Gastric secretion in suckling pigs and early-weaned pigs given a dry cow's-milk formula *ad lib.*

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1. Twelve gastric-cannulated litter-mate pigs were used to study secretion and proteolytic activity in the stomach of suckling and early-weaned pigs in relation to age and food intake.

2. Results demonstrate that from the first observation at day 8, piglets were able to secrete acid. pH and acid concentration did not change during the first 4 weeks of life.

3. Proteolytic activity was low during the first 2–3 weeks of life and rapidly increased thereafter.

4. Two phenomena differentiated suckling pigs from pigs given dry cow's milk: (1) a low buffering capacity the gastric contents, beginning 1 h after feeding the dry cow's-milk formula, results in a low total acid concentration in the weaned pigs and (2) the increase in proteolytic activity in relation to the age is much more pronounced in the artificially-reared pigs.

5. These two phenomena are discussed and related to the formation of a hard casein clot in the stomach of the cow's-milk-fed pigs, which has a long retention time and stimulates gastrin release.

Difficulties encountered in rearing of pigs after early weaning are most frequently associated with gastro-intestinal disorders resulting finally in a bacterial over-growth of the gastro-intestinal tract by *Escherichia coli*. These gastro-intestinal disorders are primarily due to inappropriate food ingredients combined with poor management.

Some years ago a technique was developed at this Institute for weaning pigs at 5–6 d of age (Van der Heyde, 1969). The success of the technique depended on a dry *ad lib.* feeding system based on cow's milk powder and on hygienic and controlled housing conditions, pre-requisites which are also documented in the literature (Manners, 1976; Braude, 1972).

However, practical limitations of the system were the high cost of the food and the inability to feed pigs younger than 5 d of age on the dry food, thus preventing the realization of the economic advantages of early weaning as described by Braude (1972). Such limitations may be minimized by further understanding of the digestive physiology of the very young pig, hence the present work was carried out to investigate: (1) if there was an effect of early weaning on gastric acid secretion, by a comparison of the gastric secretory response to histamine stimulation in suckled and early-weaned pigs of different ages; (2) the conflicting information on the age of onset of gastric acid secretion and the evolution of gastric proteolytic activity by a comparison of both suckled pigs and pigs eating a dry diet based on cow's milk powder.

EXPERIMENTAL

Animals and housing

At 2 d before farrowing the sow (Belgian Landrace; mated with a Belgian Landrace boar) was washed and placed in a thoroughly-cleaned and disinfected farrowing pen. The sow farrowed thirteen pigs without assistance. After farrowing the sow was retained in a farrowing crate and was moved for 30 min each day to a paddock which had not been used previously for pigs, where she was fed and where she normally defaecated. During this period the farrowing house was cleaned. The piglets were provided with floor heating and

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Table 1. *Composition of the dry cow's-milk food fed to pigs*

Ingredients (kg)	
Full-cream-milk powder (spray-dried)	50
Skim-milk powder (spray-dried)	50
Vitamins and minerals*	0·340
Proximate composition (g/kg)	
Dry matter	953
Ash	70
Crude protein (nitrogen × 6·38)	306
Crude fat	124

* Contained (g/0·340 kg): 0·344 retinyl acetate, 0·025 cholecalciferol, 1·0 riboflavin, 6·0 nicotinic acid, 2·0 calcium pantothenate, 0·2 pyridoxine, 0·1 pteroylmonoglutamic acid, 0·004 cyanocobalamin, 0·01 biotin, 50·0 α-tocopherol, 100 choline nitrate, 64 $FeSO_4.7H_2O$, 8·2 $CuSO_4.5H_2O$, 23·8 $ZnSO_4.7H_2O$, 62·5 MgO, 24·3 $MnSO_4.4H_2O$.

straw bedding. No creep feed was available. Twelve of the pigs were each prepared with a cannulated gastric fistula between the fifth and eighth day after birth and were returned to the sow after operation. Four of them were used to study the gastric secretory response to histamine and its evolution with age, and two of the four pigs were also used to study the influence of weaning on gastric secretion. The remaining eight pigs were used to study gastric secretion in relation to food intake, when reared naturally (two pigs) or artificially (six pigs). Allocation to the previously-mentioned treatments was random. After 14 d eight of the twelve pigs were weaned and placed in individual wire-meshed cages in an environmentally-controlled room (28°, relative humidity 70%).

When not under experiment they had free access to water via a nipple, and dry feed (see Table 1), which was based mainly on milk powder, was given *ad lib.*

Gastric fistulation

The gastric fistulation technique was essentially as described by Decuypere, Vervaeke, Henderickx & Dierick (1977).

Sampling

Histamine experiments. The pigs were fasted for 5 h, and were then lightly anaesthetized with halothane (Fluothane; ICI (Pharmaceuticals Division), Alderley Park, Cheshire, UK). The stomach contents were removed by suction and the stomach was then washed out five times with warm distilled water. Samples were removed 30 and 60 min after the intramuscular injection of histamine dihydrochloride (0·25 mg/kg body-weight).

Feeding experiments. For the study of gastric secretion after feeding, the first sample was taken after a fast of 2 h. Then the sow-reared pigs were allowed to suckle. The artificially-reared piglets were allowed to eat for a period of 30 min. The first sample was taken 30 min after the start of the suckling or feeding period, thereafter samples were taken by suction every 30 min for 2 h. The samples were cooled in ice and transferred to the laboratory where, after measurement of pH, they were stored at −20° until required for further analysis. All samples of a yellow colour were considered to be contaminated with duodenal contents and rejected.

Analytical procedures

pH. This was determined with a pH meter (Radiometer, Copenhagen, Denmark).

Free and total acid. Free acid was determined by titrating (Titrator TTT 1C; Radiometer) the sample with 0·1 M-sodium hydroxide to pH 3·5, and total acid by titration to pH 7·0

Table 2. *pH and free acid concentration in histamine-stimulated gastric secretion in suckling pigs and early-weaned pigs given a dry cow's-milk formula**

(Mean values with their standard errors for two pigs/treatment)

Age (d)...	10		14		16–17		22–23		28–29	
Feeding regimen	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
pH										
Suckled	2·63	0·06	2·65	0·01	2·75	0·01	2·58	0·03	2·40	0·00
Weaned	—	—	—	—	2·70	0·01	2·30	0·01	2·50	0·01
Free acid (mmol/l)										
Suckled	49	6	76	6	71	8	41	3	57	5
Weaned	—	—	—	—	71	6	74	7	53	4

* For details, see Table 1.

(Dragstedt & Lawson, 1964; Lubran, 1966). The titration curves of the individual samples were recorded automatically (Titrigraph SBR 2c; Radiometer). Mean titration curves were obtained by calculating the average pH/20 mmol base added per l fresh sample.

Proteolytic activity. This was determined by a modified Anson procedure (Anson, 1939; Second report of the Federation Internationale de Pharmacie Commission, 1966) with purified haemoglobin (Serva, Heidelberg, Germany) as the substrate. Activity was expressed as pepsin units/ml per min, where one unit was that amount of enzyme per min which released sufficient peptides from the haemoglobin–enzyme substrate to produce a colour with Folin–Ciocalteu phenol reagent equivalent to that produced by 1 μmol pure tyrosine. Incubation was for 30 min at 37°. Each sample was analysed in triplicate, and from each sample a portion was inactivated by immediate addition of 10 ml tricloroacetic acid (40 g/l) to provide a blank.

Lactic acid. Lactic acid was determined using the microdiffusion technique of Conway (1957).

RESULTS

Histamine-stimulated gastric secretion

Although with the sampling technique used it was impossible to collect the entire gastric contents, special care was taken to collect as much secretion as possible so that differences in volume of secretion after histamine administration could be observed. To ensure that the secretory responses were due to histamine, injections of distilled water were given on occasion. These 'sham' injections had no effect on volume, pH or free acid concentration of the contents. Histamine, on the other hand, nearly doubled the quantities of secretion, while the pH dropped from 4·0 to 2·7. Free acid concentration increased from 0·0 to approximately 60·0 mmol/l. The magnitude of this response may have been influenced by Fluothane administration which is known to influence gastric function (Zontine, 1973), but as proven by the 'sham' injection did not stimulate secretion.

Sampling was done at 30 and 60 min after histamine injection, this being the period after which maximal secretion could be expected (Cranwell & Titchen, 1974). The pH and free acid concentration after histamine stimulation are presented in Table 2, each value being the mean of four observations. As values at 30 min did not differ from values at 60 min the mean value for both sampling periods was taken and plotted *v.* age.

The mean pH of the stomach contents of suckling pigs after histamine stimulation, was 2·60 and the mean free acid concentration 60·0 mmol/l, without any age-dependent variation during the experimental period from 10 to 29 d of age. No difference could be observed

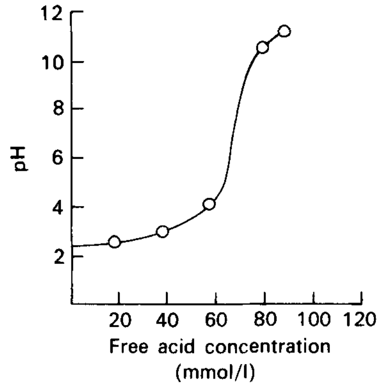

Fig. 1. Mean titration curve of stomach contents 30 min after histamine stimulation (0·25 mg/kg body-weight, intramusculary) in suckling pigs (*n* 10). For details of experimental procedures, see p. 92.

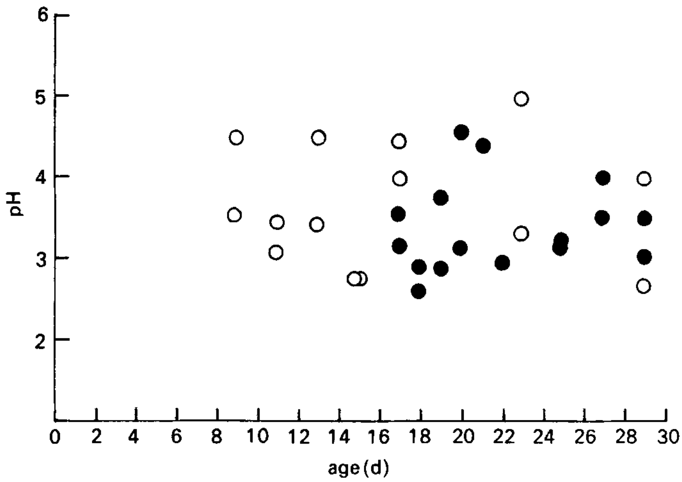

Fig. 2. Fasting pH of stomach contents at different ages (d) of suckling pigs (○) and early-weaned pigs (●) given a dry cow's-milk formula (for details of composition, see Table 1).

between suckling and early-weaned pigs. Titration curves of stomach contents after histamine stimulation were typical of those for strong acids (Fig. 1).

Gastric acid secretion in relation to food intake

To study gastric secretion after feeding pigs were removed from the sow for 2 h. After sampling the stomach contents, they were allowed to suckle. The first sample was taken 30 min after the onset of suckling and then every 30 min for 2 h.

For the pigs reared artificially food was not available for 2 h before the first sampling. They were then allowed to eat for 30 min after which the first sample was taken. Further sampling was done as for the suckling pigs.

pH values determined before suckling or eating (fasting values) are presented in Fig. 2.

The mean (±SE) pH values for the entire experimental period were 3·65±0·19 and 3·40±0·14 for suckling and weaned pigs respectively. The values did not differ significantly and there was no age-related variation.

Free acid was detected in some of the pigs, both suckling and early-weaned pigs. The

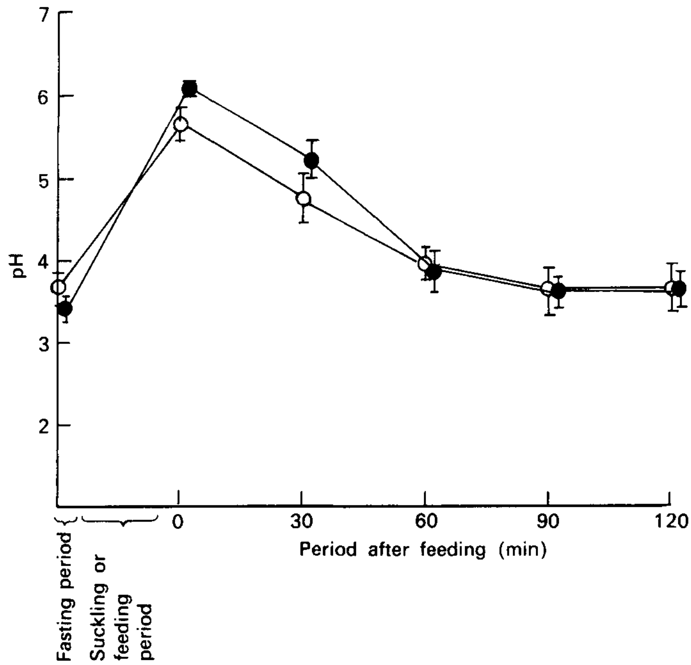

Fig. 3. pH of stomach contents in suckling pigs (○) (*n* 14) and early-weaned pigs (●) (*n* 16) given a dry cow's-milk formula (for details of composition, see Table 1) in relation to feeding. Points are mean values with their standard errors represented by vertical bars.

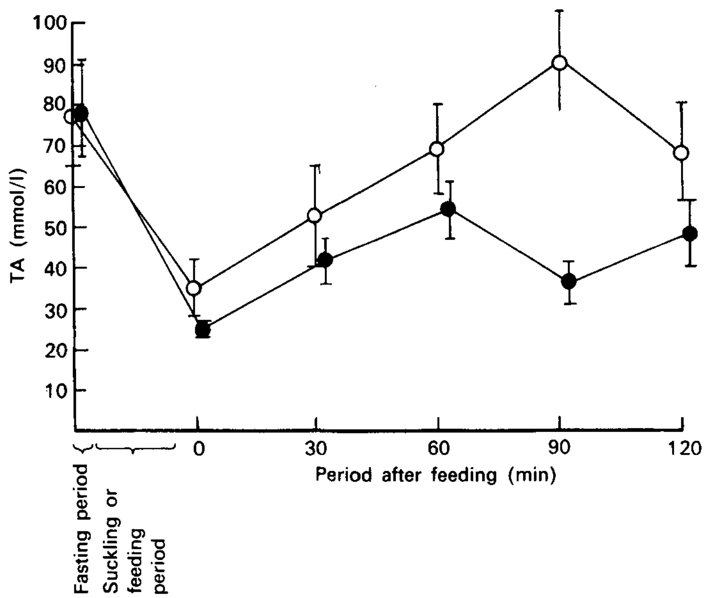

Fig. 4. Concentration of total acid (TA; mmol/l) in stomach contents of suckling pigs (○) (*n* 14) and early-weaned pigs (●) (*n* 16) given a dry cow's-milk formula (for details of composition, see Table 1) in relation to feeding. Points are mean values with their standard errors represented by vertical bars.

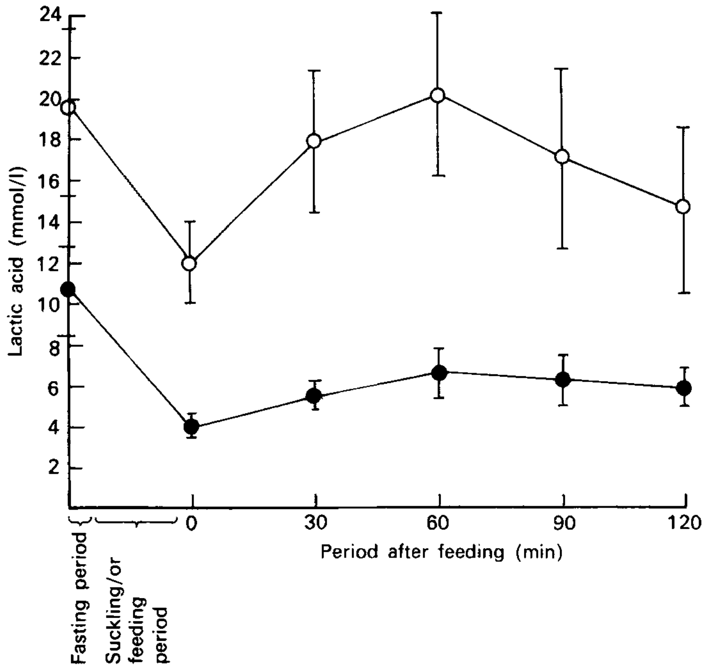

Fig. 5. Mean lactic acid concentration (mmol/l) in stomach contents of suckling pigs (○) (*n* 14) and early-weaned pigs (●) (*n* 18) given a dry cow's-milk formula (for details of composition, see Table 1) in relation to feeding. Points are mean values with their standard errors represented by vertical bars.

mean concentration (mmol/l) was 25 ± 7 and 32 ± 7 in suckling and early-weaned pigs respectively, whilst the mean concentration of total acid, 78 ± 12 mmol/l, was comparable in both groups.

As no age-dependent variation was observed in the present work, the results for gastric acid secretion in relation to feeding were pooled and presented as a mean value for the entire period, as shown in Fig. 3 which compares suckling (age-range 9–29 d) with artificially-reared (age-range 16–29 d) pigs.

In both groups the highest pH value was recorded directly after feeding. Subsequently there was a gradual decrease until after 60–90 min the fasting pH value was regained and remained constant. In the weaned pigs the pH at 0 and 30 min after feeding was higher than in suckling pigs, although the differences were small and did not reach statistical significance.

The concentration of total acid in relation to feeding is presented in Fig. 4. In suckling pigs acid concentration was lowest directly after feeding and the concentration gradually increased until maximal values were obtained after 90 min. At 2 h after feeding the concentration of total acid did not differ significantly from the fasting value.

In artificially-reared pigs a similar pattern was recorded during the first hour after feeding, but concentrations of acid were slightly lower. At 90 and 120 min total acid concentrations were considerably lower in the weaned pigs although pH values were exactly the same. As the acidity of the stomach contents is affected by hydrogen ions originating both from the secretion of the gastric glands and the production of organic acids by bacterial fermentation, of which lactic acid is the most important (Friend, Cunningham & Nicholson, 1963; Cranwell, Noakes & Hill, 1976), lactic acid concentration was measured in each sample. The titration curves were also recorded.

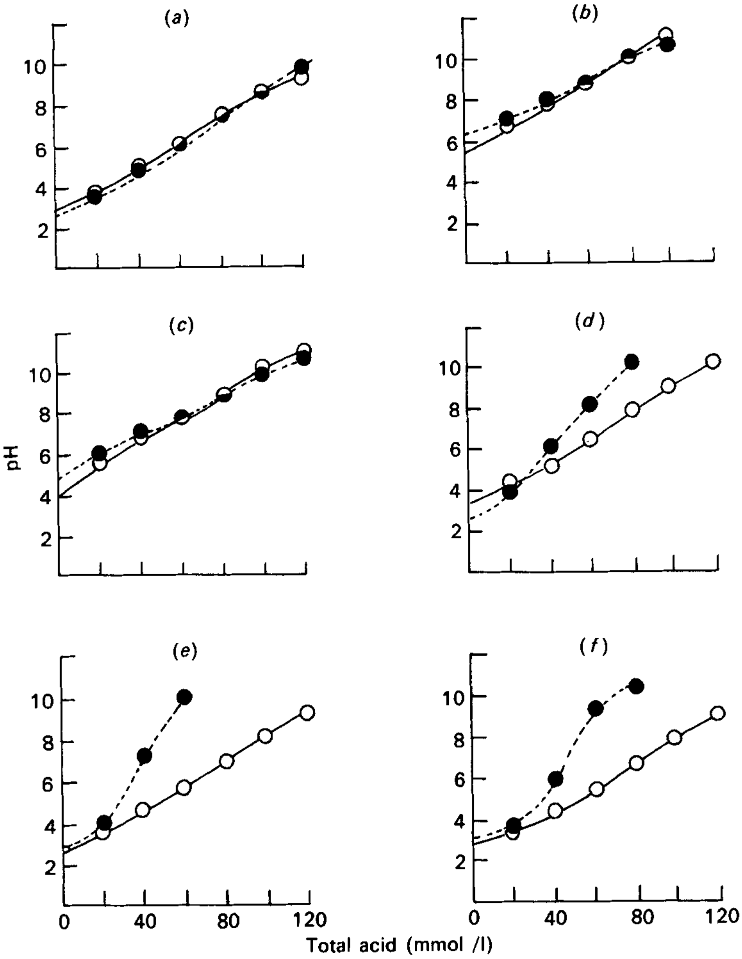

Fig. 6. Buffering capacity of stomach contents of suckling pigs (○) (*n* 14) and early-weaned pigs (●) (*n* 16) given a dry cow's-milk formula (for details of composition, see Table 1) when (*a*) fasting and at (*b*) 0, (*c*) 30, (*d*) 60, (*e*) 90 and (*f*) 120 min after feeding.

Lactic acid concentration in relation to feeding is shown in Fig. 5. Fasting concentrations were 19·7 and 10·6 mmol/l in suckling and early-weaned pigs respectively.

As total acid concentration was 78 mmol/l there was ample indication of active HCl secretion.

The milk which entered the stomach after suckling caused a decrease in lactic acid concentration, thereafter the concentration gradually increased until, 1 h after feeding, maximal values occurred. Between 1 and 2 h after feeding, lactic acid concentration decreased slightly, indicating that pH or substrate concentration may become critical for lactic acid fermentation during the second hour after feeding.

The same pattern in relation to feeding was observed in artificially-reared pigs although lactic acid concentrations were significantly lower.

As differences in lactic acid concentration between suckling and weaned pigs sampled at 90 and 120 min cannot account for the differences observed in total acid concentration, the acid-buffering capacity of the stomach contents was determined. The mean titration curves for each sample are presented in Fig. 6.

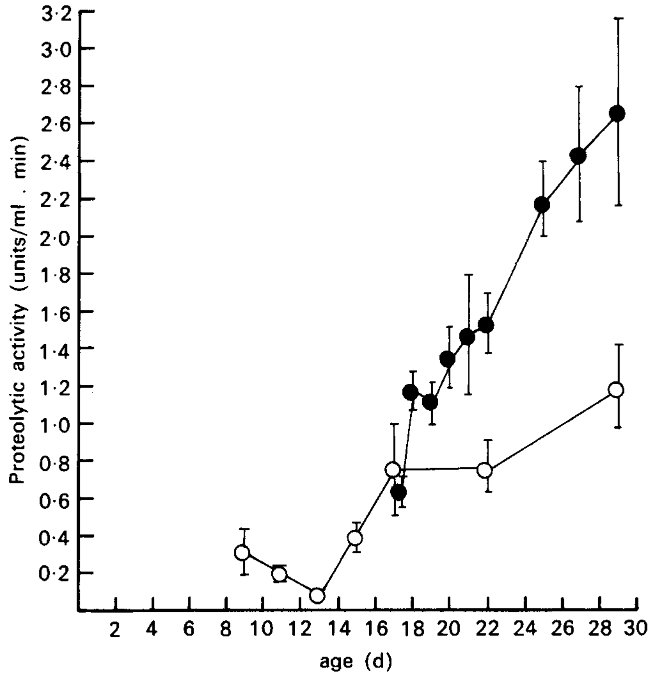

Fig. 7. Proteolytic activity (units of pepsin activity/ml per min) in stomach contents of suckling pigs (○) and early-weaned pigs (●) given a dry cow's milk formula (for details of composition, see Table 1) in relation to age (d). Points are mean values with their standard errors represented by vertical bars, for six observations from two pigs. One unit of enzyme activity was that amount of enzyme per min which released sufficient peptides from the haemoglobin–enzyme substrate to produce a colour with Folin–Ciocalteu phenol reagent equivalent to that produced by 1 μmol pure tyrosine. Incubation was for 30 min at 37°.

While there was no difference for fasting contents and at 0 and 30 min after feeding, it is clear that beginning 1 h after feeding, stomach contents of artificially-reared pigs had little acid buffering capacity, less, in fact, than contents collected from fasting pigs.

Proteolytic activity

Fig. 7 represents mean values of proteolytic activities determined 60, 90 and 120 min after food intake in two pigs. Activities were plotted *v.* age, comparing suckling and weaned pigs given the milk-powder diet.

As expected proteolytic activity was low until the age of 14 d and increased gradually thereafter. However the increased activity in artificially-reared pigs was far more pronounced, the values being nearly twice those found in suckling pigs.

DISCUSSION

Histamine-stimulated gastric secretion

Results obtained with histamine stimulation clearly indicate that the gastric mucosa of pigs was able to secrete acid from the first observation at 10 d of age and that there was no age-dependent evolution in the acid secretory capacity until the age of 4–5 weeks. Neither was there any effect of early weaning. The results support the findings of Forte, Forte & Machen (1972) who demonstrated in vitro that, even at the age of 5 d, pig gastric mucosa was able to secrete acid after histamine stimulation. Cranwell & Titchen (1974), using Heidenhain fundic pouches, even observed hydrochloric acid secretion 17 h after birth; it seems likely, therefore, that the relatively high pH normally observed in fasting pigs of comparable age cannot be attributed to the inability of the pig gastric mucosa to secrete acid, as suggested by Kwasnizkij & Bakejewa (1940), Cranwell, Noakes & Hill (1968) and Noakes, Cranwell & Hill (1968) but to the fact that fasting gastric contents have a relatively high buffering capacity (saliva, mucus, duodenal reflux).

Kutas & Szabo (1974) studied gastric acid secretion after histamine stimulation comparing suckling and early-weaned pigs of approximately the same age as in our experiment and reared in quite comparable conditions. Histamine stimulation was done after a fast of 6–8 h. These authors noted that in suckling pigs histamine only exceptionally (two of twenty pigs) could provoke acid secretion, while this occurred in nearly all the weaned pigs. The pH recorded in histamine-sensitive piglets was similar to the value obtained in our experiment. This difference between suckling and early-weaned pigs led Kutas & Szabo (1974) to conclude that early weaning shortened the physiological period of achlorhydria in very young pigs. This effect of early weaning could not be confirmed in our experiment, pH values and free acid concentrations being identical in suckling and early-weaned pigs, suggesting that weaning had no influence on the histamine-stimulated secretory capacity of the gastric mucosa.

On the other hand, the results of Schulman (1973), who weaned pigs on a commercial diet at the age of 4–6 weeks, suggest that weaning should have an inhibitory effect on gastric acid secretion, as the decrease in pH in relation to the period after food intake was more pronounced in suckling pigs. However it is rather difficult to interpret the results of the latter author as possible influences of suckling and cow's milk on the buffering capacity of stomach contents, and especially on stomach emptying, both factors which greatly regulate stomach secretion, are not taken into account.

Gastric acid secretion in relation to food intake

Fasting pH values recorded in the present experiments are comparable with those noted by Lewis, Catron, Liv, Speer & Ashton (1955), Walker (1959), Maner, Pond, Loosli & Lowrey (1962), Braude, Mitchell, Newport & Porter (1970) all of whom recorded fasting pH values in suckling pigs aged 1–5 weeks, and also with the values mentioned by Schulman (1973) in weaned pigs of 4–7 weeks.

Most authors however, state that a pH value of 3·5 is about the lowest value recorded during the first month of life (Kwasnizkij & Bakejewa, 1940; Cunningham & Brisson, 1957; Walker, 1959) whilst free acid was detected only in the exception. In the present experiments, free acid was present in half the pigs examined.

Concerning the pH values and total acid concentrations after food intake, it is rather difficult to compare our results with available information in the literature as experimental procedures and diets vary considerably. Hartman, Hays, Baker, Neagle & Catron (1961), using a very comparable experimental procedure (2 h fasting, 30 min suckling or feeding) but feeding a 400 g dry skim-milk/kg diet to the weaned pigs, recorded pH values of 3·9–4·3 in suckling pigs and 4·1–4·7 in weaned pigs 1 h after feedings; values which are comparable to those found in our experiments.

As lactic acid concentrations were much lower than total acid concentrations both in suckling and weaned pigs in the present experiments, there is no indication that acidification of stomach contents depends on lactic acid production by the gastric flora as suggested by Friend *et al.* (1963) and Cranwell *et al.* (1976). The concentration of lactic acid in suckling pigs was also much lower than the value recorded by Cranwell *et al.* (1976), although the animals in the present experiment were kept in a conventional environment. It was, however, comparable to that found by the latter authors in pigs reared in a clean environment. Cranwell *et al.* (1976) also observed an inverse relationship between lactic acid and total acid concentration, a relationship which was not observed in the present experiment.

The difference between the lactic acid concentration in suckling and artificially-reared pigs was most probably related to differences in the gastric bacterial flora. Indeed, previous experiments (Decuypere & Van der Heyde, 1972) in which the gastro-intestinal flora of suckling pigs was compared with the flora of early-weaned pigs reared in identical circumstances to those used in the present experiment revealed that, whilst in the stomach of suckling pigs only lactobacilli could be detected, the numbers of coliforms found in the stomach of the early-weaned pig were considered sufficient to catabolize lactic acid to a significant extent.

The slightly lower total acid concentration observed in the artificially-reared pigs during the first hour after feeding may be explained by the difference in lactic acid concentration. However, at 90 and 120 min total acid concentration differed considerably between both groups, differences which could not be accounted for by variations in lactic acid concentrations. So the acid-buffering capacity of the stomach contents could be important.

Indeed the low acid-buffering capacity of stomach contents of weaned pigs from 1 h after feeding most probably is the cause of the relatively low concentration of acid, as acid-buffering capacity of ingesta is the most important factor stimulating gastric acid secretion (Saint Hilaire, Lavers, Kennedy & Code, 1960).

In suckling pigs, titration curves registered after 60 min are nearly the same as the fasting curve so it can be assumed that the stomach has been emptied 90 min after suckling. This rapid emptying after suckling was also demonstrated by Kidder & Manners (1974).

Titration curves from stomach contents after 90 and 120 min in artificially-reared pigs closely resemble those published by Manners (1970) which were determined on the supernatant fluid of acid-precipitated early-weaning diets, mainly based on skim-milk powder. This leads us to the suggestion that in the pigs given dry cow's-milk powder a firm casein clot was formed in the stomach, contrary to the relatively soft casein precipitate obtained normally with sow's milk (Cranwell *et al.* 1976). This difference in clot consistency between suckling and artificially-reared pigs on a cow's-milk diet was also observed by other authors (White, Wenham, Sharman, Jones, Pattrey & McDonald, 1969; Braude, Newport & Porter, 1970).

This hard casein clot is rather difficult to sample and, most probably, had not left the stomach after 120 min. This is further supported by the fact that Braude, Mitchell *et al.* (1970) recorded comparable pH values in the whey fraction of stomach contents 1 h after feeding early-weaned pigs with cow's milk, the pH of the clot being considerably higher.

Comparing the titration curve of the stomach contents of fasting pigs with the curve obtained after histamine stimulation (Fig. 1) a totally different picture is observed, suggesting that in normal circumstances some buffering substances are present in the stomach, most probably originating from the cardiac secretion (Höller, 1970*a*). Histamine and all gastrin-stimulating compounds strongly inhibit this secretion (Höller, 1970*b*). This can explain the typical strong acid shape of both the titration curves in histamine-stimulated contents and of the curves obtained at 90 and 120 min in artificially-reared pigs as the hard casein clot most probably is a strong stimulating factor for gastrin release.

Proteolytic activity

As mentioned earlier by Hartman *et al.* (1961) and Cranwell & Titchen (1974), proteolytic activity is low and decreases during the first 2 or 3 weeks of life after which a gradual increase is observed. However, the increased activity in artificially-reared pigs, as observed in the present experiments, is far more pronounced, the values being nearly twice those found in suckling pigs. Hartman *et al.* (1961), on the other hand, weaning pigs on a soya-bean-protein-based diet, found that although proteolytic activity of stomach tissue did not differ between suckling and early-weaned pigs there was a very pronounced inhibition of proteolytic activity in the stomach contents of weaned pigs. However, Cranwell & Titchen (1975) noted that the highest increase in proteolytic activity occurred when pigs started eating solid food and related this phenomenon to hormonal influences, suggesting a changed gastrin secretion.

The role of solid food was also demonstrated by Zelenkova & Gregor (1971) who studied gastrin activity in extracts of rat stomach in relation to age. Using a bioassay they were not able to detect gastrin activity until the young rats started eating solid food at approximately 21 d of age, after which a gradually increasing activity was observed.

The firm casein clot in the stomach of the pigs given dry cow's milk may also be a potent stimulus for gastrin release.

As the increase of gastrin activity observed by Zelenkova & Gregor (1971) and the increase in peptic activity noted by Cranwell & Titchen (1975) both seem to be induced by the same factor, that is the stimulus of solid food, the hypothesis can be put forward that gastrin has some trophic or stimulatory effect on the development of the proteolytic-enzyme-secreting cells, although the trophic effect of gastrin on the adult fundic mucosa of rats is limited to the parietal cells only (Crean, Marshall & Rumsey, 1969; Willems, Vansteenkiste & Limbosch, 1972).

It seems worthwhile to investigate further the effect of solid food and of clot formation in the stomach of baby pigs, in the light of these findings and the suggestions of various authors that clot formation and gastric secretory regulation are closely linked (Walker, 1959; Braude, Mitchell *et al.* 1970; Braude, Newport *et al.* 1970). The results of the present experiments suggest that, as in calves (Roy & Ternouth, 1972), some of the problems associated with early weaning and the artificial rearing of piglets may be associated with food formulations which do not form a firm curd in the stomach.

The relative success of early-weaning techniques based on dry feeding systems, on the other hand, most probably could be associated with the regulatory function of solids on gastric physiology.

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