THE EFFECT OF VARIOUS DIETS ON THE HISTOLOGICAL DEVELOPMENT OF THE STOMACH IN THF CALF

THE EFFECT OF VARIOUS DIETS ON THE HISTOLOGICAL DEVELOPMENT OF THE STOMACH IN THF CALF*

By

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Introduction

In the previous study of this series, several techniques were used to observe the development of the calf's stomach under the influence of various substances administered experimentally (1). Stomach development in the experimental animals was demonstrated by changes in the reticulo-rumen capacities, forestomach-tissue weights, topography of the stomach in the abdominal cavity (gross anatomy), and papillary growth in the rumen. Forestomach growth, mainly of the rumen, was prominent in the calves fed hay and starter in addition to whole milk, whereas it was not found in the animals fed only whole milk up to 12 weeks after birth. Administration of large amounts of volatile fatty acids into the reticulo-rumen of calves fed with whole milk, but no hay or starter, resulted in both papillary and muscular development of the forestomach. The critical amount of the acids was estimated to be approximately 8 molar equivalents per week per individual.

The above techniques were also employed by several other workers. The gross observations reported by Trautmann (2) were followed by the studies of Blaxter *et al.* (3) and Tamate (4). Stomach capacity, relative either to live or empty body weight of the animals, has been used as a criterion of development by several workers (5, 6, 7, 8, 9, 10, 11, 12). Tissue weight increase, especially

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the ratio between the mucous membrane and muscular layer, has proved valuable (5, 6, 7, 11, 13). However, the papillary growth evaluation initiated by Brownlee (13) has been favored by most of the recent workers (1. 5, 6, 7, 8, 9, 11, 14, 15, 16, 17). The direct papillae-counting method used by the present authors (1) was tested recently by Harrison *et al.* (7) with success. Some other techniques, such as cellulose digestion, volatile fatty acid concentraion in rumen contents, and rumen motility have proved less sensitive for measuring the development of the rumen (6).

In spite of the various experiments conducted, little attention has been directed toward the histological evaluation of stomach development. The senior author reported that the postnatal development of the reticulo-rumen in the goat, observed grossly and by its capacity measurement, coincided with the marked cornification of the stratum corneum, an increase in numbers of the primarily swollen cells, and a rapid decrease in the thickness of the forestomach epithelium (15). A similar result was obtained later by Thompson *et al.* (18) by administering a completely pelleted ration containing 50 percent roughage to growing lambs. Recently, the senior author reported that retarded cornification of the forestomach epithelia was observed in calves fed concentrates alone (16). Wardrop also found that cornification was less rapid in the rumen of milk-fed lambs than in lambs fed lucerne chaff (17).

The present authors (1) have suggested that the brownish-green color of the forestomach mucosa of calves probably is derived from rumen fermentation products. This hypothesis is supported by Sinclair and Kunkel (14) who made a histological study of this pigment in the rumen mucosa of weaned lambs. Brownlee and Elliot (19) reported that such pigmented epithelia in the forestomachs of early-weaned calves showed a positive reaction for iron.

The only information available on the postnatal change in abomasal histology comes from studies made with very young lambs (20). The fact that little change occurs in the size and shape of the abomasum after birth probably is responsible for this dearth of information.

The present investigation was planned to provide information on the histological changes in the stomach mucosa taken from the same calves used in the previous study (1), and to clarify further the mechanism by which various substances administered intraruminally stimulate the forestomach growth. Special attention was given to the histology of the abomasum to determine whether the changes occurring in this organ could possibly be associated with those in the forestomach.

Materials and Methods

Twenty-seven male Holstein, Ayrshire, and Brown Swiss calves, were divided into three experimental groups. Group A received whole milk by nipple pail;

Group B received hay and starter (ad libitum) in addition to whole milk; and Group C was divided so that six calves (4698, 4736, 4737, 4761, 4792, 4793) received, in addition to whole milk, various amounts of acetic, propionic, and butyric acids, either as free acids or as salts, while three calves received, respectively, carbonated water (4706), sodium and potassium chlorides (4716), and sodium and potassium bicarbonates (4718), and one calf (4769) received a large amount of whole milk administered intraruminally. The last four calves were also fed whole milk. Polyvinyl plastic sponges were given to three calves (4737, 4792, 4793) receiving the volatile fatty acids. In Groups A and B, two calves were sacrificed at 4, 8, and 12 weeks of age except that group B had three 4-week-old calves. One newborn calf, two 1-day-old calves and one 3-day-old calf also were included in Group A. All animals in Group C were sacrificed at 8 weeks of age (Table 1). The records of individual calves, i. e. the initial and final body weights and breeds, and a description of the methods used, are described in the previous paper (1).

Table 1. Average total intake of feed and other materials per animal.

Group	No. of calves	Age at sacrifice	Calf No.	Whole milk (by nipple)	Starter	Hay	Fatty acids (intraruminally)	Other substance (intraruminally)
		(wk.)		(kg.)	(kg.)	(kg.)	(kg.)	
	4	0–3 (days)		—	—	—	—	—
A	2	4		111.1	—	—	—	—
	2	8		273.1	—	—	—	—
	2	12		506.6	—	—	—	—
	3	4		104.8	3.5	0.1	—	—
B	2	8		258.8	23.2	3.6	—	—
	2	12		436.2	60.8	13.6	—	—
	1	8	4698	241.3	—	—	2.5*1	—
	1	8	4737	328.3	—	—	2.5*1	5040 sponges
	1	8	4736	294.6	—	—	2.5*2	—
	1	8	4706	231.2	—	—	—	138.6 liters carbonated water*6
	1	8	4716	208.3	—	—	—	1.6 kg. NaCl and 1.6 kg. KCl
C	1	8	4718	223.5	—	—	—	1.6 kg. $NaHCO_3$ and 1.6 kg. $KHCO_3$
	1	8	4761	314.4	—	—	16.1*3	138.6 liters whole milk
	1	8	4769	177.8	—	—	—	138.6 liters whole milk
	1	8	4792	233.4	—	—	6.7*4	1120 sponges
	1	8	4793	225.7	—	—	6.0*5	1120 sponges

*1 70% acetic–30% propionic acid
*2 Butyric acid
*3 70% acetate–30% Na propionate
*4 Na butyrate
*5 Na propionate
*6 Concentration- 4 volumes %

Tissue samples were taken from the four divisions of the stomach as follows: the rumen, from the anterior dorsal blind sac; the reticulum, from a part of its

fundus where the reticular plicae were tallest; the omasum, from the middle part opposite the omaso-abomasal sulcus; the abomasum, from the middle part of its fundic region along the greater curvature.

All samples were fixed in 10 percent buffered formalin, embedded in paraffin (Bioloid, American Hospital Supply Co.), and cut into 6 to 9 μ sections. The sections were stained by the following techniques: hematoxylin-eosin and Crossman's trichrome modification (21) for general histology; PAS-Light Green stain for glycogen with or without saliva digestion; PAS-Aurantia stain of Kametaka and Imai (22), and improved PAS-Luxol Fast Blue stain of Tamate and Kondo (23) for differential demonstration of the fundic gland cells of the abomasum. The numbers of chief and parietal cells (in ten squares, X 1000) at the middle part of the glands were counted, following the technique of Kametaka and Imai (22). The minimum thickness of the forestomach epithelia and the length of the terminal portions and secretory ducts of the fundic glands were also measured by using an ordinary micrometer. The thickness of the muscular layer (Tunica muscularis) was not measured in this study because of the difficulty of determining its degree of contraction in the histological preparations.

The terminology used by Henriksson and Habel (24), and subsequently by Tamate (16), for the forestomach epithelia is also used in this study.

Results

The following results were obtained from each of the three dietary groups, A, B, and C.

1. Group A. (Whole milk or colostrum)

Forestomach. The forestomach epithelia of newborn to 3-day-old calves receiving colostrum were characterized by extremely well developed pegs extending into the submucosa. The pegs occasionally consisted of more than 20 rows of the epithelial cells. The stratum corneum was hardly distinguishable from the thin inner layer, stratum transitionale. It did not stain typically by the dyes employed in Hematoxylin-eosin stain or in Crossman's trichrome modification.

The cells in the stratum granulosum contained only a small number of keratohyalin granules. These granules were also present in some prickle cells in the stratum spinosum. The prickle cells in the rumen and reticulum, but not in the omasum (Figures 1-2), contained a considerable amount of glycogen. The intercellular spaces and bridges were already present among these cells.

After 4 weeks of age, the stratum corneum was either thin or frequently absent from the forestomach epithelia of the calves fed only milk. The stratum transitionale showed an affinity to the dyes employed in the hematoxylin-eosin stain and trichrom stain. The epithelial pegs became shorter, containing no more than a few rows of cells in most cases, though longer ones were present occasionally. The glycogen in the prickle cells disappeared completely from the reticulo-

Table 2. The minimum thickness[*1] of the forestomach epithelia of the young calves in Groups A and B.

Group[*2]	Age at sacrifice	Average thickness of epithelium (μ)								
		Rumen			Reticulum			Omasum		
		Stratum corneum[*3]	Stratum germinativum[*4]	Total	Stratum corneum[*3]	Stratum germinativum[*4]	Total	Stratum corneum[*3]	Stratum germinativum[*4]	Total
A	0–3 days[*5]	10.9±1.4[*6]	34.2±4.2[*6]	45.1	10.8±1.5[*6]	30.5±4.4[*6]	41.3	11.3±1.4[*6]	29.7±3.2[*6]	41.0
A	4 weeks	9.4±1.3	36.0±3.5	45.4	8.8±1.5*	24.9±2.7	33.7	10.2±2.4	24.4±1.3**	34.6
A	8 〃	12.2±0.8*	35.0±2.1	47.2	8.2±1.3	28.5±2.2	31.7	11.4±1.0	22.7±3.0	34.1
A	12 〃	11.8±1.4	31.7±2.8*	43.5	8.3±1.6	27.6±2.9	35.9	5.9±0.8**	28.5±3.5**	34.4
B	4 〃	11.6±1.6	33.1±4.9	44.7	8.3±1.2	31.7±2.0	40.0	8.5±1.5	29.7±2.7	38.3
B	8 〃	21.3±2.6**	32.8±5.1	54.1	25.4±3.2**	14.0±2.8*	39.4	8.8±1.6	26.5±3.0	35.3
B	12 〃	27.0±4.7*	22.6±3.9**	49.6	29.3±3.5	14.4±2.5	43.7	18.4±2.6**	46.5±3.7*	64.9

*1 Thickness of epithelium which covers the secondary papilla of submucosa.
*2 Group A, whole milk; Group B, Whole milk, hay, and starter.
*3 Stratum corneum and stratum transitionale.
*4 Stratum granulosum, stratum spinosum and stratum basale.
*5 Received colostrum.
*6 Confidence interval ($\alpha=0.05$, n=10)
* Significant differences from the preceding stage at the 5% level of probability.
** Significant differences from the preceding stage at the 1% level of probability.

rumen of 4-week-old calves in this group (Figures 3, 4, 6).

The thickness of the forestomach epithelia showed relatively small changes during the first 12 weeks. The stratum corneum of the rumen in the newborn and 12-week-old calves had an average thickness of 10.9 μ and 11.8 μ, respectively (Table 2). The statistically significant decrease (in comparison with younger calves in the same group) in the thickness of the stratum corneum in the reticulum and omasum of 12-week-old calves was probably due to the heavy sloughing. A slight decrease in the thickness of the stratum germinativum, including basale, spinosum, and granulosum (24), was observed in the rumen and reticulum but not in the omasum of the older animals in this group.

Abomasum. The fundic glands in the newborn and 1-day-old calves consisted of immature gland cells which differed from the mature types in the older calves in the following ways; the chief cells contained only a small amount of the zymogen granules; their cytoplasm could be stained by hematoxylin, showing that they were not typically acidophilic; their nuclei were much larger than those in older animals. In contrast to this, the parietal cells were relatively smaller and had weakly acidophilic cytoplasm. The mucous cells at the neck of the glands contained a smaller amount of mucin than those in the older animals (Figures 5, 8).

In the fundic glands of the 3-day-old calf, most of the three cell types mentioned above had become characteristic of the more mature forms. The chief cells contained a large amount of the zymogen granules. The parietal cells increased in size and had typical acidophilic cytoplasm. The mucous neck cells contained much cytoplasmic mucin which was not stained by hematoxylin (Figure 7).

After 4 weeks of age, no marked differences were obserbed in the cytological aspects of the three types of gland cells present in the calves of this group. The cells were entirely typical of those found in 3-day-old calves (Figure 9).

The fundic glands in the newborn and 1-day-old calves consisted of short tubular glandular bodies connected with relatively longer excretory ducts. The lengths of the glandular bodies and ducts averaged 116 μ and 312 μ, respectively (Table 3). The parietal cells comprised only 8.4 percent of the total gland cells in the middle part of the fundic glands. In 3-day-old calves, the length of the fundic glands increased considerably, averaging 210 μ. The middle part of the glands consisted of parietal and chief cells which occupied 13.8 and 86.2 percent of the total number, respectively.

In 4- to 12-week-old calves, the length of the fundic glands and the excretory ducts was again nearly equal to that observed in the newborn and 1-day-old calves (Figure 9). The proportion of parietal cells to total cells in the middle part of the glands increased as much as 30.6 percent in 4-week-old calves, and thereafter remained almost at the same level up to 12 weeks of age (Table 3).

Table 3. The average lengths of the fundic glands and the ratios of their cellular components in the abomasum of the calves in Groups A and B.

Group	Averagelength (μ)*2				Ratios of gland cell*4		
	Age at sacrificee	Excretory duct*3	Glandular body	Total	Parietal cells	Chief cells	Numbers of cells counted
A*1	0–1 day	312±21*5	116±19*	428±73*5	8.4	91.6	393
A*1	3 days	362±75	210±22**	572±129	13.8**	86.2	347
A	4 weeks	224±51**	136±28**	360±94	30.6**	69.4	299
A	8 〃	251±11	122±12	373±44	36.7**	63.3	278
A	12 〃	363±65**	123±21	486±94	27.0**	73.0	396
B	4 〃	279±76	261±19*	540±116	20.2*6	79.8	331
B	8 〃	130±29**	226±42	356±64	30.6*	69.4	255
B	12 〃	173±34*	436±47**	609±28	33.5	66.5	239

*1 Received colostrum.
*2 Average of five observations.
*3 Including the depth of the gastric pits.
*4 Total of 10 observations at the middle of the glands.
*5 Confidence interval ($\alpha=0.05$, n=5)
*6 Difference from 3-day-old calves in Group A.
* Significant difference from the preceding stage at the 5% level of probability.
** Significant difference from the preceding stage at the 1% level of probabilitiy.

2. Group B (Whole milk, hay and starter)

Forestomach. In 4-week-old calves, the forestomach epithelia histologically showed relatively small differences from those in the 4-week-old calves in Group A, though the stratum corneum and stratum granulosum became more or less conspicuous. The stratum germinativum was as thick as that found in the 4-week-old calves in Group A (Table 2). The only marked difference was that a large number of the primarily swollen cells appeared in the epithelium of the reticulo-rumen (Figure 10).

In 8- to 12-week-old calves, a prominent cornification occurred in the epithelia of the forestomach. The stratum corneum in these animals was approximately two times as thick as that in the 4-week-old calves in the same group (Table 2). The outer three layers of the epithelium became as conspicuous as in mature cows. The primarily swollen cells also became numerous in the rumen and reticulum at 8 weeks of age, and in the omasum at 12 weeks of age. The stratum germinativum became thinner in the rumen and reticulum while the epithelial pegs became shorter with age. Thus, the forestomach epithelia in the older calves appeared very thin in contrast to their developed papillae and plicae (Figures 11-13).

Abomasum. In 4-week-old calves, the fundic glands of the abomasum consisted of the mature gland cells. The ratio of the parietal cells at the middle

of the glands increased from 20.2 percent in 4-week-old calves to 33.5 percent in 12-week-old calves (Table 3). Marked growth of the glands and shortening of the excretory ducts were observed in the abomasum of the older calves in this group. The growth was most prominent in 12-week-old calves. At this age, the glandular bodies were about three and one-half times longer than the ducts, and these glands were more than three times as large as those of the 12-week-old calves in Group A (Figure 14).

3. **Group C (Whole milk plus various treatments)**

Calves 4698, 4736, 4737, (subgroup a). Heavy sloughing of the stratum corneum was observed in the forestomach epithelia of each of the three calves fed 2.5 kg. of volatile fatty acids intraruminally during the 8-week period. The stratum corneum was absent in most cases. When the sloughing was moderate, the outer layer of the epithelia consisted mainly of thick, deeply stained transitionale. Generally, the stratum germinativum of the forestomach epithelia was as thick as that of the 8-week-old calves in Groups A and B. A considerable number of the primarily swollen cells were present in the stratum corneum (Figures 15-16).

It was also noticed that the histological aspects of the forestomach epithelia in the calf receiving a large number of plastic sponges (4737) did not differ greatly from those in the other two animals receiving no sponges (Figure 15).

The fundic glands of the abomasums were approximately two times as long as those in the 8-week-old calves fed only milk, whereas their excretory ducts appeared to be shorter (Figure 17). This indicated that although the administration of 2.5 kg. of the volatile fatty acids stimulated the growth of the glands, it did not produce growth of the forestomach epithelia typical of that of calves fed hay and starter.

Calves 4761, 4792, 4793 (subgsoup b). Calves in this group received large amounts of the volatile fatty acids. Generally, an advanced stage of cornification was observed in the forestomach epithelia of these animals. The stratum corneum was thicker than that of 8-week-old calves in Group B, but the outer layer of the stratum corneum was not so conspicuous. A relatively small number of the primarily swollen cells were found in the forestomach epithelia (Figures 18-19). The thickness of the stratum germinativum was approximately equal to that in Groups A and B. Peculiar folds were observed in the rumen mucosa of all calves receiving the volatile fatty acids. The histological observations of these folds revealed that they consisted of epithelium and submucosa, and that the lamina muscularis mucosa was absent (Figure 15).

Calf 4769 (subgroup c). Heavy sloughing of the forestomach epithelia with an invasion by leucocytes was observed. The stratum corneum and stratum transitionale were absent. A large number of leucocytes, mainly the neutrophiles, were found in the lamina propria and lamina submucosa (Figure 21). The same

Table 4. The minimum thickness[*1] of the forestomach epithelia in the 8-week-old calves in Group C.

Sub-group	Calf no.	Average thickness of epithelium (μ)								
		Rumen			Reticulum			Omasum		
		Stratum corneum[*2]	Stratum germinativum[*3]	Total	Stratum corneum[*2]	Stratum germinativum[*3]	Total	Stratum corneum[*2]	Stratum germinativum[*3]	Total
a	4698	13.4±1.9[*4]	36.6±4.1[*4]	50.0	8.7±1.7[*4]	31.0±5.8[*4]	39.7	7.7±1.8[*4,*]	24.5±2.6[*4]	32.2
a	4737	18.2±3.0[*+]	35.5±3.8	53.7	20.2±4.6[*+]	48.2±6.4[*+]	68.4	7.7±1.9[*]	20.2±2.6	27.9
a	4736	18.2±4.1[*]	27.3±3.8[*+]	45.5	16.6±7.3[*]	26.7±2.9[+]	43.3	5.4±0.8[*+]	23.5±2.6	28.9
b	4761	22.6±2.9[*]	45.0±4.6[*+]	67.6	—	—	—	13.6±2.6[+]	25.9±4.6	39.5
b	4792	14.9±2.1[*+]	18.9±3.3[*+]	33.8	18.8±2.8[*+]	25.8±4.0[+]	44.6	8.3±2.1[*]	23.8±2.9	32.1
b	4793	32.4±4.1[*+]	38.8±6.6	71.2	39.4±10.1[*+]	27.0±5.2[+]	61.4	6.3±1.2[*+]	19.3±1.8[*]	25.6
c	4769	—	—	58.0±2.7[*3]	—	—	86.0±8.1[*3]	—	—	60.3±5.3[*3]
d	4706	10.3±3.7[+]	26.3±5.1[*+]	36.3	10.2±4.9	27.0±3.6[+]	37.2	6.8±1.1[*+]	25.0±1.8	31.8
d	4716	16.8±1.2[*+]	38.5±3.5	55.3	33.1±2.0[*+]	34.8±1.9[*+]	67.9	11.2±1.0[+]	18.7±1.4[*+]	29.9
d	4718	13.0±1.8[+]	28.6±4.2[*]	41.6	9.2±1.5[+]	23.6±1.7[*+]	32.8	8.4±2.1[*]	30.6±3.5[*+]	39.0

[*1] Thickness of epithelium which covers the secondary papilla of submucosa.
[*2] Stratum corneum and stratum transitionale.
[*3] Stratum granulosum, stratum spinosum, and stratum basale.
[*4] Confidence interval ($\alpha=0.05$, n=10)
[*] Significant differences from the 8-week-old calves in Group A at the 5% level of probability.
[+] Significant differences from the 8-week-old calves in Group B at the 5% level of probability.

growth of the abomasal fundic glands observed in the former group was noticed in this calf.

Calves 4706, 4716, 4718 (subgroup d). It was generally observed that the forestomach epithelia, especially of the reticulo-rumen, did not show any conspicuous growth in this group. The thickness of the stratum corneum did not exceed that found in Group A, except for the reticulum of calf 4716. The primarily swollen cells were few in this group (Figure 22). The thickness of the inner three layers of the epithelia was approximately equal to that found in Group A.

The length of the fundic glands of the abomasum varied from approximately the same length as, to shorter than, that of their excretory ducts, demonstrating that glandular growth did not occur in this group. The extreme decrease in the ratios of the parietal cells was probably due to the effects of the salts or carbonated water given to these animals (Table 5). However, these effects are not the same as those observed in subgroups a and b which resulted from the presence of the volatile fatty acids.

Table 5. The average length of the fundic glands and the ratios of their cellular components in the abomasums of the 8-week-old calves in Group C.

Subgroup	Average length (μ)*1				Ratios of gland cells*3		
	Calf no.	Excretory duct*2	Glandular body	Total	Parietal cells	Chief cells	Numbers of cells counted
a	4698	164±49*4,*	240±21*4,*	404±51*4	20.5*+	79.5	361
a	4737	256±34+	297±34*	453±74	27.8*	72.2	378
a	4736	177±31*	261±16*	438±53	29.2	70.8	318
b	4761	181±46*	429±71*+	610±92	22.7*+	77.3	286
b	4792	316±65*+	346±71*+	662±112	29.1*	70.9	337
b	4793	134±39*	234±21*+	368±54	29.2*	70.8	415
c	4769	232±67*+	320±20*+	552±59	27.9*	72.1	398
d	4706	254±57+	199±53*	453±83	26.3*	73.7	376
d	4716	264±51+	233±27*	497±96	22.2*+	77.8	352
d	4718	171±22*	187±42	358±75	22.1*+	77.9	371

*1 Average of five observations.
*2 Including the depth of the gastric pits.
*3 Total of 10 observations at the middle of the glands.
*4 Confidenece interval (α=0.05, n=5)
* Significant difference from the 8-week-old calves in Group A at the 5% level of probability.
\+ Significant difference from the 8-week-old calves in Group B at the 5% level of probability.

Discussion

The histological changes, with increasing age, in the forestomach epithelia

of the calves fed with whole milk, hay, and starter included a rapid cornification of the epithelia, an increase in the number of primarily swollen cells in the corneum, a decrease in the thickness of the stratum germinativum, and a marked shortening of the epithelial pegs extending into the submucosa. Accordingly, the epithelia appeared to become thinner with age, while the capacity of the forestomach increased greatly in the same animals. The process of cornification in the forestomach epithelia may be readily observed by the presence of continuous stratum corneum and strongly chromophilic stratum transitionale.

Generally, the changes were similar to those previously observed in the forestomachs of post-weaning young goats (15) and in the young lambs (17). An increase in the numbers of primarily swollen cells and parakeratotic changes in the forestomach epithelia of growing calves also were observed by Henriksson and Habel (24). They reported, however, that the thickness of the stratum germinativum increased with age on normal feed, a conclusion which was inconsistent with the findings of this study. Thompson and his co-workers (18) reported that the increased folding of the basal layer (Malpighian layer) occurred with the thickening of the corneum in the rumen epithelia of pellet-fed lambs. This also would appear to be in contradicition with the results obtained by the present authors, who hold that such folding should be regarded as an extension of the epithelial pegs. The reasons for these difference in interpretation are not clear.

The developmental changes in the forestomach epithelia described above were not observed in the calves fed only whole milk (Group A), whereas the replacement of hay and starter by the higher amounts of the volatile fatty acids resulted in the aforementioned changes in the forestomach epithelium. The presence of a thicker corneum in the calves in Group A was probably due to retarded sloughing of this layer as the result of a friction between stomach contents and epithelia. The reason the administration of 2.5 kg. of volatile fatty acids to one 8-week-old calf resulted in the thickening of only the stratum transitionale was not clear. However, it may be the result of heavy sloughing of the outer layers of the epithelia, since nearly the same parakeratotic condition was found in the forestomach epithelia of the calves fed concentrates alone (16).

It was previously reported that the forestomachs of the calves receiving various materials such as plastic sponges, carbonated water, sodium and potassium bicarbonates, and sodium and potassium chlorides showed no indication of the development which was observed grossly and by papillary evaluation (1). Histological examinations of the forestomachs in these animals revealed that the epithelia remained as undeveloped as in the calves fed only milk. The absence of the conspicuous stratum corneum was again one of the most marked characteristics showing the undeveloped state of the epithelia.

The forestomach epithelia of the calf (4769) receiving 138 l. of whole milk

intraruminally showed a sloughing of the outer layers. Most of the invading leucocytes were neutrophiles. The epithelia of the calf (4761) receiving the same amounts of milk with 16 kg. of the volatile acid mixture appeared almost the same as those of the calves fed hay and starter. Therefore, the unusual histology of the forestomach epithelia of Calf 4769 was probably due to the stimuli of milk in the rumen.

Sander *et al.* (8) suggested that the peculiar folds of the rumen mucosa present in the calves receiving large doses of the volatile fatty acids might have functional relations to the plicae of the reticulum. The fact that these folds contained no muscular tissues should not be regarded as conflicting with the fact that the plicae usualy contain a mucosal muscle layer (lamina muscularis mucosa), since such fold-like, muscle-free plicae were always present in the rumino-reticular fold region of the reticulo-rumen (15).

Hill (20) reported that the parietal cells, which were very sparse in the abomasal glands until term, increased rapidly in sheep during the first two days of life. In agreement with this finding, the present authors noted the same increase and rapid development of the cells of the fundic glands during the first three days after birth. It may be concluded, therefore, that the unusually rapid maturation of the fundic glands is one of the morphological characteristics of the ruminant stomach.

It was demonstrated that rumen fermentation had a significant effect on glandular development in the abomasum. The growth of the fundic glands, indicated by the marked extension of their glandular bodies, was stimulated by the addition of hay and starter to the diet, whereas no significant development was observed in the animals fed only milk to 12 weeks. The fact that development was stimulated by the intraruminal administration of the lower amounts of the volatile fatty acids suggests that the acids probably were the factors which promoted growth of the fundic glands in the abomasum of the calves receiving hay and starter in addition to milk.

When stomach growth was observed, grossly and by papillary evaluation, in the calves fed large amounts of volatile fatty acids as replacement for hay and starter, these observations were clearly substantiated by subsequent histological examination. In other words, the growth stimulated experimentally by the acids was essentially identical, macroscopically as well as microscopically, to that occurring in animals fed a normal diet.

Summary

Twenty-seven dairy calves from newborn to 12 weeks of age were assigned to different dietary groups. The growth of their stomachs, previously observed grossly and by papillary evaluation, was examined histologically. The results were as follows:

1. The histological changes in the forestomachs of the calves fed whole milk, hay, and starter (Group B) included a rapid cornification of their epithelia together with an increase in the number of primarily swollen cells, a decrease in thickness of the stratum germinativum, and a shortening of the epithelial pegs. However, these changes were never observed in the forestomach epithelia of the calves fed only whole milk up to 12 weeks of age (Group A). The stratum spinosum contained a large amount of glycogen in the newborn, but not at 4 weeks of age.

2. The replacement of hay and starter by the higher levels of volatile fatty acids (6.7 kg. to 16.1 kg.) resulted in forestomach tissue growth similar to that in calves of Group B. The administration of 2.5 kg. of the acids caused a thickening of the stratum transitionale, but failed to produce a consistent stratum corneum.

3. The administraton of plastic sponges, Na and K bicarbonates, Na and K chlorides, and carbonated water failed to stimulate epithelial growth of the forestomach.

4. A prominent maturation of the glandular cells of the abomasal fundic glands occurred during the first three days after birth. The glands remained undeveloped in the calves in Group A up to 12 weeks of age, whereas they showed marked growth in the animals in Group B.

5. The administration of 2.5 kg. of volatile fatty acids to one 8-week-old calf resulted in growth of the fundic glands as mentioned above. The administration of bicarbonates, or carbonated water resulted only in a decrease in the number of the parietal cells in the glands.

6. The intraruminal administration of whole milk did not produce typical cornification of the forestomach epithelia. However, the fundic glands in the abomasum showed growth similar to that found in the calves in Group B.

7. It was concluded that the stomach growth stimulated by the substitution of fatty acids for hay and starter was essentially identical, microscopically as well as macroscopically, to the growth occurring in young calves fed normally.

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References

1) Tamate, H., A.D. McGilliard, N.L. Jacobson and R. Getty (1962). J. Dairy Sci., **45**, 408.
2) Trautmann, A. (1932). Arch. Tierernahr. u. Tierzucht, **7**, 400.
3) Blaxter, K.L., M.K. Hutcheson, J.M. Robertson and A.L. Wilson, (1952). Brit. J. Nutrition, **6**, i.
4) Tamate, H. (1956). Tohoku J. Agr. Res., **7**, 209.

5) Flatt, W.P., R.G. Warner and J.K. Loosli (1958). J. Dairy Sci., **41**, 1593.
6) Flatt, W.P., R.G. Warner and J.K. Loosli (1959). Cornell Univ. Memoir, **361**, 3.
7) Harrison, H.N., R.G. Warner, E.G. Sander and J.K. Loosli (1960). J. Dairy Sci., **43**, 1301.
8) Sander, E.G., R.G. Warner, H.N. Harrison and J.K. Loosli (1959). J. Dairy Sci., **42**, 1600.
9) Flatt, W.P., R.G. Warner and J.K. Loosli (1956). J. Dairy Sci., **39**, 928.
10) Warner, R.G., C.H. Grippin, W.P. Flatt and J.K. Loosli (1955). J. Dairy Sci., **38**, 605.
11) Warner, R.G., H.N. Harrison and E.G. Sander (1959). Proc. Cornell Nutrition Conference for Feed Manufactures, 91.
12) Tamate, H. (1957). Tohoku J. Agr. Res., **8**, 65.
13) Brownlee, A. (1956). Brit. Vet. J., **112**, 369.
14) Sinclair, J.H. and H.O. Kunkel (1959). Proc. Soc. Exptl. Biol. & Med., **102**, 57.
15) Tamate, H. (1960). Ph. D. Thesis. Tohoku University, Sendai, Japan (In Japanese).
16) Tamate, H., K. Ishida, Y. Kondo, F. Kondo, T. Hoshino, and Y. Toryu (1962). Tohoku J. Agr. Res. **13**, 351.
17) Wardrop, I.D. (1961). J. Agri. Sci., **57**, 343.
18) Thompson, G.B., L.D. Kitner and W.H. Pfander (1958). J. Animal Sci., **17**, 1220.
19) Brownlee, A. and J. Elliot (1961). Vet. Rec., **73**, 384.
20) Hill, K.L. (1956). Quart. J. Exptl. Physiol., **42**, 72.
21) Crossman, G. (1937). Anat. Rec., **69**, 33.
22) Kametaka, M. and K. Imai (1956). Jap. J. Zootech, Sci., **27**, 43. (In Japanese).
23) Tamate, H. and Y. Kondo (1961). Stain Technol., **36**, 254.
24) Henriksson, K.B. and R.E. Habel (1961). Anat. Rec., **139**, 499.

Explanaton of Figures

Staining: Crossman's trichrome modification except Figures 1, 7-9, 14, 17, and 20.

Plate I

Fig. 1. Rumen. 1-day-old calf (4875). PAS-hematoxylin stain. X 100. Prickle cells contain abundant glycogen.

Fig. 2. Rumen. Newborn calf (6034). X 100. Thick epithelium with long pegs extending into submucosa.

Fig. 3. Rumen. 4-week-old calf in Group A (4722). X 100. Undeveloped papilla is covered with relatively thicker epithelium.

Fig. 4. Rumen. 8-week-old calf in Group A (4733). X 100. Stratum corneum is absent in papillary epithelium.

Fig. 5. Abomasum. 1-day-old calf (4875). X 100. Fundic glands consist of short glandular bodies and longer excretory ducts.

Fig. 6. Reticulum. 8-week-old calf in Group A (4750). X 100. Stratum corneum is thin and discontinuous, and primarily swollen cells are few.

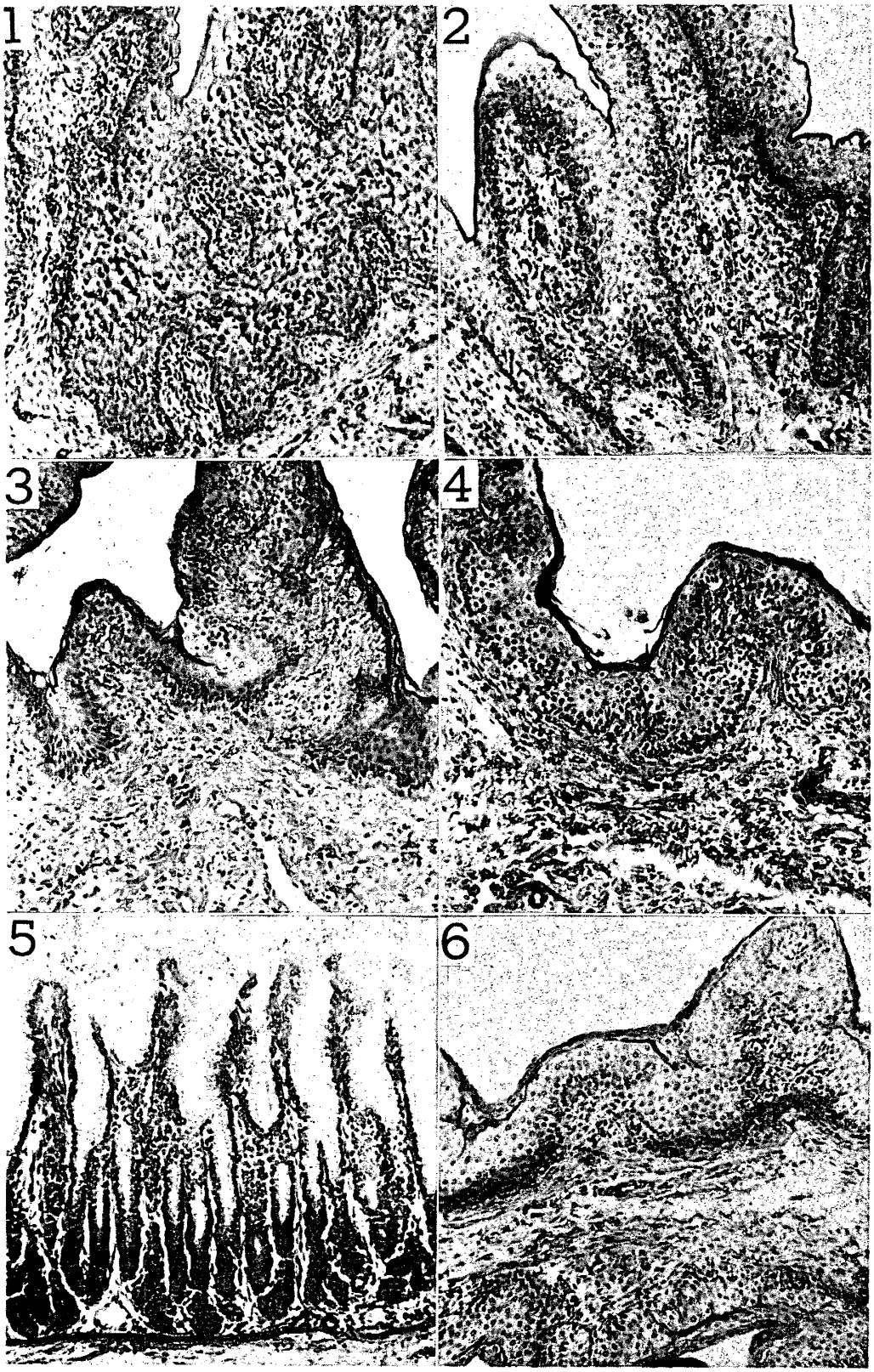
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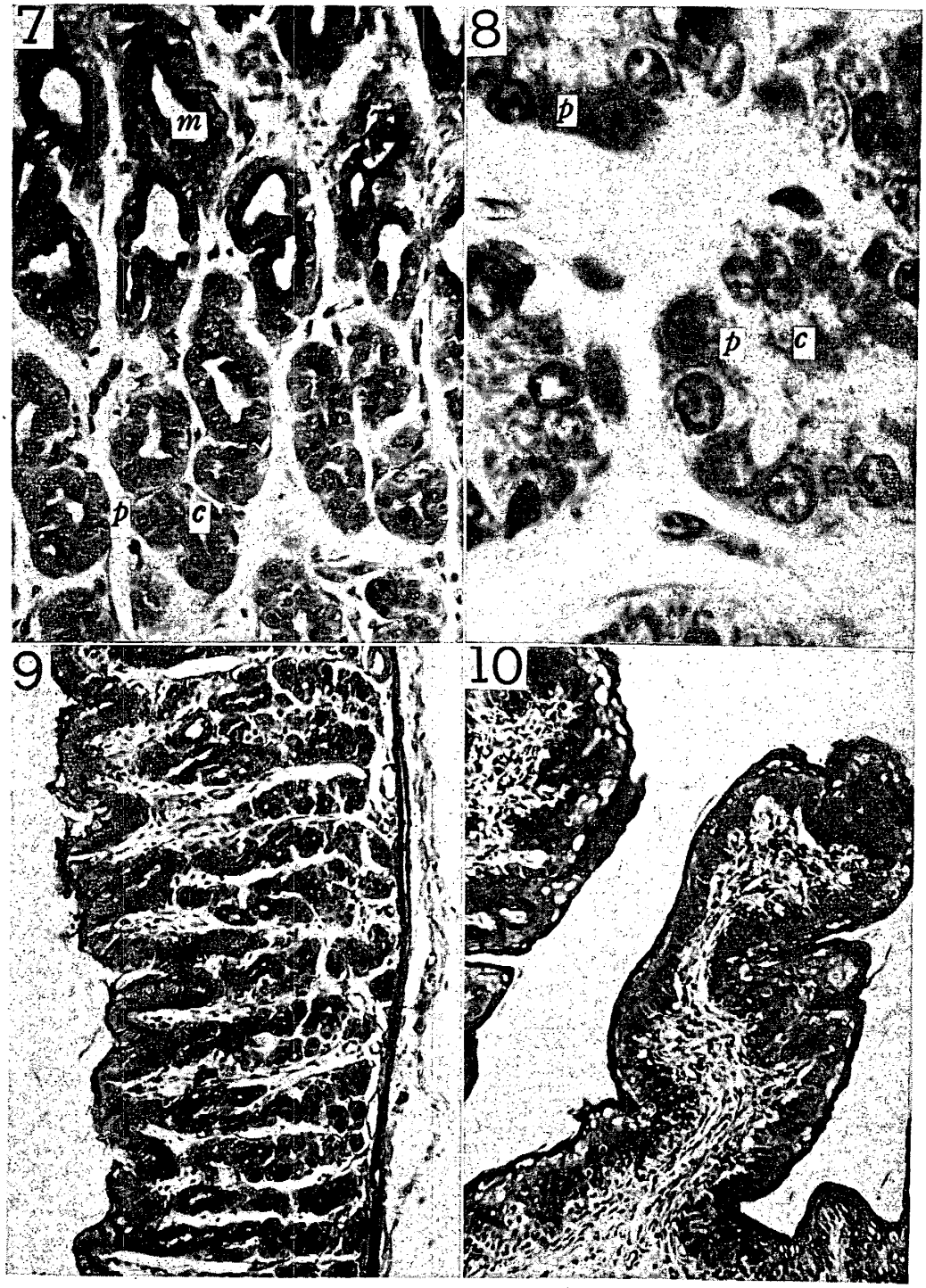
Plate II

Fig. 7. Abomasum. 3-day-old calf (4828). Tamate and Kondo's modification of LFB-PAS stain. X 400. Zymogen granules are present in chief cells (c). Typical parietal cells (p), and mucous neck cells (m) are noted.

Fig. 8. Abomasum. Newborn calf (6034). Tamate and Kondo's modification of LFB-PAS stain. X 1000. Chief cells with few zymogen granules (c) and smaller parietal cells (p) are present.

Fig. 9. Abomasum. 8-week-old calf in Group A (4750). Tamate and Kondo's modification of LFB-PAS stain. X 100. Undeveloped fundic glands with shorter glandular bodies.

Fig. 10. Rumen. 4-week-old calf in Group B (4692). X 100. Developing papillae and appearance of numerous primarily swollen cells in epithelium are noted.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m
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Plate III

Fig. 11. Rumen. 8-week-old calf in Group B (4680). X 100. Many primarily swollen cells appear in typically cornified epithelium.

Fig. 12. Omasum. 8-week-old calf in Group B (4680). X 70. Stratum corneum is mostly continuous.

Fig. 13. Rumen. 12-week-old calf in Group B (4673). X 100. Heavy cornification of papillary epithelium, numerous primarily swollen cells, and thick stratum corneum are noted.

Fig. 14. Abomasum. 8-week-old calf in Group B (4685). Tamate and Kondo's modification of LFB-PAS stain. X 100. The fundic glands consist of typical glandular cells.

Fig. 15. Rumen. Calf 4737 in Group C-a. X 70. A peculiar fold contains no muscular layers. Sloughing occurs in stratum corneum.

Fig. 16. Omasum. Calf 4737 in Group C-a. X 100. Stratum corneum is absent, while stratum transitionale is thicker.

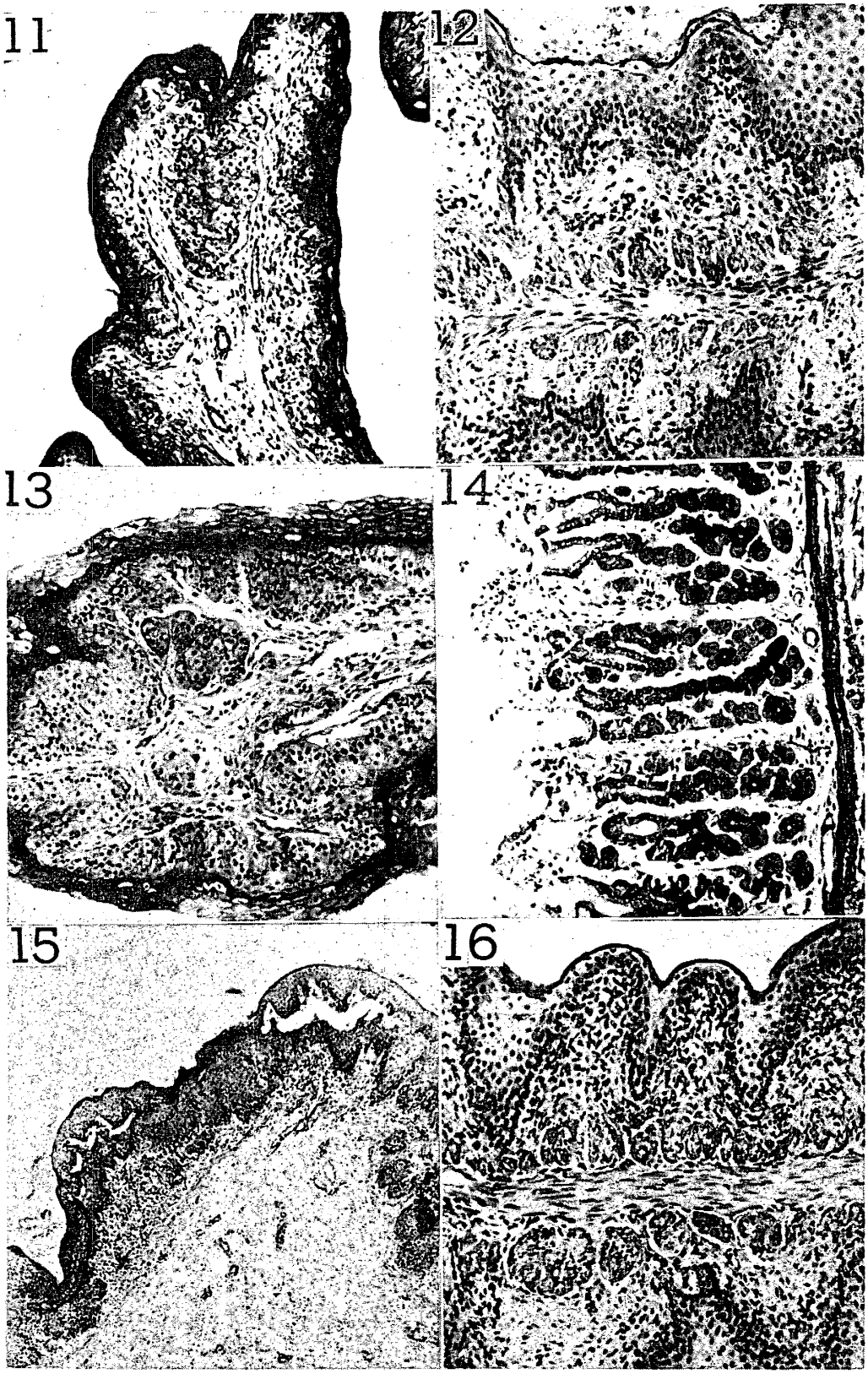
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Plate IV

Fig. 17. Abomasum. Calf 4737 in Group C-a. Tamate and Kondo's modification of LFB-PAS stain. X 100. A significant increase in the length of glandular bodies is noted.

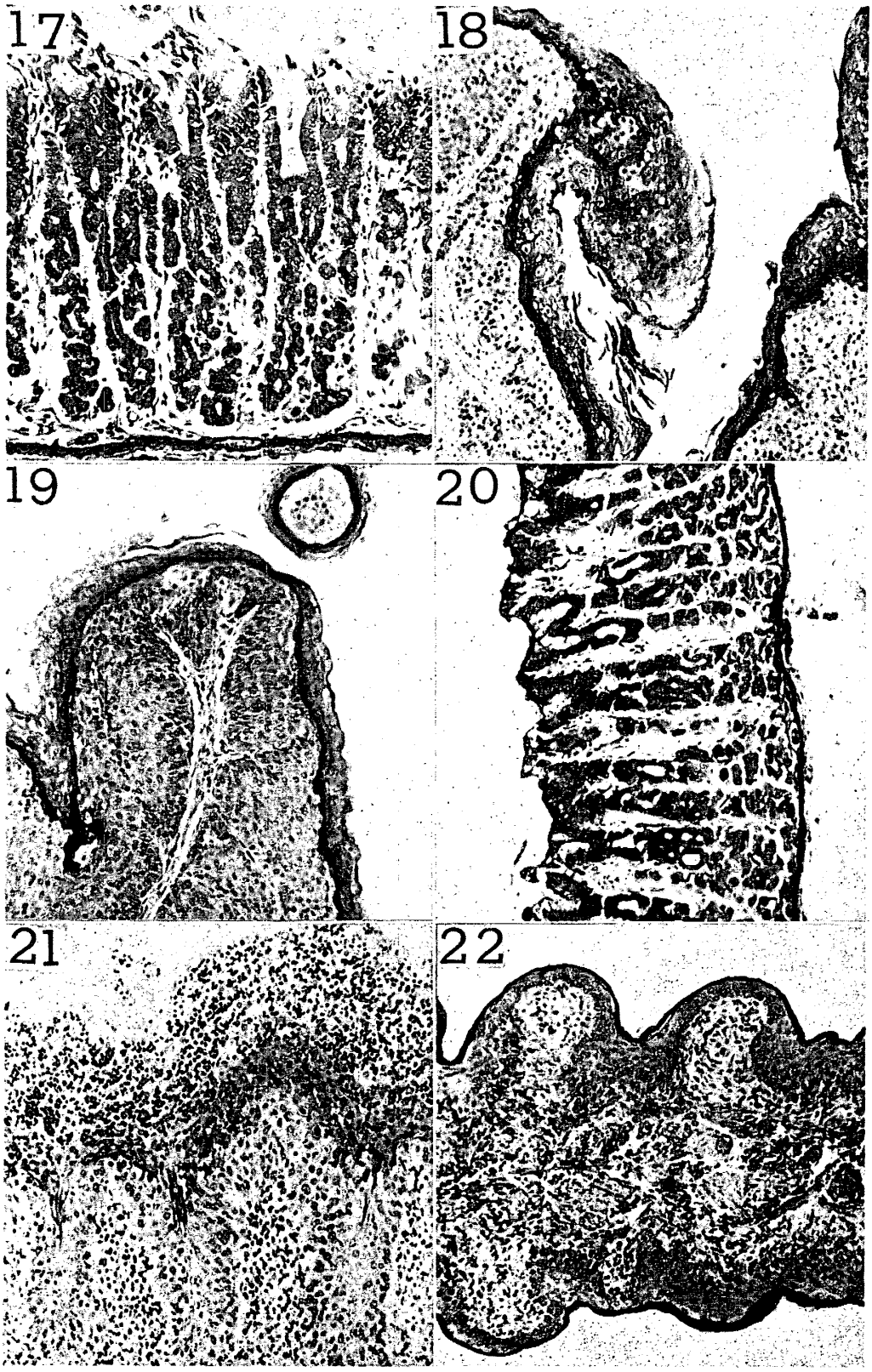
Fig. 18. Rumen. Calf 4761 in Group C-b. X 100. Numerous primarily swollen cells appear with sloughing of stratum corneum.

Fig. 19. Rumen. Calf 4793 in Group C-b. X 100. Cornification of epithelium is indicated by the presence of stratum corneum.

Fig. 20. Abomasum. Calf 4792 in Group C-b. LFB-PAS stain. X 100. Growth of fundic glands is indicated by their longer glandular bodies.

Fig. 21. Reticulum. Calf 4769 in Group C-c. X 100. Stratum corneum is absent, and leucocyte invasion is noted.

Fig. 22. Omasum. Calf 4706 in Group C-d. X 100. Absence of conspicuous stratum corneum indicates that true cornification does not occur in epithelium.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