ABSTRACT
The role of pregastric esterase was investigated in four abomasally-cannulated calves during a sequence of ten 3-day periods beginning when calves were 2 to 5 days of age. Two calves were fed whole milk from nipple bottles to maximize exposure to pregastric esterases, and two other calves were given milk by infusion directly into the abomasum to minimize exposure. The initial system of feeding each calf was continued for eight periods after which treatments were reversed. Postfeeding lipolytic activity of samples of abomasal contents and of intragastric lipolysis of milk fat were markedly greater for orally-fed calves than for abomasally-fed. Systems of feeding effected no significant differences in a) digestibility of dry matter, fat, crude protein, and nitrogen-free extract, b) retention of nitrogen, and c) percentage weight gains. Digestibility of dry matter, crude protein, and nitrogen-free extract of milk and retention of nitrogen were greater in later periods than in initial. Although age of calves was a positive factor associated with utilization of milk, decreased exposure of milk to pregastric esterase and the resulting reduction of lipolysis in the abomasum did not effect a detectable lowering of milk utilization by calves in age groups studied.

INTRODUCTION
Salivary lipase, designated as pregastric esterase (PGE) (21, 22), is a potent lipolytic enzyme complex secreted mainly from palatine glands (7) and from tissues in other regions of the oral cavity of young calves (21, 22). Early observations (30) of pronounced in vitro lipolysis in and rapid rennet coagulation of whole milk that had been sham-fed to calves prompted speculations relative to the physiological significance of PGE in nutrition of calves (15). Among many subsequent investigations of this enzyme system are results showing that in milk-fed calves approximately 70% of dietary long-chain fatty acids were absorbed in the absence of pancreatic lipase (6). A recent study (3) revealed that about a third of esterified fatty acids ingested was catabolized to absorbable products by salivary lipase activity in the abomasum. Although PGE has been detected in intestinal digesta (19), its activity was minimal (4).

Pregastric esterase characteristically is secreted in large amounts into milk consumed by healthy young calves (7, 30, 31), but secretions may be maintained in similar concentrations to more advanced ages (11, 31). Pancreatic lipase activity normally is low at birth but subsequently increases (4, 10). Inasmuch as pregastric and pancreatic secretions are the principal sources of lipolytic enzymes in calves (29), PGE possibly serves an important contributory function in lipolysis, particularly during neonatal life.

In the study that follows, the role of PGE in utilization of whole milk by calves during early postnatal stages was explored.

MATERIALS AND METHODS
Basically, the procedure was designed to determine the relative effect of PGE on milk
utilization by calves when exposure of administered milk to salivary lipases was maximized by oral ingestion and minimized by abomasal infusion. Experimental subjects were male Holstein calves that had been cannulated abomasally within 1 to 4 days after birth. The feed was raw Holstein-milk, which was removed in batches (adequate for 3 to 4 days of feeding) from a bulk tank and subsequently stored at 4°C. The methods of feeding milk were a) by sucking from a nipple feeder and b) by infusion directly into the abomasum via a cannula. Criteria of calf responses included lipolytic activity in the abomasum, digestibility of the proximate components of milk, retention of nitrogen, and daily gains.

Abomasal Cannulation

Calves that had been fasted 12-h were anesthetized by i.v. injection of Nembutal (Abbott, Inc., North Chicago, IL). The abomasum was exposed by a right laparotomy. A stab wound was made through a relatively avascular region of the dorsal surface of the abomasum, an area situated midway between the omasal-abomasal orifice and the pylorus. The cannula was 30-cm silastic tubing (Dow Chemical Co., Midland, MI), .95 cm i.d. by 1.59 cm o.d. Two collars, made of the same type tubing, were glued to the cannula by type-A medical adhesive (Dow Chemical Co., Midland, MI). The collars were attached approximately 1 cm apart at a point 4 cm from the end that was inserted into the abomasum. After the cannula was inserted through the stab wound, the serosal surface of the abomasum was drawn snugly around the cannula in the region between these collars by purse-string sutures. Additional anchoring of the cannula was effected by several sutures attaching the serosal surface of the abomasum to the cannula, which was exteriorized (slightly posterior to the diaphragm) through a stab wound between the 10th and 11th ribs. Skin was sutured around the cannula in a manner similar to that described for abomasal anchor sutures.

During the 1- to 2-day recovery period between surgery and initiation of digestion trials, colostrum was fed from nipple bottles at 6-h intervals in quantities at each feeding equal to 2% of body weight (BW). This frequent feeding in small amounts minimized distention of the abomasum and any consequential leakage and, thus, facilitated healing.

Experimental Plans

The a) efficacy of abomasal infusion in minimizing exposure to PGE and b) the choice of postfeeding time most appropriate for aspirating samples of abomasal contents were determined in a preliminary trial. An 8-wk-old abomasally-cannulated calf was fed nonfat milk in two trials, first by direct infusion into the abomasum and 12 h later by sucking from a nipple. A series of 10-ml samples of abomasal contents, aspirated prior to feeding, immediately afterward, and at various predetermined intervals during the subsequent 6-h postfeeding period, were assayed for PGE activity (11, 24).

For the digestion and nitrogen balance trials that followed, four abomasally-cannulated calves, ranging in age initially from 2 to 5 days, were assigned as they became available, in the order and for the periods indicated in Table 1, to oral and abomasal feedings.

Feeding Program

Daily amounts of milk were based on BW of

<table>
<thead>
<tr>
<th>TABLE 1. Schedule of feeding whole milk, orally by nipple feeder and abomasally by infusion, to abomasally-cannulated calves.</th>
</tr>
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<tbody>
<tr>
<td>Periods</td>
</tr>
<tr>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 - 8</td>
</tr>
<tr>
<td>9 - 10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three-day digestion trial periods.

<sup>b</sup>Calves randomly assigned initially.
calves at the beginning of each 3-day period. The feeding schedule in total quantities of milk (% BW) and frequency of feeding (meals per day), respectively, for sequential periods were: period 1, 8% and 4; periods 2 to 3, 8% and 3; period 4, 10% and 3, and remaining periods, 10% and 2. Milk, warmed to 39°C and fed by a nipple feeder and by abomasal infusion, was given at approximately the same rate for the respective procedures.

**Collecting, Sampling, and Storing Procedures**

Samples of milk and of urine and total outputs of feces were kept at 4°C until the end of each 3-day period. Then the respective collections were composited, sampled, sealed in plastic containers, and stored at −12°C for subsequent analyses.

*Milk.* Each batch of milk, stored in a 40-liter container, was mixed and sampled immediately prior to each feeding.

*Excreta.* Feces and urine were collected separately. Calves were confined in special elevated cages having floor screens that retained feces but permitted urine to be funneled into beakers packed in ice. Fecal- and urine-collection periods had a 12-h lag after initiation of a 3-day milk-feeding period. The quantity of feces eliminated by a calf during 3 days was so small that the entire amount was stored, at −12°C, whereas the volume of urine was such that 10% aliquots were taken daily and composited at the end of each period.

*Abomasal Contents.* Ten-milliliter samples (primarily fluids) of abomasal contents were aspirated 30 min after the 0700-h feeding of the 2nd day of each 3-day period. These samples were packed in ice and transported immediately to a laboratory for measurements of pH (Radiometer, Copenhagen, Denmark) and of butyric acid.

**Chemical Assays**

Lipolytic activity of abomasal contents was measured by butyric acid released. In the preliminary trial, in which nonfat milk was fed, samples were incubated with a tributyrin emulsion (11, 24), whereas in the digestion trials, in which whole milk was fed, butyric acid freed by lipolysis in the abomasum was determined by a modification (17) of the gas-liquid chromatographic assay described by D’Orio (1).

**RESULTS AND DISCUSSION**

**Lipase Activity of Abomasal Fluids**

*Postfeeding Trends.* Although the preliminary trial lacks the replications necessary for a definitive study, the marked differences (Figure 1) indicate further (18, 19, 23) that abomasal feeding effectively prevents marked PGE activity in gastric contents. Except for the 240-min collection, activities for all samples following infusion of milk were less than those for the initial sample.

Factors that probably affected the ascending phase of this curve are concentration of PGE in the ingested milk, initial distribution of ingesta in abomasal fluids, time of coagulation, physical characteristics of coagulum, and rate of its disintegration, which released entrapped PGE. Factors that might have affected the descending phase are inactivation of PGE by decreasing pH (24) and by proteolysis, dilution by gastric fluids, and passage of the enzymes with chyme into the duodenum (4, 6, 18, 19).
There was a significant \( P<.04 \) increase of butyric acid concentration in abomasal samples from orally-fed calves as they advanced in age. Reasons for this response are obscure, but changes in feeding schedule, involving a decrease in frequency of feeding and increases in amounts of milk per meal, probably were factors. Range of pH (4.7 to 6.0), Figure 2B, of postprandial samples of abomasal contents, aspirated at 30 min, was similar to results from collections at 1 h (29) but wider than at 3 h (23). Method of administering milk, in accord with previous findings (23), effected no significant difference \( P>.07 \) in pH during the first eight 3-day periods, but in the crossover phase the pH for the orally-fed calves was significantly lower \( P<.01 \) than for the abomasally-fed. This change from a marginal statistically significant difference, independent of characteristics of individual subjects, possibly could be due to a transitional stimulation of gastric secretions resulting from change of feeding system in older calves (27).

Lipolysis of Milk Fat. In digestion trials, concentration of free butyric acid in samples of abomasal fluids was the primary indicator of PGE activity in milk digesta, and pH was an ancillary measurement.

Butyric acid concentrations for both systems of feeding (Figure 2A) varied from period to period, but, throughout, concentrations for orally-fed calves were consistently higher than those for the abomasally infused \( P<.03 \) for the first eight periods and \( P<.002 \) for the reversal phase. These distinct differences further demonstrated that abomasal infusion effectively minimizes PGE in abomasal contents.

Causes of lipolysis in abomasal aspirates from calves fed by infusion are subject to conjecture. Inasmuch as lipolytic activity was not detected in secretions of innervated pouches of the abomasum (29), normal intragastric lipolysis is attributed to salivary lipases (18, 19, 23, 29). Though pancreatic lipase might enter the abomasum via regurgitation of duodenal contents, the gastric acidity is less favorable for activity of this enzyme than for PGE (24). Hence, the presumption is that secretion of PGE was stimulated, possibly by infusion of milk into the abomasum, and subsequently was passed with saliva into the abomasum.
Digestion of Milk

The principal criterion of utilization of milk was apparent digestibility of dry matter (DM) and its proximate organic components, fat (ether extract), crude protein, and nitrogen-free extract (NFE) (Table 2).

Dry Matter. Digestibility of DM of milk was similar for the two feeding procedures but was improved significantly \( (P<.002) \) with advancing age. This response, similar to that reported by Parrish et al. (20), probably is a reflection of corresponding changes in digestibility of crude protein.

Fat. Neither method of feeding nor age of calves affected digestibility of milk fat. Coefficients (Table 2), however, are higher than those in (16, 20). Inasmuch as the procedures to determine the fat content of feces employed only one ether extraction, which was after acidification, some of the ether-soluble substances initially present possibly were not detected (28).

High apparent digestibility of fat by all calves indicates adequacy of lipolysis. This sufficiency in abomasally-fed calves might be due either to pancreatic lipase per se or to traces of PGE in combination with pancreatic lipase. Even though secretions of pancreatic lipase are relatively low in young calves (4, 6, 10), the amounts normally secreted might have been adequate, sans major PGE support, to hydrolyze the milk fat fed. Also, increases in cortisone secretions as a result of surgical stresses might have prematurely stimulated sufficient output of pancreatic lipase (8) for maximum lipolysis.

Actions of PGE in the abomasum (3, 4, 18, 19, 23) and of pancreatic lipase in the intestines generally are considered to be independent, but recent in vitro studies revealed that incubation of whole milk with salivary lipase enhanced subsequent release of fatty acids by pancreatic lipase (2). If this relationship occurs in vivo, perhaps a small amount of lipolysis by PGE, such as noted in abomasally-fed calves (Figure 2A), was adequate to enhance pancreatic lipase activity. Studies with rats (9) indicate that the observed improvement associated with partial intragastric lipolysis probably is due to facilitation of emulsification of the fat. Thus, it is possible that fineness of the fat emulsion in Holstein milk, which was fed to calves during digestion trials, was such that differences in lipolysis in the abomasum did not affect emulsification characteristics sufficiently to be detectable in postgastric digestion.

Results from analysis of feces for fatty acids (data not shown) were unsatisfactory for absolute quantitation, but the comparative distribution revealed no differences attributable to either method of milk administration or to age of calf. The predominant fatty acid in the feces was palmitic, which was followed in declining order of relative quantities by stearic and oleic (in approximately equal amounts) and by myristic.

Crude Protein. There were no statistically significant differences in digestibility of nitrogenous constituents of milk attributable to method of feeding, but there was an increase \( (P<.003) \) with age of calves, particularly during early postnatal periods. This response, similar to that reported by Parrish et al. (20), probably was related to increases in pancreatic proteases (10).

Acceleration of in vitro coagulation of whole milk as a consequence of lipolysis (30) had no detectable beneficial effect on digestion of the protein. Formation of clots, however, protects PGE and prolongs its action in the gastric environment. (Unpublished observations by Leidy, Russel, and Wise, 1974, showed that PGE activity of the interior of a milk clot removed from the abomasum of a young calf about 4 h after whole milk was consumed was greater than that either on the surface or in surrounding fluids.) Edwards-Webb and Thompson (3) found that concentration of butyric acid in the fat of a clot removed from the abomasum of a calf 12 h after feeding was “. . . present as the free fatty acid and its concentration was only 12 m mol/mol of total fatty acid, compared with 110 m mol/mol total fatty acids in the milk fat given.”

Nitrogen-Free Extract. The NFE digestibility coefficients, within ranges reported (16, 20), were not significantly different for the two feeding procedures but were significantly higher \( (P<.03) \) with increasing age. There is no obvious physiological explanation for this age trend.

Nitrogen Retention

Lipolysis of milk fat and the resulting availability of absorbable lipids for energy possibly could affect nitrogen metabolism. Average nitrogen retention (Figure 3) for the
TABLE 2. Effect of method of feeding milk to calves, by sucking from nipple feeder or by abomasal infusion, on digestion of proximate organic components of milk.

<table>
<thead>
<tr>
<th>Periods</th>
<th>Oral Dry matter</th>
<th>Abomasal Dry matter</th>
<th>Oral Lipids</th>
<th>Abomasal Lipids</th>
<th>Oral Crude protein</th>
<th>Abomasal Crude protein</th>
<th>Oral NFE</th>
<th>Abomasal NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.5 ± 2.25</td>
<td>94.0 ± 1.29</td>
<td>98.4 ± 1.25</td>
<td>96.6 ± 2.79</td>
<td>81.2 ± 8.15</td>
<td>83.4 ± 8.46</td>
<td>97.5 ± .36</td>
<td>98.5 ± .24</td>
</tr>
<tr>
<td>2</td>
<td>97.0 ± .62</td>
<td>97.2 ± .23</td>
<td>99.1 ± .08</td>
<td>99.4 ± .13</td>
<td>92.3 ± 1.49</td>
<td>92.7 ± .50</td>
<td>98.4 ± .56</td>
<td>98.5 ± .30</td>
</tr>
<tr>
<td>3</td>
<td>96.9 ± 3.10</td>
<td>98.3 ± .17</td>
<td>99.5 ± .43</td>
<td>99.7 ± .07</td>
<td>92.4 ± 7.38</td>
<td>95.3 ± .64</td>
<td>98.1 ± 1.96</td>
<td>99.1 ± .06</td>
</tr>
<tr>
<td>4</td>
<td>96.5 ± .18</td>
<td>97.4 ± .07</td>
<td>99.3 ± .28</td>
<td>99.1 ± .20</td>
<td>90.3 ± .63</td>
<td>92.9 ± .15</td>
<td>98.3 ± .87</td>
<td>99.1 ± .22</td>
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<tr>
<td>5</td>
<td>97.8 ± .75</td>
<td>98.9 ± 1.58</td>
<td>99.4 ± .10</td>
<td>99.4 ± .85</td>
<td>94.3 ± 1.67</td>
<td>97.3 ± 3.81</td>
<td>98.9 ± .53</td>
<td>99.6 ± .58</td>
</tr>
<tr>
<td>6</td>
<td>98.1 ± .01</td>
<td>99.2 ± 1.10</td>
<td>99.5 ± 1.33</td>
<td>99.6 ± .61</td>
<td>94.8 ± .16</td>
<td>98.1 ± 2.63</td>
<td>99.1 ± .01</td>
<td>99.7 ± .42</td>
</tr>
<tr>
<td>7</td>
<td>98.0 ± 1.07</td>
<td>98.0 ± 1.39</td>
<td>99.6 ± .04</td>
<td>99.0 ± .93</td>
<td>94.9 ± 2.65</td>
<td>95.3 ± 2.94</td>
<td>99.0 ± .66</td>
<td>99.1 ± .71</td>
</tr>
<tr>
<td>8</td>
<td>99.2 ± 1.18</td>
<td>98.9 ± 1.51</td>
<td>99.8 ± .29</td>
<td>99.6 ± .53</td>
<td>97.7 ± 3.20</td>
<td>97.2 ± 3.98</td>
<td>99.6 ± .58</td>
<td>99.5 ± .64</td>
</tr>
<tr>
<td>9</td>
<td>98.9 ± .27</td>
<td>99.1 ± .86</td>
<td>99.5 ± .15</td>
<td>99.8 ± .26</td>
<td>97.4 ± .91</td>
<td>97.8 ± 2.51</td>
<td>99.2 ± .03</td>
<td>99.5 ± .27</td>
</tr>
<tr>
<td>10</td>
<td>99.5 ± .69</td>
<td>98.0 ± .30</td>
<td>99.7 ± .41</td>
<td>98.9 ± .98</td>
<td>98.8 ± 1.64</td>
<td>95.7 ± 1.12</td>
<td>99.8 ± .25</td>
<td>98.9 ± .42</td>
</tr>
</tbody>
</table>

a Three-day periods.
b First period of reversal.
Figure 3. Trends in nitrogen retentions, expressed as percent of total intake (A) and of absorbed (B), by calves fed whole milk either by nipple feeders or by abomasal infusions.

Figure 4. Average daily weight changes, by 3-day periods, of calves fed whole milk either by nipple feeders or by direct abomasal infusions.

Gains in Body Weight

Average daily gains (Figure 4) were erratic, particularly during early stages of the study. For most of the periods prior to the changeover, orally-fed calves gained more in absolute BW than did those abomasally fed, but the percent change was similar for the two groups. Inasmuch as there was no indication of differences in efficiency of milk utilization (Table 2), the apparent advantage of orally-fed calves in absolute weight gains probably is not significant biologically.

General Discussion

High PGE activity in suckling calves has been interpreted as temporary adaptation to the relatively large intake of fat in milk diets (9). Inasmuch as the principal site of fat hydrolysis by PGE is the abomasum (3, 4, 6, 18, 19, 23, 29), the working hypothesis was that a reduction in exposure of milk fat to PGE during feeding and the consequent decrease of intragastric lipolysis would affect adversely efficiency of milk fat digestion and absorption. This conjecture is supported by studies with rats (9) but not by our results from calves. Digestion of neither fat nor other proximate components of first eight periods was not affected significantly by method of feeding. Although results from abomasal infusion were markedly higher than for oral feeding during periods 2 and 3, great variability of retentions rendered differences insignificant statistically. Trends with age, particularly during the early postnatal stages, showed increases ($P<.003$ for A, total N retention, and $P<.007$ for B, absorbed N retention). Relatively low retentions during the initial period might be attributed to 1) use of amino acids to supply energy (25), 2) losses of N associated with tissue damage during surgery (14), and 3) lower digestibility of protein (Table 2). Subsequent increases indicate retention of N for healing and growth. Utilization of N for synthesis of body protein is reflected in the marked parallelism of curves A and B. After period 4, the curves indicate approximate N equilibrium. For a combination of periods, there was a significant ($P<.004$) positive correlation, $r = .43$, of weight gains and N retentions, but this was not true for all individual periods.
milk was decreased by reduction in exposure of whole milk to PGE.

Validity of the assumption that PGE normally is of value in utilization of milk by calves, however, merits further critical evaluation. Limitations in experimental methodology possibly precluded detection of any enhancing in vivo roles of PGE: First, as a result of the time required for surgical preparations, the calves were from 5 to 8 days of age when the initial digestion trials were completed. This age might be beyond the developmental stage at which the complementary activity of PGE would be most beneficial and detectable. Second, surgical stresses might have stimulated pancreatic secretions prematurely (8), thus masking any normal expression of supporting PGE activity. Third, reductions of gastric lipolysis might have fostered compensatory increases of pancreatic lipase secretions. Fourth, frequent feeding of small amounts of milk, particularly during the early periods of the experiment, probably did not present a lipid-load challenge that would need PGE enhancement. In the absence of large intakes of fat in a given meal, pancreatic lipases per se probably would be adequate to hydrolyze effectively the amounts ingested.

In view of the importance of fatty acids as a source of energy for the neonate, another concept of the role of PGE is that of an emergency auxiliary that might serve an essential function in the digestion of colostrum and in the consequent enhancement of endogenous mobilization of lipids (5) when secretion of pancreatic lipase is insufficient. Intragastric lipolysis is important in total fat digestion by premature infants (9), but such information on similar abnormalities in calves is not available. Observations, however, indicating absorption of short-chain fatty acids within the abomasum (3) lend credence to the view that PGE activity might be beneficial when intestinal absorption is impaired.

Future studies of the roles of PGE in nutritional physiology of calves probably should begin with neonates and include colostrum and other dietary variables such as amounts, kinds, and physical forms of fats. The experimental challenge is that of developing techniques that would a) permit regulation of exposure of ingesta to PGE in the neonatal calf and b) provide more sensitive measures of determining digestion of fat and absorption of different lipid metabolites.

REFERENCES


