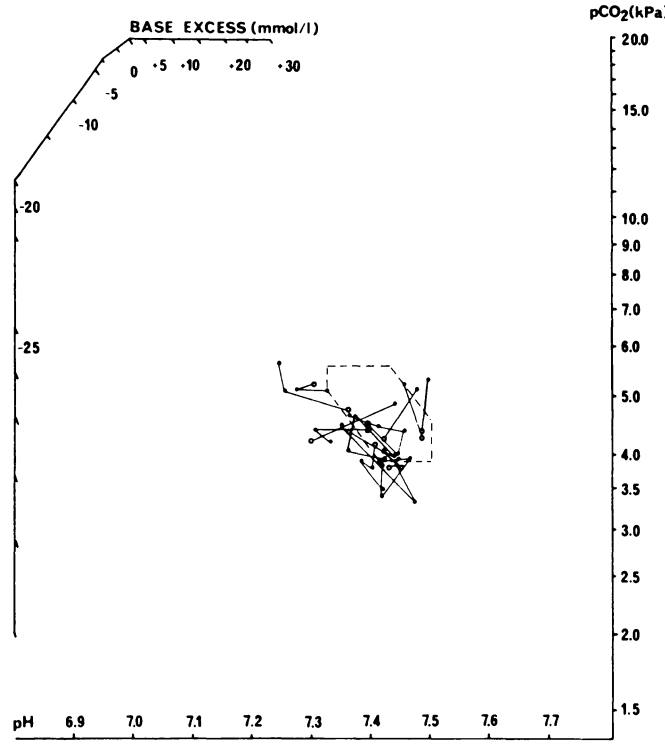

Figure 2. The individual development of the acid-base status of piglets of group DR (surviving piglets) after experimental infection with enteropathogenice E. coli bacteria, cf. Fig. 1.

of group D (Fig. 1) were reduced concurrently with the decrease of the BE values and there were significant differences ($P<0.001$) between the mean terminal values and those of group N. The pCO_2 values from group DR (Fig. 2) were also moderately lower than group N but to a lesser extent than group D.

The BE values of the respective groups are shown in Fig. 3. The terminal values of group D were mostly less than —10 mmol/l and in a few cases as low as —25 mmol/l. In group DR no value below —10 mmol/l occurred. The HCO_3^- values were also reduced throughout the disease and this was most evident in group D.

Figs. 4 and 5 show the changes of the Hct and serum protein concentrations of the respective groups. Both the Hct and protein concentrations of group D increased during the diarrhoea while minimal changes occurred in group DR.

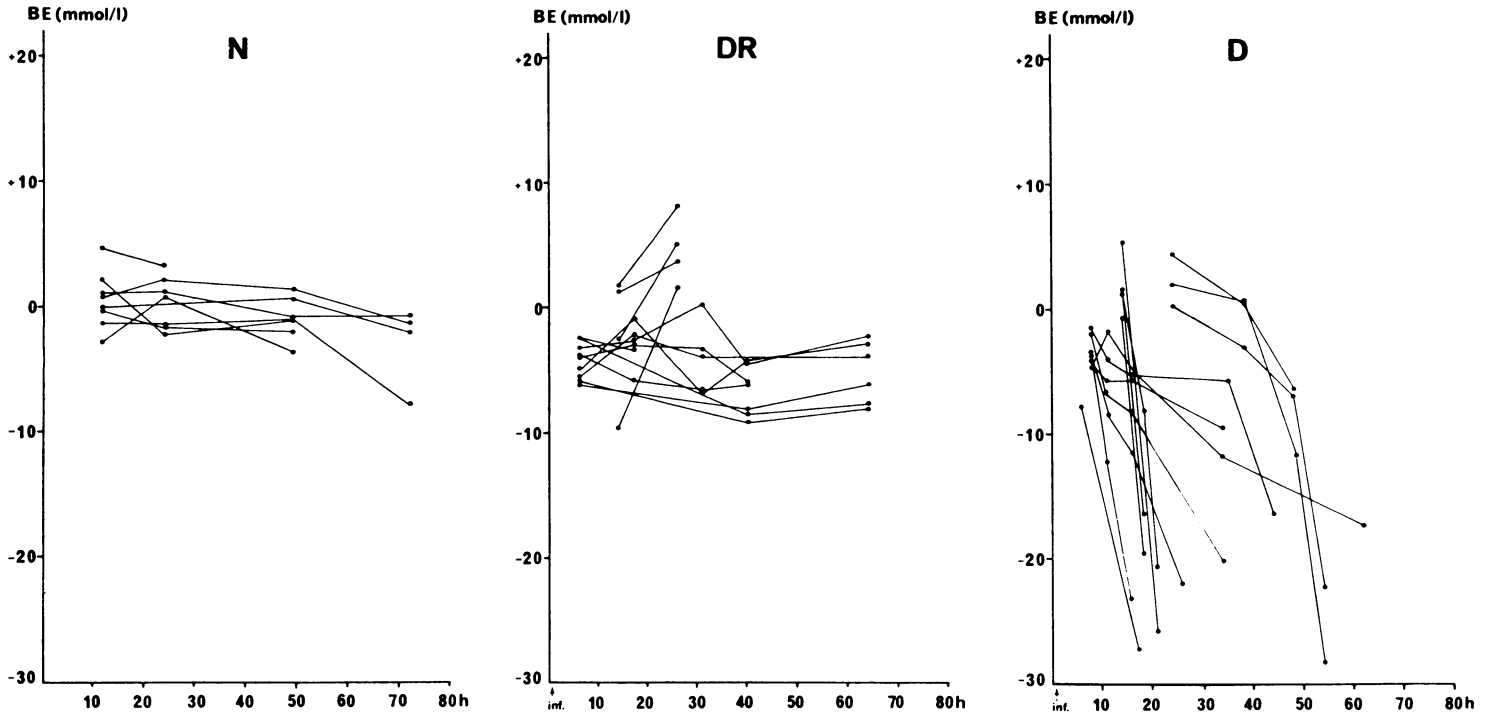

Figure 3. Base excess (BE) values of piglets in the different groups during the first 3 days of life.
N = normal piglets.
DR = piglets that recovered spontaneously after experimentally induced E. coli diarrhoea.
D = piglets that died after experimentally induced E. coli diarrhoea.

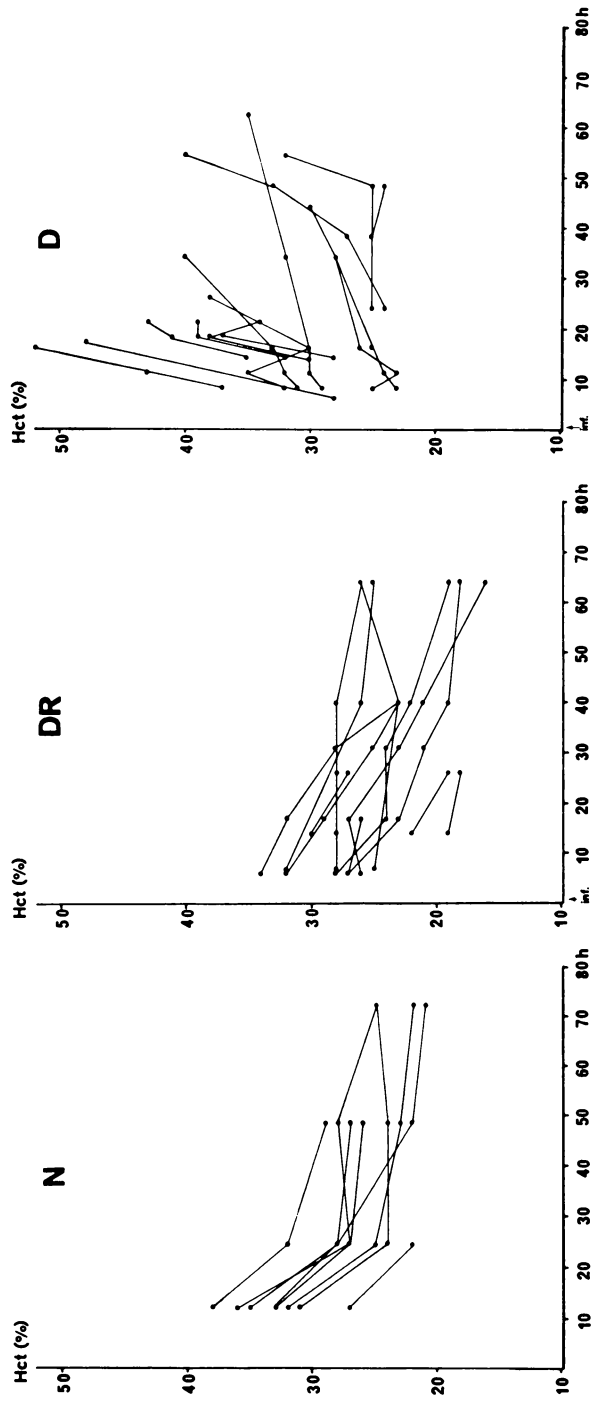

Figure 4. Hct values of piglets during the first 3 days of life. N, DR and D as in Fig. 3.

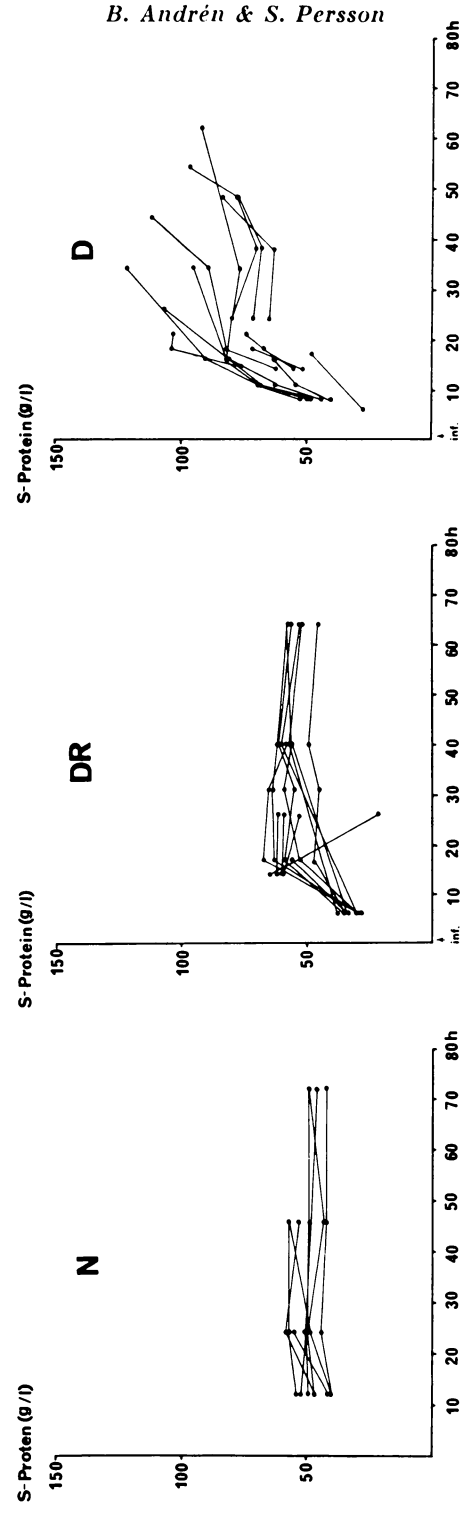

Figure 5. Serum protein concentrations in piglets during the first 3 days of life. N, DR and D as in Fig. 3.

The LA-values of group D were high in the terminal stages of the disease and there was a significant negative correlation between the degree of acidosis (BE value) and the corresponding LA-values (corr. coeff. = —0.6). No marked changes in LA values of group DR were observed. The mean terminal values of group D and DR were 6.5 and 1.8 mmol/l respectively and the mean value of group N 2.0 mmol/l.

Most piglets of group D developed high serum urea concentrations during the disease and the mean terminal value was 9.9 mmol/l. The mean terminal value of group DR was 7.4 mmol/l compared to the mean value of group N which was 6.7 mmol/l.

The Hb values increased in group D reaching a mean terminal value of 113 g/l whereas the values of group DR largely agreed with the values of group N.

The deviations from the normal Na^+ concentration were very small in group DR and in group D a further reduction of a few mmol/l during the diarrhoea was noticed.

The serum Cl^- values did not deviate from the normal value in either of the groups during the disease.

The serum K^+ showed an irregular pattern with values varying between 2.8 and 10.2 mmol/l in group DR and between 2.3 and 14.9 mmol/l in group D, whereas the values of group N were less variable with a mean value of 5.2 mmol/l. There was a significant inverse relationship between the degree of acidosis (BE value) and the serum K^+ level ($r = -0.4$, $P < 0.05$) in group D.

Spontaneous diarrhoea

Of the E. coli strains isolated from 11 different litters with diarrhoea the serotypes from 3 cases were identified as 0—149, 0—141 and 0—45 while the remaining 8 strains were classified as 0—? according to *Söderlind & Möllby* (1978).

The acid-base values of piglets with diarrhoea are plotted in the Siggaard-Andersen nomogram (Fig. 6) and compared with the normal values obtained by percutaneous sampling (*Andrén* 1982). Most values were within the normal range but moderate or severe non-respiratory acidosis (BE < —10 mmol/l) was observed in 25 % of the piglets. Table 1 shows the comparison between the values of normal and diarrhoeal piglets. Significantly lower values for pH, pCO_2 and BE were found in the diarrhoeic piglets while Hb, Hct, K^+, protein and urea were higher than normal. No differences were found in the Na^+, Cl^- or LA values.

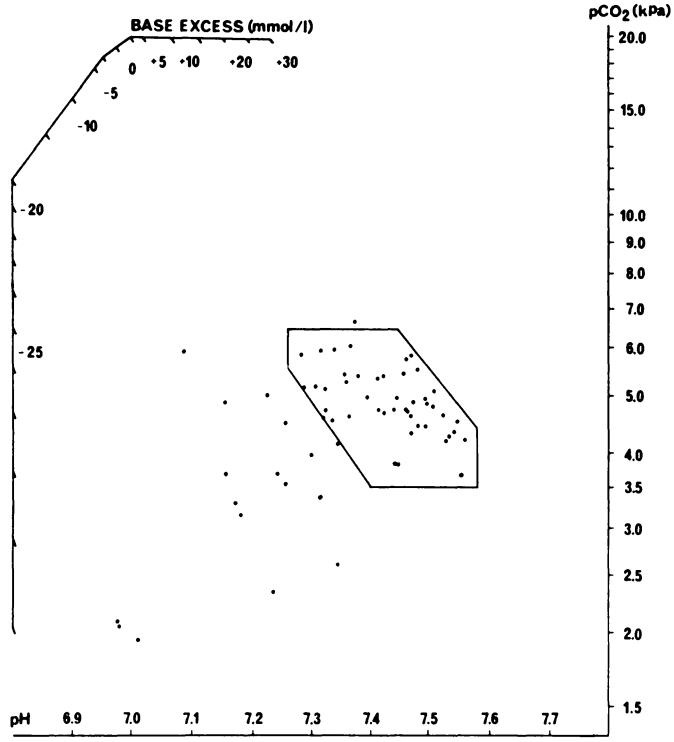

Figure 6. The individual arterial acid-base values of percutaneously sampled piglets with spontaneous diarrhoea. The hexagon represents the reference values (*Andrén* 1982).

Table 1. Comparison between mean values of pH, pCO_2, BE, Hb, Hct, K, serum protein and urea in normal piglets (*Andrén* 1982) and piglets with spontaneous diarhoea.

		pH	pCO_2 kPa	BE mmol/l	Hb g/l	Hct %	K mmol/l	Prot g/l	Urea mmol/l
Normal	mean	7.423	4.99	0.4	88	28	6.0	68	7.9
	s	0.081	0.75	4.1	14	5	1.8	11	4.3
	n	113	113	113	112	112	104	108	99
	P	< 0.005	< 0.005	< 0.001	< 0.005	< 0.001	< 0.001	< 0.05	< 0.001
Diarrhoea	mean	7.367	4.55	—3.8	96	32	7.4	72	12.5
	s	0.141	1.01	9.1	20	8	2.2	13	8.2
	n	63	63	63	64	58	58	64	33

P: level of probability of equal means.

DISCUSSION

The incidence and severity of diarrhoea after experimental infection are influenced mainly by the variations in amounts and antibody capacity of the colostrum suckled after infection (*Moon et al.* 1970). Colostrum deprived piglets infected with strain (BD 3027/76) of E. coli all developed a lethal watery diarrhoea (*Lönnroth et al.* 1978). Newborn piglets have a relatively high body water content (80 %) and are therefore extremely sensitive to changes in water balance (*Pownall* 1970).

Piglets of group D lost 10—15 % of their bodyweight and they developed the clinical signs of severe dehydration such as dryness of the mucous membranes, sunken eyes, greyish discoloration and poor turgor of the skin, weakness and finally death. The clinical signs were less severe in piglets of group DR and they were all able to suckle their dam throughout the disease.

The enterotoxin induced fluid secreted into the intestines is alkaline and isotonic with serum and high in Na^+, K^+, Cl^- and HCO_3^- (*Moon* 1974). The non-respiratory acidosis with low pH and BE values that developed (Fig. 1) during the disease is mainly due to the great losses of HCO_3^- via faeces (*Kildeberg* 1968, *Argenzio & Whipp* 1980) while the decreased pCO_2 reflects the respiratory compensation of this metabolic acidosis (*Rooth* 1974). This compensation was, however, insufficient in piglets of group D. Piglets of group DR (BE values above —10 mmol/l) seemed capable to compensate for their acidosis and by continuing to suckle replaced their fluid losses sufficiently to survive. The degree of acidosis and fluid losses are reflected by the clinical signs only in severe cases. The rapid progress of the acidosis in piglets of group D (Fig. 3) in combination with dehydration caused death within a few hours in some cases.

The degree of dehydration during severe diarrhoea is evident from the increase of Hct (Fig. 4) and Hb, variables which normally decrease during the first day of life (*Pownall* 1970, *Tumbleson & Kalish* 1972). Another indication of severe dehydration is a rise in the serum protein concentration (Fig. 5). This increase is additional to the natural increase occurring after the first absorption of colostral protein (*Pownall, Tumbleson & Kalish*). A low serum protein content decreases the buffer capacity of serum and thus makes very young piglets more susceptible to changes in the acid-base balance.

Dehydration and hypovolemia with impaired microcirculation and tissue perfusion might lead to acumulation of LA (*Kildeberg* 1968) aggravating the severe acidosis in late stages of the diarrhoea as indicated by the negative correlation between the BE and LA values.

The increase in urea values are probably due to a reduced glomerular filtration rate (*Kildeberg*). Small changes in serum concentrations of Na^+, K^+ or Cl^- are poor reflections of their disturbed equilibria, but high serum K^+ values of severe acidosis reflects its intracellular exchange with H^+ and losses during dehydration (*Fisher & McEwan* 1967, *Rooth* 1974, *Argenzio & Whipp* 1980).

As expected the blood changes in piglets with spontaneous diarrhoea correspond well with those found after experimental infection. The same changes were observed by *Moon et al.* (1970) and *Kutas & Szabó* (1971). The greatest differences between normal and diseased piglets were noted in BE, Hct and K^+ values which seem to be good indicators of both the acid-base status and water balance and also of the prognosis of the individual diseased piglet. A BE value of —10 mmol/l (Figs. 1 and 3) seems to be a critical limit below which the prognosis should be considered poor with piglets unlikely to survive without therapy. The degree of dehydration is best determined from the weight losses (*Boyd* 1981) but as this is usually impossible to measure in clinical practice Hct and serum protein values together with the clinical signs give sufficient information about the amount of fluid needed for replacement (*Boyd* 1981). Since the course of the disease is very rapid in some cases the importance of an early and adequate replacement therapy must be emphazised.

The replacement fluid should be isotonic, contain the electrolytes Na^+, K^+, Cl^- and HCO_3^- and glucose since the absorption of glucose is unaffected in piglets with enterotoxic E. coli diarrhoea (*Whipp & Moon* 1973). Oral rehydration has been widely used in human cholera and other diarrhoeic diseases (*Pierce & Hirschhorn* 1977) and the mortality has been shown to be lower in diarrhoeic piglets with access to a glucose electrolyte mixture ad lib (*Bywater* 1980). Suitable commercial electrolyte solutions for oral therapy are now available and should be used immediately as diarrhoea occurs. In severe cases of piglet diarrhoea with dehydration of 10 % or more or when the piglets are unable to suckle or drink, rehydration by the subcutaneous or intraperi-

toneal route is indicated. Intraperitoneal infusion amounting to 8 % of bodyweight would be well tolerated in piglets 1—2 days of age (*Staples* 1981).

In conclusion this experimental procedure is also a useful model in therapeutic studies of piglet diarrhoea.

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SAMMANFATTNING

Syra-bas och elektrolytförändringar hos 1—3 dagar gamla spädgrisar infekterade med enteropatogena Escherichia coli och hos spontana fall av spädgrisdiarré.

Nyfödda spädgrisar (57 st) infekterades peroralt med enteropatogena E. coli (ST + LT) bakterier. Av dessa utvecklade 50 st (88 %) en vattentunn diarré med dödlig utgång i 17 fall (34 %). Diarrégrisarna utvecklade en delvis kompenserad metabolisk acidos, vilket manifesterades genom signifikanta sänkningar av pH, BE och pCO_2 värden. Acidosen (BE-värdena) var signifikant korrelerad till ökade LA värden i blod och till ökade serum K^+-värden. Dehydreringen under sjukdomen bekräftades av ökade Hb, Htk, serumprotein och urea värden i kombination med viktsförlust. Förändringarna var mest uttalade hos de grisar som dog. Ett BE-värde på —10 mmol/l föreföll vara en gräns under vilket prognosen kunde förutsägas vara dålig.

Förändringarna i syra-bas status och vätskebalans bekräftades hos 64 st spädgrisar med spontana fall av E. coli diarré.

(Received December 23, 1982).

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