THE OCCURRENCE OF PARANUCLEAR VACUOLES IN THE EPITHELIAL CELLS OF RUMEN MUCOSA AFTER TREATMENT WITH DISTILLED WATER IN VITRO

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Introduction

Henriksson and Habel (1) reported that peculiar vacuoles appeared frequently ı the epithelial cells of the rumen mucosa of calves and mature cow. The acuoles were in contact with the nucleus, and when larger compressed the :tter into a narrow crescent in section. They occurred in sections fixed and :ained by widely different methods, and with the shortest interval between :unning and fixation.

A similar type of paranuclear vacuoles was observed by the senior author 2) in the histological preparations of the rumens of calves, sheep, and goats of arying ages. In those animals, the rumens were rinsed with a large amount : cold tap water before histological sampling. Dobson and Phillipson (3) :ported that the paranuclear vacuoles were more abundant in the samples of ımen mucosa taken from sheep when the rumen contained tap water than the ımples taken when the rumen contained isotonic solutions. Their findings ›gether with the experience of the senior author suggest that the vacuoles ıay develop by the stimuli of water in which intact rumen mucosa is immersed :fore histological sampling and fixation.

The occurrence of the paranuclear vacuoles in the sections of rumen mucosa as also reported by Hayashi *et al.* (4). They were also seen in the photo- ıicrographs of the rumen tissues of Wardrop(5, 6).

It is not yet certain whether the paranuclear vacuoles actually exist in the ving cells of intact rumen tissues. The Cornell workers(1) suggested that the acuoles are probably related with the rumen absorption, and are something ıore than a simple artefact. No direct evidence was, however, given by them ›ncerning the above suggestion.

In the present study, a pronounced occurrence of the paranuclear vacuoles was observed in the epithelial cells of the rumens of goats and sheep when the tissues were incubated, in vitro, with distilled water before histological sampling. A possible relation of the vacuoles to the absorptive properties of the rumen epithelium was discussed.

Materials and Methods

Rumen samples, approximately 10^2 cm, were taken from the ventral part of the anterior dorsal blind sac of this organ. One sheep and two goats, all adult, were used. The rumen samples were collected shortly after the death of the animals. After a brief rinse in saline water (0.85 % NaCl solution) to remove the rumen contents from mucosa, a small piece of the tissue was fixed in Helly's fluid as the initial control.

The samples were then divided into two halves, which were incubated at room temperature (20—22°C) in saline and distilled water, respectively. The incubation media were renewed several times during the incubation. Tissue samples were collected at 30, 60, 120 minutes after the initial sampling. In one goat, the sampling was also made at 240 minutes of incubation. In the sheep the sampling was made at 150 minutes, but not at 30 minutes of incubation. In the two goats, oesophageal mucosa was taken from the middle portion of this organ, and treated in the same manner as done for the rumen samples. The samples of oesophageal tissues thus treated were also collected at the times of incubation corresponding to those of the rumen mucosa.

All tissues collected were fixed in Helly's fluid for overnight. After washing in running water for a few hours, the tissues were passed through a series of alcohol and dioxane, and embedded in paraffin. Sections of 6 to 7 μ thick were stained either by hematoxylin-eosin or by Crossman's modification of trichrome stain.

Results

Occurrence of paranuclear vacuoles in the rumen epithelial cells was noted in the sections of the rumen mucosa after incubation for 30 minutes in distilled water. They generally appeared quite similar to what was described as "paranuclear vacuoles" by the Cornell workers(1). For convenience of comparison, the vacuoles were arbitarily classified into three transitional phases, based on their size and the pycnotic condition of their nuclei.

Phase I (Figs. 1—3) The vacuoles are much smaller than the nucleus; the nucleus is not pycnotic.

Phase II (Fig. 4) The size of the vacuoles is nearly equal to the nucleus; the nucleus is more or less pycnotic.

Phase III (Figs. 5—7) The vacuoles are larger than the nucleus which is

Table 1. Average numbers of paranuclear vacuolations in three loci of epithelia treated with saline or distilled water in vitro (20°—22°C).

Loci of epithelium*1	Treatment*2	Incubation time in minutes																							
		0 +++				30 ++				60 +++				120 ++				150 +				240 +			
		Paranuclear vacuolation per 1000 epithelial cells *3																							
		I	II	III	I–III	I	II	III	I–III	I	II	III	I–III	I	II	III	I–III	I	II	III	I–III	I	II	III	I–III
Upper	Saline	2.0	1.0	0	2.5	3.0	1.0	0.5	4.5	2.5	2.5	0.5	5.5	1.5	2.5	1.5	5.5	3.0	2.0	0	5.0	1.0	4.0	1.0	5.0
	Dist.					3.0	0.5	0	3.5	4.0	1.7	0	5.7	4.0	1.5	0.5	6.0	4.0	1.0	0	5.0	6.0	6.0	1.0	13.0
Middle	Saline	1.3	0.3	0	4.0	4.0	1.5	0	5.5	3.5	0.5	0.5	4.5	2.0	1.5	0.5	4.0	4.0	3.0	1.0	8.0	4.0	4.0	0	8.0
	Dist.					6.0	4.5	0.5	11.0	6.0	2.3	1.3	9.6	11.5	5.5	5.0	22.0	7.0	2.0	2.0	11.0	10.0	13.0	6.0	29.0
Lower	Saline	4.0	0.7	0	4.7	4.5	2.5	0	7.0	6.0	2.0	0.5	8.5	2.0	2.5	0	4.5	3.0	1.0	0	4.0	3.0	2.0	1.0	6.0
	Dist.					14.5	12.0	6.0	32.5	12.0	10.3	4.3	26.6	17.0	9.5	9.0	35.5	18.0	5.0	4.0	27.0	3.0	14.0	49.0	66.0

*1. Upper: stratum granulosum and upper spinosum
Middle: stratum spinosum
Lower: stratum basale

*2. Saline: 0.85% NaCl
Dist. : distilled water

*3. I : Phase I; the size of vacuole is much smaller than that of nucleus. The nucleus is not pycnotic.
II: Phase II; the size of vacuole does not exceed that of nucleus which is more or less pycnotic.
III: Phase III; the size of vacuole is larger than that of nucleus.
+++: Average of three trials
++ : Average of two trials
+ : Value of one trial

highly pycnotic and is compressed into a narrow crescent in section.

The numbers of the paranuclear vacuoles, described above, were counted in the upper (stratum granulosum and upper spinosum), middle (stratum spinosum), and lower (stratum basale) loci of the rumen epithelium. The counts were made on the three phases of the vacuoles per 100 cells randomly selected in the three loci. The results are shown in Table 1.

1. Distilled water incubation of the rumen tissues.

a) Relation between the incubation time and the counts of the vacuoles.

The numbers of the paranuclear vacuoles were large at 30 minutes of incubation in distilled water. They were mostly of Phases I and II. The numbers of the vacuoles did not particularly increase after the prolonged incubation for as long as two hours. The only difference was that the number of the Phase III vacuoles increased slightly in the lower loci of the epithelia. At four hours of incubation, the Phase III vacuoles became numerous. The nuclei of the epithelial cells appeared more or less pycnotic, and were less well-defined in the stained sections (Table 1).

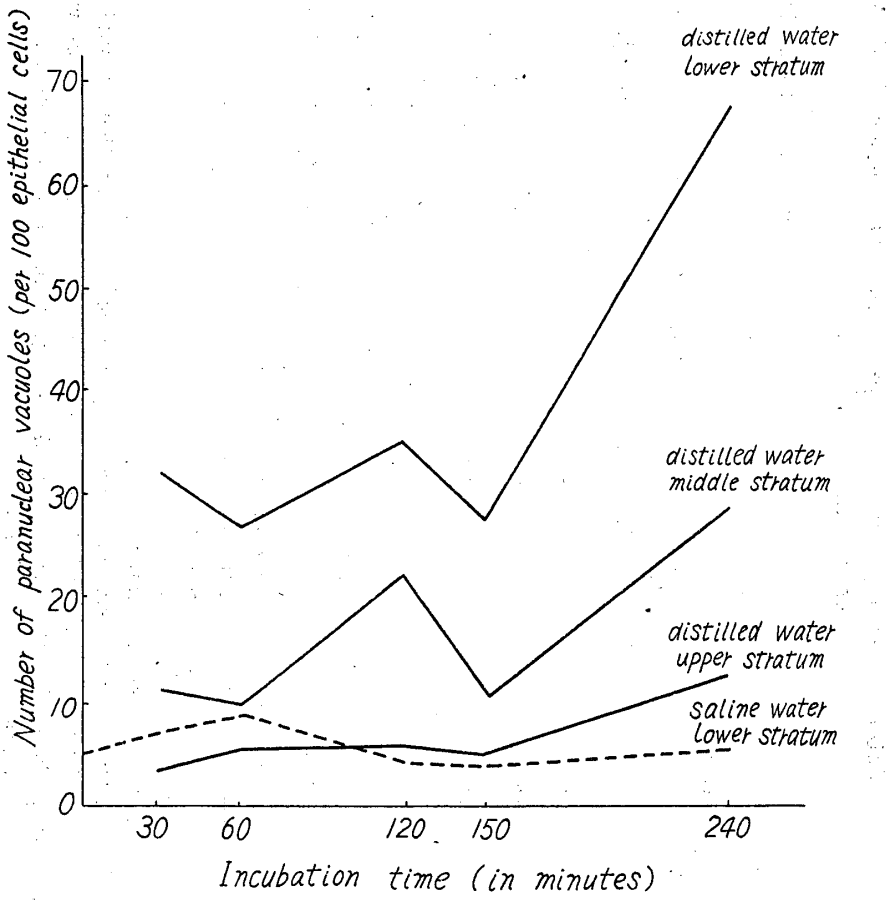

Fig. 8. Relation between the numbers of the paranuclear vacuoles and the incubation time at the three loci of the rumen epithelium.

b) Occurrence of the vacuoles in the different loci of the epithelium.

It will be noted, from Table 1 and from Fig. 8, that the paranuclear vacuoles increased in number towards the lower locus of the rumen epithelium. Namely, the numbers at 30 minutes of incubation were 32.5, 11.0 and 3.5 per 100 cells in average in the lower, middle and upper locus of the epithelium, respectively. At four hours of incubation, the cells with the vacuoles occupied 66 percent of the epithelial cells in the lower locus, whereas only 13 percent in the middle. The occurrence of the heavier type of the vacuoles, Phase III, was also seldom in the latter locus (Figs. 1—2).

2. The incubation of the rumen tissues in saline water.

As shown in Table 1, only a few numbers of the smaller vacuoles were present in the epithelia of the rumens at the time of the initial smapling. During the incubation for as long as four hours, the vacuoles did not increase in number and Phase III vacuoles were few in contrast with the tissues incubated in distilled water (Fig. 9).

There were no differences in the numbers of the vacuoles in the three loci of the rumen epithelium. The vacuoles, when present in relatively larger numbers, were found almost uniformly throughout the epithelium.

Generally the rumen tissues incubated in saline water showed a shrinkage of the cellular components. The intercellular space of the epithelium was more or less clearer than in the tissues treated in distilled water (Fig. 10).

c) Occurrence of the vacuoles in the papillary epithelium.

Table 2 and Fig. 11 show the counts of the paranuclear vacuoles in the papillary and non-papillary epithelia of the rumen of the goat (no. 2). No difference was found in the counts between the papillary and non-papillary epithelia during the four-hour incubation in distilled water. A slight difference was found between the two counts at 30 minutes of incubation, but this was statistically not significant.

3. Incubation of the oesophageal tissues in distilled and saline water.

As shown in Fig. 12, no typical paranuclear vacuoles were observed in the epithelial cells of the oesophageal mucosa, even after the four-hour incubation in distilled water. The Phase III vacuoles were never seen. The change observed was a shrinkage of the general tissue components of the tissue after the prolonged incubation.

4. Swelling of the capillaries in the submucosa of the rumen.

A prominent swelling or dilation of the capillaries was noted in the secondary dermal papillae of the rumen tissues. This was clearly seen in the cross sections of the rumen papillae (Fig. 13) incubated either in distilled or in saline water. The swellings of the capillaries were prominent after the shortest time of incubation. Such swellings were not seen in the submucosa of the oesophageal tissues incubated in distilled water (Fig. 12), and less prominent in the rumen

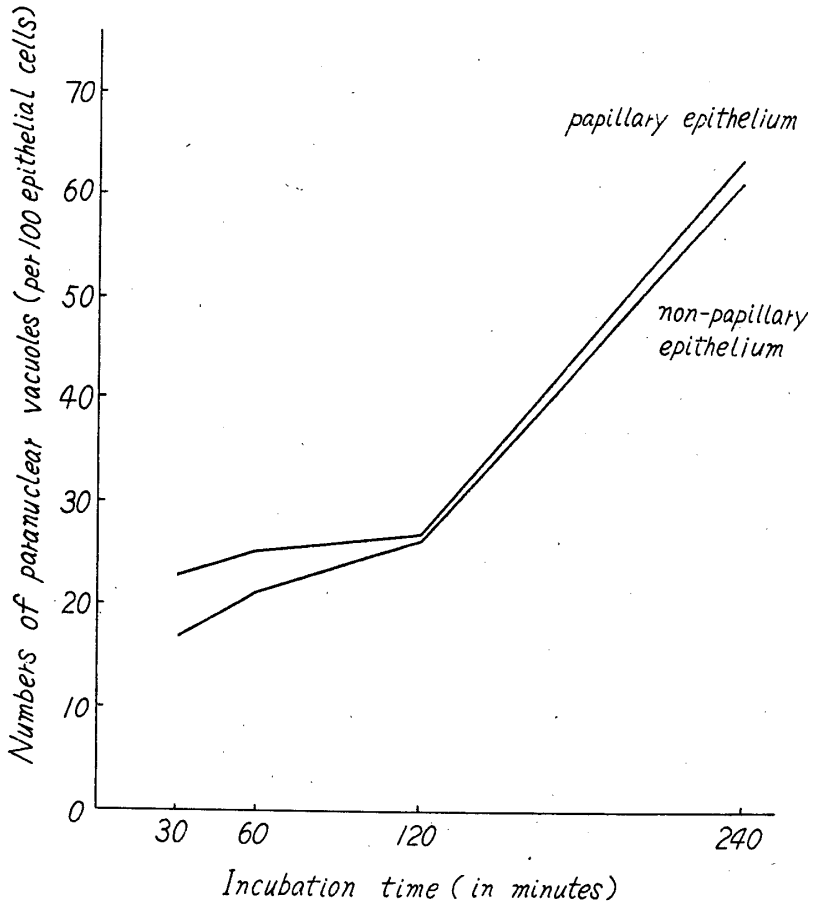

Fig. 11. The number of the paranuclear vacuoles in the lower locus (Stratum basale) of the papillary and non-papillary epithelia of the rumen mucosa incubated in distilled water in vitro.

Table 2. Appearance of paranuclear vacuoles in the lower locus of papillary and non-papillary epithelia of goat rumen after incubation in distilled water (Goat no. 2).

Locality of epithelia	Cell counts*1	Incubation time in minutes			
		30*3	60	120	240
Non-papillary epithelium	Normal	83	79	76	37
	Phase I*2	11	8	13	11
	Phase II*2	2	9	7	20
	Phase III*2	4	4	4	32
	Total	100	100	100	100
Papillary epithelium	Normal	77	75	74	39
	Phase I*2	7	12	11	11
	Phase II*2	7	8	10	17
	Phase III*2	9	5	5	33
	Total	100	100	100	100

*1 Counts of 100 epithelial cells at stratum basale

*2 Same as in Table 1.

*3 Difference in the counts of paranuclear vacuoles in papillary and non-papillary epithelia is statistically not signicant ($x_2=1.12$, $0.30>P>0.20$)

tissues incubated in saline water (Fig. 10).

Discussion

The data of the present study indicate that the so-called paranuclear vacuoles occur when the rumen tissue is exposed to the hypotonic medium before histological fixation. The fact that the numbers of the vacuoles did not increase during from 30 minutes to two hours of incubation in distilled water suggests that the vacuoles probably appear at a time earlier than 30 minutes. When the rumen was incubated in isotonic solution, i. e., in saline water, the paranuclear vacuoles were few in number, although the shrinkage of the cellular components and the swellings of the capillaries in the secondary dermal papillae were evident. The above facts indicate that the rumen mucosa is highly sensitive to the osmotic changes of the medium in which its free surface is exposed, unusal to the stratified squamous epithelium which covers the non-absorptive surface of the body, such as in skin and oesophagus.

The swelling of the submucosal capillaries indicated that a large quantity of water had entered their cavities via epithelium. The question arises then that how the water could pass through several layers of the epithelial cells so rapidly. There are two possible pathways of water through the rumen epithelium, intra-cellular and extra-cellular. In the former pathway, it is likely that the epithelial cells may suffer severe damage by the water, and the damage may be heavier in the upper loci of the epithelium. However, this is not in accord with the results obtained in this study, since the paranuclear vacuoles as indications of such cellular damage were abundant in the lower loci of the epithelium, but scarce in the upper. Thus it may be assumed that the pathway of water through the rumen epithelium is extra-cellular, probably through its well-developed intercellular spaces.

It has been pointed out that the passage of water or other substances of lower molecular weight via rumen epithelium may be handicaped if they should pass through the cell membranes of stratum germinativum (7). This handicap will be overcome if we assume that the absorption of such substances is done via intercellular canal system. The result of this study seems to provide a direct evidence to this hypothesis.

The question still remains why such active absorpton does not occur in other epithelia of the stratified squamus type. The paranuclear vacuoles, as indication of such active intra-epithelial transport, does not appear in the oesophageal epithelium. This suggests that such transporting mechanism is actually lacking in the oesophageal epithelium. The present authors are inclined to believe that this is due to the structural difference of stratum corneum and of the intercellular space or canal in stratum germinativum. It is now well-known that the absorptive epithelia are equipped with developed cyto-membrane systems

and that the intercellular space of the stratified squamous epithelium has extensive microvilli (8). In his preliminary report, Mikami (9) observed a desmosome-like structure in the intercellular spaces of the rumen epithelium of the cow. However, his work appears to fail in providing any definite evidence for the extensive development of such sealing structure in the rumen epithelium which may resist the absorption of water through the epithelium.

The present authors hope that further studies on the ultrastructure of the rumen epithelium in reference to its absorptive function, unusal to the stratified squamous type, may clarify the question why the rapid transport of water could take place through the epithelium. The authors are also inclined to believe that the paranuclear vacuoles are not only something more than a simple artefact, but also the best indication of the functional property of the rumen mucosa, i.e., intra-ruminal absorption.

Summary

Rumen tissues from one adult sheep and two goats were incubated, in vitro, in saline (0.85 % NaCl aq. soln.) or distilled water for as long as four hours. The tissue samples were collected at 0, 30, 60, 120, 150, and 240 minutes of incubation. They were fixed in Helly's fluid and were examined histologically. The results are as follows:

1. An occurence of the paranuclear vacuoles, recently reported by Henriksson and Habel, was prominent in the preparations of the rumen tissues incubated in distilled water. The vacuoles were few in the tissues incubated in saline water for as long as four hours.

2. The paranuclear vacuoles were already numerous at 30 minutes of incubation in distilled water, and their number did not increase during the incubation up to two and a half hours. This suggests that they probably appear at a time of incubation much earlier than 30 minutes.

3. The vacuoles increased in number towards the lower loci of the rumen epithelium, i.e., stratum basale. They were relatively few in the upper stratum spinosum.

4. Oesophageal mucosa taken from the goats were treated in the same manner as done for the rumen tissues. The paranuclear vacuoles were scarcely seen in the sections of this mucosa, and the heavier type of the vacuoles (Phase III) was never seen.

5. Prominent swellings of the capillaries in the secondary dermal papillae of the rumen were noted after the shortest time of incubation, either in distilled water or in saline water.

6. The above data indicate that the paranuclear vacuoles in the rumen epithelium probably are produced by the stimuli of water passing through the mucosa.

7. An existence of the extracellular, via-intercellular-space pathway of water as well as other substances such as the volatile fatty acids in the rumen epithelium was discussed in relation to the absorptive characteristics of the epithelium.

Acknowledgement

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Plate I

Explanation of Figures

All photomicrographs were taken with the sections of the goat rumen tissues stained by hematoxylin-eosin. The tissues are incubated in distilled water in vitro, for two hours (Figs. 1-4, 7) or for 30 minutes (Figs. 5-6).

Fig. 1. Paranuclear vacuoles(p) are abundant in the lower locus of the epithelium, stratum basale. ×1000.

Fig. 2. Paranuclear vacuoles are few in the upper locus of the epithelium; the cells containing a few keratohyalin granules show no paranucear vacuoles. ×1000.

Fig. 3. Phase I vacuole in upper stratum spinosum. ×2000.

Fig. 4. Phase II vacuoles are seen in stratum basale. The cells contain inclusions which are probably cytoplasmic debris. The nuclei are pycnotic. ×1600.

Fig. 5-7. Phase III vacuoles in stratum basale. A series of the compression of nuclei by the enlarging vacuoles are shown. ×2000 (Figs. 5-6); ×1600 (Fig. 7).

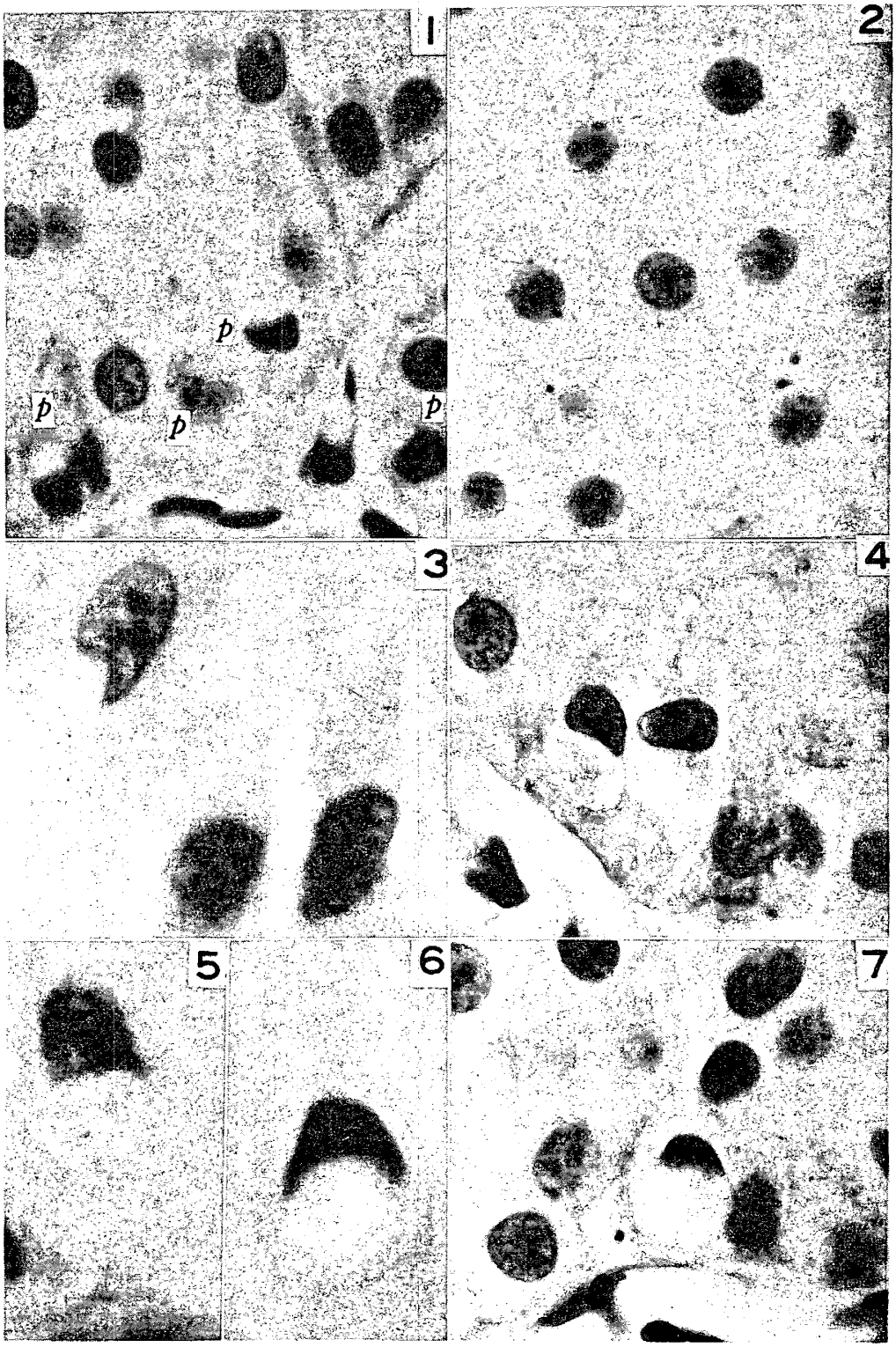
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Plate II

Explanation of Figures

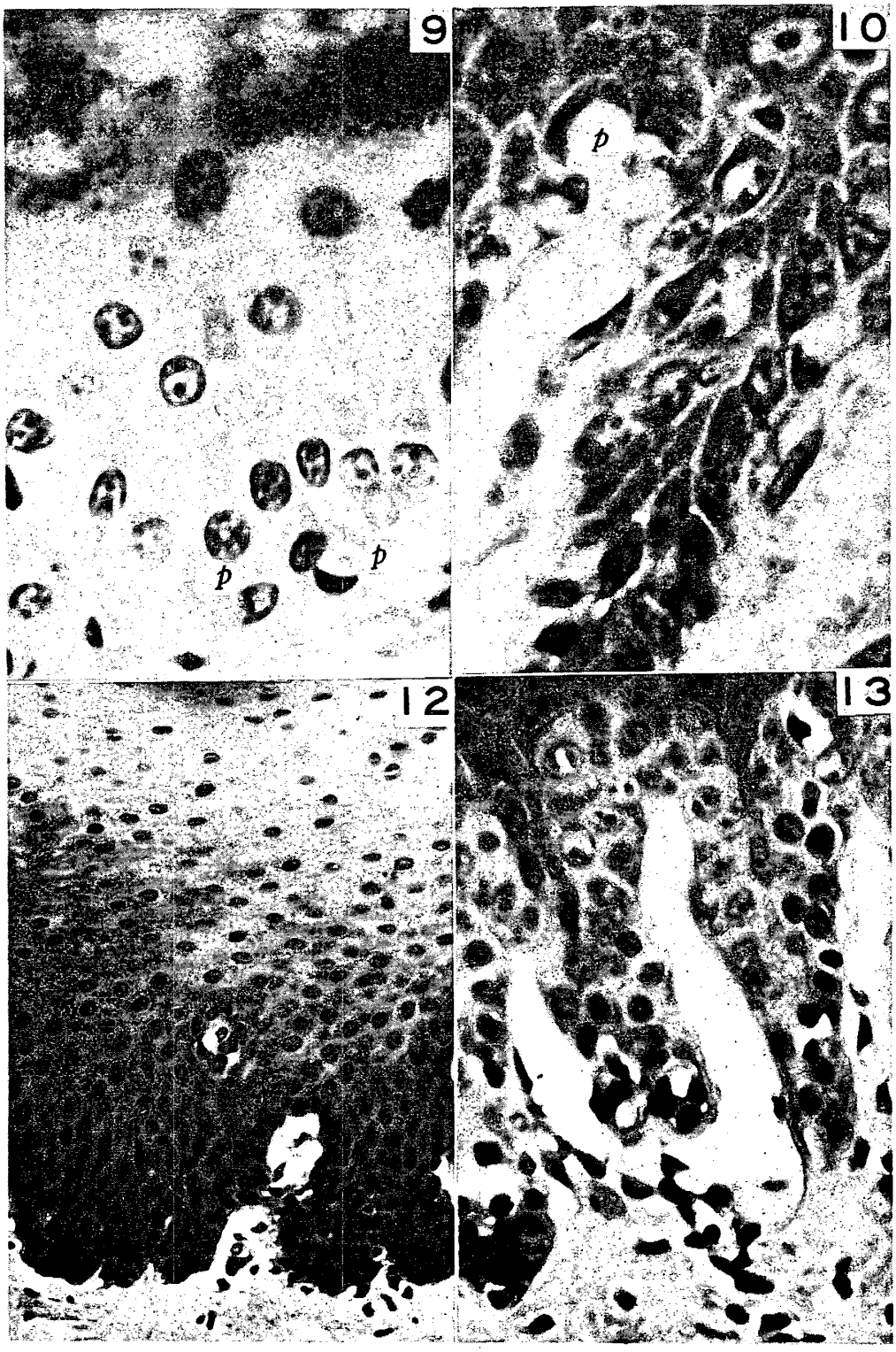
All photomicrographs were taken with the sections of the goat tissues stained by hematoxylin-eosin. The tissues are the rumen mucosa except in Fig. 12.

Fig. 9. Saline water incubation for 30 minutes. Only a few paranuclear vacuoles (p) are seen in the lower strata of the epithelium. ×1000.

Fig. 10. Saline water incubation for four hours. The paranuclear vacuoles (p) do not increase in number. The dilation of the capillaries in the second dermal papillae is less prominent than in the distilled water treatment (Fig. 13). ×1000.

Fig. 12. The oesophageal mucosa incubated in distilled water for four hours. The paranuclear vacuoles are absent. ×250.

Fig. 13. Distilled water incubation for four hours. The dilation of the capillaries in the secondary dermal papillae is noted. ×640.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