HISTOCHEMICAL STUDIES ON THE FEED TISSUES IN THE RUMINAL FLUIDS (REPORT II), ESPECIALLY ON THE ALTERATION OF THE CORN-SEED PUT IN THE ARTIFICIAL RUMINAL FISTULA OF THE GOAT

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By

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Introduction

Our previous report (1) described that the onset of autolytic phenomena with necrobiosis, coagulation and necrosis of the cell walls, degeneration of the plastids such as lipophanerosis, decrease or disappearance of protein and starch, nuclear changes of DNA-decomposition or -disappearance, and the multiplication of the microorganisms in the intercellular spaces and within the cells of the clover-leaves used as feed in the rumen, from the side of histochemistry.

Histochemical studies on the feed tissues in the ruminal fluids has been done on the digestive alterations of the leaves, corn grain, barley, potato, turnip, pea, corn silage and dry fish put in the artificial ruminal fistula of the goat. Through these results, the morphological changes of the digestible feed was established from histochemistry and pathology. No one has investigated the alteration of the digestible feed in the ruminal fluid from the histochemical view. These investigative methods might be effective for the ruminological studies, and it seemed to be important to clarify the mechanism of digestion of the feed tissues in the rumen. To the effect that the lesions of autolysis and fermentation occurred in the feed under different medium conditions such as pH effect, salt concentration effect, water effect, ionic effect, heat effect and molecular effect, it might be important to observe the relationship between the damages of the cytoplasms and nuclei in the feed tissues and the multiplication of the microorganisms in the feed-fermentation.

In the present study it was planned to examine the histochemical changes of the maize or corn-seed, put in the artificial fistula, as a kind of the principal

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components in the concentrates. Other feed, grasses and silage as the roughages; and barley, potato and fishes as the concentrates, will be reported on another occasion.

Materials and Methods for Studies

Corn grains bought from the market were cut slightly on the external coat to the interior by the safe blade, and immediately put in silk-bag-nets, and then kept in the artificial ruminal fistula of the goat in the Animal Physiological Laboratory. As the goat had been given various feeds, the ruminal fluids might contain fragments of the various feeds. The materials for the studies in the silk-bag-net, were pulled up in their original form, and the ones broken beyond recognition were removed. At the time of 0, 4, 8, 12, 16, 20, 24 and 36 hours after administration of the materials, these corn grains were gathered from the silk-bag-net, and fixed with CARNOY's solution for DNA, RNA, starch, cellulose, pectin, cutin and protein, and with buffered formol for lipids and fat. The corn-grains fixed in CARNOY's fluid were embedded in paraffine and then cut with the microtome. The corn-grains fixed in formol, were cut with the frozen-microtome for fat-staining. The stains employed were hematoxylin-eosin and Azan stain for general histological views, PAS-hematoxylin stain with or without saliva-digestion for starch and other polysaccharides, acrolein-SCHIFF reaction for protein, pyronine-methyl green stain with or without ribonuclease digestion for RNA, depolymerized DNA and polymerized DNA, and FEULGEN's reaction counterstained with light green for DNA, and carbol-thionine staining for bacteria.

Results

1. Histopathological changes of the corn grains in the ruminal fluids.

Two structural types of fruit walls are recognized, the parenchymatous freshy, often succulent fruit walls and the sclerenchymatous dry fruit wall. The latter may be dry dehiscent fruits, if the fruit wall splits open at maturity, or dry indehiscent, if the fruit wall remains closed. when the ovary contains a single ovule, it usually develops into an indehiscent fruit. The pericarp of many indehiscent one-seeded fruits resembles a seed-coat in structure. If the pericarp and seed coat are adherent in the fruit, the fruit is a grain or caryopses. The caryopses of the Gramineae show certain conspicuous differences in the development of their fruit coats. The ovary wall of wheat consists of the following cell layers, such as the outer epidermis (one cell layer), colorless parenchyma cells (many layers), grooved region (several layers), and small cells of the inner epidermis (one layer). The nucellus also is present and consists of several layers of thin-walled cells bounded by a distinct nucellar epidermis. The nucellar tissue, with the exception of the epidermis, is absorbed by the enlarging endosperm and embryo. The inner layer of the inner integument also becomes compressed.

The outer layer of this integument is crushed into a hyaline membrane covered with cuticula. The outer integument disintegrates. On the contrary, in the grain of Zea the integuments disintegrate completely, but the nucellar epidermis is retained as a thick-walled layer showing a fatty reaction and covered with a cuticle according to RANDOLPH (2) (1936). Adjacent to the grain coats lies the proteinaceous endosperm layer, the aleuron layer, which in turn encloses the starchy endosperms.

The corn-grains were put into the ruminal fluids through the artificial fistula. As the living corn-grains were immersed in the gastric juice, there were found the onset of the autolytic phenomena with necrobiosis, foamy degeneration, discharges of the cytoplasms such as plasmoptysis, coagulation and necrosis of the cytoplasms, degeneration of the plastids like lipophanerosis and hydrolysis, nuclear changes, vacuolization of the nuclei and cytoplasms, immersion with the gastric juice, and the invasion of microorganisms into the intercellular spaces and cell bodies. Postmortem self-digestion is the decomposition of the cells, tissues and organs of the dead body. At first these processes played in the tissue a kind of necrosis caused by the immersion within the gastric mucous juice, and the postmortem digestion occurs in the form of cellular decomposition of the dead body. This decomposition begins just after death, and then it developes to fermentation by the participation of the microorganisms.

Patho-histological changes with the progress of time are indicated in Table 1. The alterations of the damaged cut-surface in the artificial wound are stronger than those of the non-damaged cut-surface of the corn-grain from the cortex to the interior. The pathological findings consist of the following alterations: swelling, desquamation, erosion and isolation of the cuticular layer; swelling of the crushed nucellar cells; swelling, deformation, isolation and cavity formation of the aleuron layer and starchy layer: swelling of the epithelium in the scutellum: and swelling and vacuolization of the parenchyma in the scutellum. Furthermore in the damaged cut-surface there were observed the following changes: invasion of gastric mucin to the aleuron layer and starchy layer, destruction and disolution of the aleuron grain and starch grain, disociation, desquamation, and disolution of the epithelium and parenchyma in the scutellum.

It is interesting to find the nuclear hydrops of the aleuron layer, swelling of the aleuron- and starchy endosperm, and seed-coat, cytoplasmic vacuolization of the parenchyma of the scutellum, isolation and disolution of starchy layer parenchyma, hematoxylin-stained zone over the cut-surface of the starchy layer and scutellum-parenchyma with the process of invasion of the gastric juice. These alterations are indicated in Fig. 1.

(a) At four to eight hours after putting the corn-grain into the fistula, the cuticular layer of both non-damaged and damaged cut-surface indicates slight swelling, desquamation and mucous invasion, and at 12 to 24 hours shows a

Table 1. Histopathological changes of corn-grains in the ruminal fluids.

	Structure		Periods	Progress of time after putting corn-grains into the fistula				
				0 hr.	4～8 hrs.	12～16 hrs.	20～24 hrs.	36 hrs.
Non-damaged cut-surface from cortex to interiors	Seed-coat	Cuticular layer		—	Swelling desquamation invasion of mucin	Swelling, desquamation, net-work formation	Swelling, desquamation net-work formation	Swelling, desquamation, erosion
		Crushed nucellar cells		—	Swelling	Swelling	Swelling	Swelling
	Aleuron layer			—	Swelling	Swelling	Remarkable swelling	Remarkable swelling
	Starchy layer			—	—	Deformation, Swelling	Swelling, isolation, cavity formation	Swelling cavity formation
	Scutellum	Epithelium		—	—	Swelling	Swelling	Swelling
		Parenchyma		—	Slight vacuolization	Swelling, vacuolization	Swelling, remarkable vacuolization	Swelling, remarkable vacuolization
Damaged cut-surface in artificial wounds :	Seed-coat	Cuticular layer		—	Swelling, mucin invasion	Swelling, desquamation, mucin invasion	Swelling, desquamation, mucin invasion	Swelling, desquamation, erosion
		Crushed nucellar cells		—	Swelling	Swelling	Swelling	Swelling
	Aleuron layer			—	Swelling, invasion of gastric mucin, nuclear hydrops	Swelling, invasion of gastric mucin, nuclear hydrops	Swelling, invasion of gastric mucin, nuclear hydrops	Swelling, destruction, invasion of gastric mucin
	Starchy layer			—	HX-zone formation of mucous invasion, swelling, desquamation	HX-zone formation of mucous invasion, decomposition of starch swelling of starch grain, hydrops, depolymerization	Isolation destruction solution of starch grain, HX-zone formation of mucin	Isolation, destruction, solution of starchy grain, HX-zone formation of mucin, cavity formation
	Scutellum	Epithelium		—	Dissociation, vacuolization desquamation	Nuclear shrinkage, vacuolization dissociation	Dissociation, isolation remarkable vacuolization destruction	Isolation, destruction, disolution. HX-zone formation of mucin, cavity formation
		Parenchyma						

Remarks: HX is the abreviation of hematoxylin-stainability.

net-work appearance, and at 36 hours indicates erosion owing to the softening and invasion by gastric fluids and microorganisms.

(b) At four to 36 hours after putting the corn-grain in the fistula the crushed nucellar cells of both non-damaged and damaged cut-surface indicate swelling owing to the mucous invasion.

(c) In the aleuron layers of non-damaged cut-surface, slight swelling after four to 16 hours and remarkable swelling after 20-36 hours are found. The aleuron layers of the damaged cut-surface indicate swelling, mucous invasion and nuclear hydrops during all periods, especially destruction at 36 hours by the invasion of the gastric juice.

(d) Starchy layers of non-damaged cut-surface indicate swelling and deformation after 12-36 hours, isolation and cavity formation after 20 to 36 hours. In the starchy layers of the damaged cut-surface, there are found mucous invasion around and in the cells at four to 36 hours, swelling of the starch grains at four to 16 hours, isolation of the starch grains at four to eight hours, decomposition and disolution of the starch grains at 12 to 36 hours, cytoplasmic vacuolization from 12 to 36 hours, and cavity-formation in the layers at 36 hours.

(e) Both the epithelium and parenchyma in the scutellum of the non-damaged cut-surface indicate swelling from 12 to 36 hours after putting the grains into the rumen. On the contrary, on the scutellum-epithelium of the damaged cut-surface there are indicated dissociation, isolation, cytoplasmic vacuolization, from four to 24 hours and gastric mucin-invasion around and in the cells at 36 hours.

BRINK (3) described that the protoplasm can also break up into discrete pieces within the cell by acids, ammonia and temperature variations. Severe disturbance of either layer produced the damaged hyaloplasm fusing (PFEFFER). Accordingly the alterations called swelling may contain the abnormal lamination, damaged lamination and fusing of the cell membrane.

2. Histochemical changes of polysaccharides and fats in the corn grains put into the ruminal fistula.

The grain of Zea contains an indehiscent fruit wall covered with a cuticle. The corn grains shows the structures such as the cuticular layer and crushed nucellar cells in the seed-coat. The aleuron layer and starchy layer in the endosperm; and the epithelium and parenchyma in the scutellum. Accordingly the cuticular substances, pectic substances, cellulose, starch, protein and fats are located on the seed-coat, endosperm and scutellum.

From the point of histochemistry, these substances of the corn grains in the artificial ruminal fistula are shown in Table 2 and 3.

According to ESAU (4) (1953), the fatty compounds, cutin, suberin, and waxes, occur in varying amount in the walls of many types of cells; the most important fatty substances are cutin, suberin, and wax. PRIESTLEY (5) (1943) described that waxes melt readily and are easily extracted by fat solvents, whereas cutin

Table 2. Histochemical changes of polysaccharides and fats in corn-grains put into the ruminal fluids.

	Substances structure		Period	Progress of time after putting corn-grains into fistula				
				0 hr.	4～8 hr.	12～16 hrs.	20～24 hrs.	36 hrs.
Non-damaged cut-surface from cortex to interiors	Seed-coat	Cuticular layer	Cutin	++++	++++	++++	++++	++++
			Fat	−	−	−	−	−
			Starch	−	−	−	−	−
		Crushed nucellar cells	Cutin	+++	+++	+++	+++	+++
			Fat	++	++	+～−	+～−	+～−
			Starch	−	−	−	−	−
	Aleuron layer		Pectin	++	++	+	+	+
			Fat	+++	+(−)	−(+)	−(+)	−(+)
			Starch	−	−	−	−	−
	Starchy layer		Pectin	+	+～+$_s$	+～++(+Bac)	+～++(+Bac.)	+++(+Bac.)
			Fat	−	−	−	−	−
			Starch	++	++～+	+～++	+++(+Bac.)	+++(+Bac.)
	Scutellum	Epi-thelium	Pectin	++	++～+	+	+	+
			Fat	−	−	−	−	−
			Starch	−	−	−	−	−
		Paren-chyma	Pectin	+	+	+	+	+
			Fat	++++	++	++	+	+
			Starch	−	−	−	−	−
Damaged cut-surface in artificial wounds	Seed-coat	Cuticular layer	Cutin	++++	+++	+++	+++(+～−)	+++(+～−)
			Fat	−	−	−	−	−
			Starch	−	−	−	−	−
		Crushed nucellar cells	Cutin	++++	+++	+++	+++(+～−)	+++(+～−)
			Fat	++	++	+～−	+～−	+～−
			Starch	−	−	−	−	−
	Aleuron layer		Pectin	++	+	+	+	−
			Fat	+++	−	−	−	−
			Starch	−	−	−	−	−
	Starchy layer		Pectin	+	+(+Bac.++)	+(+Bac.+++)	+(+Bac.+++)	+(+Bac.+++).
			Fat	−	−	−	+	+
			Starch	++	+(+Bac.+)	+(+Bac.+++)	−(+Bac.++++)	−(+Bac.++++).
	Scutellum	Epi-thelium	Pectin	++	+	+	+(+Bac.+++)	+(+Bac.+++).
			Fat	−	−(+)	−	−	−
			Starch	−	−	−	−(+Bac.+++)	−(+Bac.+++).
		Paren-chyma	Pectin	+	+	+	+(Bac.+++)	+(+Bac.+++).
			Fat	++++	−(+)	−	−	−
			Starch	−	−	−	−(+Bac.+++)	−(+Bac.+++).

Remarks: Bac. indicates the strong intensity of stainability by the addition of bacterial multiplication.

Table 3. Histochemical changes of nucleic Acids and protein in the corns put into the ruminal fluids.

Structure \ Period				Progress of time after putting corn-grains into fistula				
				0 hr.	4～8 hrs.	12～16 hrs.	20～24 hrs.	36 hrs.
Non-damaged cut-surface from cortex to interiors	Seed-coat	Cuticular layer	Nuc.	−	−	−	−	−
			Cyt.	−	−(Protein ＋, GJ)	−(Protein ＋, GJ)	−(Protein ＋, GJ)	−(Protein ＋, GJ)
		Crushed nucellar cells	Nuc.	−	−	−		
			Cyt.	−	−(Protein ＋, GJ)	−(Protein ＋, GJ)	−(Protein ＋, GJ)	−(Protein ＋, GJ)
	Aleuron layer		Nuc.	DNA＋＋, Prot.＋＋	DNA＋＋, Prot.＋＋	DNA＋(Dep.),Prot.＋	DNA＋(Dep.),Prot.＋	DNA＋(Dep.),Prot.＋
			Cyt.	RNA＋＋, Prot.＋＋	RNA＋＋, Prot.＋＋	RNA＋, Prot.＋＋	RNA−, Prot.＋＋	RNA−, Prot.＋＋
	Starchy layer		Nuc.	DNA＋＋, Prot.＋＋	DNA＋＋, Prot.＋$_s$	DNA＋(Dep),Prot.＋$_s$	DNA＋(Dep.),Prot.−$_s$	DNA＋(Dep.),Prot.−$_s$
			Cyt.	RNA−, Prot.＋	RNA−, Prot.＋＋(GJ)	RNA−, Prot.＋＋(GJ)	RNA−, Prot.＋＋(GJ)	RNA−, Prot.＋＋(GJ)
	Scutellum	Epithelium	Nuc.	DNA＋＋, Prot.＋＋	DNA＋＋, Prot.＋＋	DNA＋＋, Prot ＋＋	DNA＝, Prot.＋	DNA＋, Prot.＋
			Cyt.	RNA＋＋, Prot.＋＋	RNA＋, Prot.＋＋	RNA＋, Prot.＋＋	RNA−, Prot.＋＋	RNA−, Prot.＋＋
		Parenchyma	Nuc.	DNA＋＋, Prot.＋＋	Prot.＋＋	Prot.＋＋(V)	Prot.＋(V)	Prot.＋(V)
			Cyt.	RNA＋＋, Prot.＋＋	RNA＋＋, Prot.＋＋	RNA＋, Prot.＋＋	RNA−, Prot.＋	RNA−, Prot.＋
Damaged cut-surface by artificial wounds	Seed-coot	Cuticular layer	Nuc.	−	−	−	−	−
			Cyt.	−	−(Prot. ＋＋, GJ)	−(Prot. ＋＋, GJ)	−(Prot.＋＋, GJ)	−(Prot.＋＋, GJ)
		Crushed nucellar cells	Nuc.	−	−	−	−	−
			Cyt.	−	Prot. ＋＋	Prot. ＋＋	Prot. ＋＋	Prot. ＋＋
	Aleuron layer		Nuc.	DNA＋＋, Prot.＋＋	DNA −	DNA −	DNA −	DNA −
			Cyt.	RNA＋＋, Prot.＋＋	RNA−, Prot.＋	RNA−, Prot.＋(V)	RNA−, Prot.＋(V)	RNA−, Prot.−(V)
	Starchy layer		Nuc.	DNA＋＋, Prot.＋＋	DNA− ＋(V)Prot.−	DNA−＋ Prot.−	DNA− Prot.−	DNA− Prot.−
			Cyt.	RNA−, Prot.＋	RNA−, Prot＋(GJ＋＋)	RNA−,Prot.＋(GJ＋＋)	RNA−,Prot.−(GJ＋＋)	RNA−,Prot.−(GJ＋＋)
	Scutellum	Epithelium	Nuc.	DNA＋＋, Prot.＋＋	DNA＋$_s$, Prot.＋$_s$	DNA＋$_s$, Prot.＋$_s$	DNA−, Prot.−	DNA−, Prot.−
			Cyt.	RNA＋＋, Prot.＋＋	RNA−, Prot.＋＋−	RNA−, Prot.−	RNA−,Prot.−(GJ＋＋)	RNA−, Prot−(GJ＋＋)
		Parenchyma	Nuc.	DNA＋＋, Prot.＋＋	RNA＋$_s$, Prot.＋$_s$	DNA＋$_s$, Prot.＋$_s$	DNA−, Prot.−	DNA−, Prot.−
			Cyt.	RNA＋＋, Prot.＋＋	RNA−, Prot.＋＋−	RNA−, Prot.−	RNA−,Prot.(GJ＋＋)	RNA−,Prot.−(GJ＋＋)

Remarks: GJ is the abrebiation of the gastric juice, Nuc. nucleus, Cyt. cytoplasm, and Prot. protein.

and suberin are not meltable and show considerable insolubility in fat solvents. Suberin and cutin and closely related, highly polymerized compounds consisted of fatty acids (FREY WYSSLING, (6), 1935). Cutin also occurs with the cellulose in the outer walls of the epidermis, and forms a continuous layer as the cuticle on the surface of the epidermis of all aerial parts. Pectic substances are derivatives of polygalacturonic acid and occur in three types, such as protopectin, pection and pectic acid (BONNER (7), 1936-1950). Pectic compounds constitute the intercellular substance with cellulose. The leucoplasts called amyloplasts are specialized as starch-stored bodies. A well-known amorphous ergastic protein is glutein, which is combined with starch in the endosperm of wheat. Crystalloids are often combined with amorphous protein in the aleuron grains which are ergastic bodies found in the endosperm (SHARP (8), 1934).

With the progress of time after putting the corn-grains in the ruminal fistula, these substances appear or disappear within the cuticle, and nucellar cells; the cell membrane, the cytoplasm, the starchy and aleuron endosperm, the epithelium and parenchyma in the scutellum by means of gastric digestion and self-digestion as shown in Table 2 and 3.

(a) Cuticular substances in the seed-coat.

Cuticular substances in the seed-coat consisted of non-fatty substance in the cuticular layer and fatty substance in the nucellar cells of the inner layer, but they reacted on the PAS reaction for polysaccharides. Owing to the results, there were found only polysaccharide in the cuticular layer, and polysaccharide and fat in the nucellar cell layer. According to PRIESTLEY (1943), waxes melt readily and are easily extracted by fat solvents, but cutin and suberin are not meltable and show considerable insolubility in fat solvents. It seemed to be cutin and suberin which were detected by the reagent Sudan III in the nucellar cells, but it is not possible to determine the existence of waxes in the cuticular layer. According to AMBRONN (4) (1888) and MEYER (9) (1938) wax is shown by the melting experiments at 100°C.

In the non-damaged cut-surface the cuticular substances in the seed-coat were not altered at four to 36 hours after putting the grains into the ruminal fistula, but in the damaged cut-surface the cuticular substances in some places of the seed-coat were disappeared at 20 to 36 hours on the erosion of the cuticle. Stainability of the PAS-staining and Sudan-staining decreased in these lesions.

(b) Pectinic substances in the cell membranes of the soft cells in the endosperms and scutellum.

According to MÜHLETHALER (10) (1961), pectinic substances are generally localized in the cell wall, and the elementary structural unit of this polymer has been to be galacturonic acid, glucuronic acid and arabinose. Cellulose is the most important constituent of the plant cell wall, forms the structural framework within which other wall substances, such as pectin, lignin, hemicellulose, etc., are

embedded. Accordingly, the cell membranes of the soft cells in the endosperms and scutellums seemed to be of pectin and cellulose.

In the non-damaged cut-surface of the corn-grains, cellulose and pectin in the cell membranes of the various soft cells did not disappear or decrease at four to 36 hours after putting the corn-grains into the ruminal fistura, however these cell walls were swollen in membranous appearance from the beginning. In the damaged cut-surface of the artificial wounds upon the corn-grains, pectin and cellulose in the cell membranes of the various soft cells became enlarged and swollen, and increase the intensity of stainability of PAS owing to the bacterial multiplication. It is easy to distinguish the difference between pectinic substances and bacterial multiplication because of the homogenous or granular appearances in PAS-staining reaction.

(c) Fat in the soft cells in the endosperms and scutellum.

The cytoplasms in the soft cells of the aleuron layer and scutellum were stained with Sudan III or Sudan Black B stains. These fats gradually decreased and disappeared from four to 36 hours after putting the grains into the ruminal fistula in both non-damaged and damaged cut-surface. It is of intetest to find the lipophanerosis of the soft cells of the starchy layer in the damaged cut-surface at 20 to 36 hours after putting the grains into the rumen. ITIKAWA *et al.* (1) (1964) described that there were found alterations such as the decomposition of the plastids with the decrease of starch grains and protein, and the increase of lipoids in the clover-leaves at four to eight hours after putting in the ruminal fistula. These increases of lipoids were called lipophanerosis.

(d) Starch in the starchy layer.

According to GRANICK (11) (1961), starch is formed in the stroma of the plastids, and two kinds of starch molecules are generally present as follows: amylose as the minor component and amylopectin as the major component. The former stains with iodine in a blue color and the latter stains red with iodine. Although starch in the starchy layer was not too much in amount, from the results of Table 2, starch decreased gradually and disappeared at four to 36 hours after putting it into the fistula.

3. Histochemical changes of nucleic acids and proteins of the corn-grains put into the ruminal fluids.

(a) Nucleic acids in the endosperms and scutellum.

Duget (12) (1961) described some problems of the structure and biochemistry of the vegetative plants. He wrote on the existence of RNA and DNA in the nucleus and that of RNA in the cytoplasms by the cellular fractionation. Proplastids in the meristematic leaf cells contained both RNA and DNA localized in the primary granum ; in addition some RNA is detectable in the stroma (SPERKERMANN (13), 1957). In mature chloroplasts the evidence for the presence of RNA was perhaps positive, but it is negative for DNA according to the studies

of LITTAU (14) (1958) on four species of monocotyledons(GRANICK, 1961). METZNER (15) (1952) could detect on DNA in Agapanthus with the FEULGEN reaction, but a methyl green-pyronine stain appeared to indicate that DNA is present in the grana. CHIBA (16) (1951) reported a positive FEULGEN reaction for DNA in chloroplasts of *Selaginella, Tradescantia and Rheo.* Both METZNER and CHIBA reported it in the grana.

From the results of Table 3, there were found RNA in the plastids of the soft cells of the association tissues and in the soft cells of the scutellum, but RNA in the aleuron layer and scutellum disappeared on and after the time of 12–16 hours in the non-damaged cut-surface and at four to eight hours in the damaged cut-surface. DNA was localized in the cells of the endosperms and scutellums. These DNA decreased or disappeared on and after the time of 20 to 24 hours in the non-damaged cut-surface, and at four to eight hours in the damaged cut-surface.

(b) Protein in the endosperms and scutellum.

GRANICK (11) described that the mature chloroplasts of the tomato and tomato leaves (GRANICK 1938), sudan-grass (HANSON, 1941) and out leaves (GALSTON, 1943) make up 35–45 percent of the total protein-N of the cell; and 35–55 percent of dry weight of the chloroplasts is protein. And according to his description about 80 percent of the total protein-N of the chloroplast is an insoluble form, probably as lipoproteins and structually built in enzymes; and a breakdown of the chloroplast structure is readily observed in the older leaves when bean plants are placed in the dark for several days.

From the results of Table 3, the protein in the endosperms and scutellum disappeared on and after the time of 20 to 24 hours in the non-damaged cut-surface and at 12 to 16 hours in the damaged cut-surface, but the protein in some endosperm and scutellum of the damaged cut-surface decreased from four to eight hours.

4. Histochemical alterations of the corn-grains in the rumen by the staining for the demonstration of tissue composition applied on the relationship between the dyes-diversion and tissue-condensity.

The alterations of the corn-grains in the rumen were stained with Azan trichrome stains which consisted of azocarmine, aniline blue and gold orange G. According to SEKI (17) if the dyes are arranged from the micromolecule, to macromolecule, there will be found the following orders: azocarmine (molecular weight 433, 921) (reddish coloring) <orange G (M. W. 452, 370) (orange coloring)<aniline blue (M. W. 732, 718 (bluish coloring). He assumed the size of the dye molecule from the point of dispersity of dyes following STOKES-EINSTEIN's law and electron-microscopic measurement of the dye-molecule. And also he divided them into two groups as minute or higher dispersity, and rough or lower dispersity of the dyes. Generally the macromolecular dyes with lower dispersity could easily

Table 4. Transition of stainability of azan-and hematoxylin-eosin-staining on the corn in the ruminal fluids.

Structure \ Period			Progress of time after putting corns in the fistula				
			0 hr.	4~8 hrs.	12~16 hrs.	20~24 hrs.	36 hrs.
Non-damaged cut-surface from cortex to interiors	Seed-coat	Cuticular layer	Eosin, blue,	Eosin, blue, green	Eosin, blue→violet	Eosin, dark blue	Eosin, light orange, yellow green
		Crushed nucellar cells	Eosin, blue	Blue, green, orange	Blue, red→violet	Violet	Eosin, light orange, yellow green
	Aleuron layer		(M) eosin, blue, HX. (CY) orange, red	(M) eosin, blue, green, HX. (CY) orange, red, blue yellow	(M) eosin, HX, blue, green, red (CY) red	(M) eosin, HX, blue, green (CY) dark red	(M) eosin, HX, blue, green (CY) dark red, blue
	Starchy layer		(M) eosin, red (CY) eosin, yellow	(M) eosin-red, blue (CY) eosin, yellow, blue	(M) eosin, blue→violet, (CY) yellow, red	(M) eosin, blue→yellow (CY) weak eosin, yellow, blue	(M) eosin, light blue (CY) weak eosin, HX, light yellow
	Scutellum	Epithelium	(M) blue, yellow (CY) eosin, HX, red	(M) blue (CY) eosin, HX, red	(M) light blue (CY) eosin, HX, light red	(M) light blue (CY) eosin, HX, light red	(M) light blue (CY) weak eosin, HX
		Parenchyma	(M) blue (CY) eosin, HX, red	(M) blue (CY) eosin, red, HX, blue	(M) light blue (CY) eosin, HX, red, blue	(M) dark violet (CY) eosin, HX, dark red, dark blue	(M) dark violet (CY) eosin, HX, dark red, light blue
Damaged cut-surface by artificial wounds	Seed-coat	Cuticular layer		Redish orange	Violet	Violet	Light orange
		Crushed nucellar cells					
	Aleuron layer			Strong eosin, orange	Weak eosin, orange, red	Loss of eosin-stainability, light violet	Loss of eosin-stainability, light blue
	Starchy layer			(M) weak eosin, (CY) blue centrum, yellow granules	(M) weak eosin (CY) blue center, red-violet granules	(M) loss of eosin-stainability (CY) blue-center, pink, violet granules	(M) loss of eosin-stainability (CY) loss of stainability
	Scutellum	Epithelium		(M) HX, weak eosin (CY) blue	(M) HX(weak), weak eosin (CY) blue	(M) weak eosin, weak HX (CY) light blue	(M) weak eosin, weak HX or loss of stainability (CY) light blue
		Parenchyma		(M) HX, weak eosin (CY) blue	(M) weak HX, weak eosin (CY) blue	(M) weak eosin, weak HX. (CY) light blue	(M) weak eosin, weak HX or loss of stainability (CY) light blue

Remarks: M is the abrebiation of the cell membrane, CY., cytoplasm, and HX. hematoxylin-stainability.

absorb the tissue substances, and also the micromolecular dyes with higher dispersity stained the same as the macromolecular dyes. But to use these dyes as the mixture, the loose structure contained wide holes stained with the macromolecular dyes such as aniline blue, on the contrary the fine structure contained narrow holes stained with the micromolecular dyes such as orange G.

(a) Stainability of the seed-coat.

The seed-coat stained with Azan stains showed variable stainability of blue, green, orange, violet, dark blue to light orange coloring. This might be considered to be the transformation of the loose structure to the fine structure by the mucous invasion from the reason of stainable changes of blue to light orange coloring. (see Table 4)

(b) Stainability of the aleuron layer.

The stainability of the aleuron layer in the non-damaged cut-surface indicates the changes from orange, red to blue coloring. Accordingly this might show the transformation of the fine structure to the rough structure owing to the decrease of RNA and nuclear protein. On the contrary, the stainability of the aleuron layer in the damaged cut-surface indicates the changes from orange, red to light blue coloring. This might be suspected to show the transformation of the fine structure to the rough structure owing to the decomposition of the aleuron grains and the disappearance of RNA, DNA and protein.

(c) Stainability of the starchy layer.

The stainability of the starchy layer in the non-damaged cut-surface indicates the changes from yellow to blue or light yellow. Accordingly this might show the transformation of the fine structure to the rough structure owing to the degeneration and decomposition of starch grains, decrease of DNA and nuclear protein. In some cells at 12 to 36 hours after putting into the fistula, there was found a falcy increase of protein, so the stainability indicates the changes to hematoxyline coloring by the gastric invasion. On the contrary the stainability of the starch layer in the damaged cut-surface indicates the change from yellow to the loss of stainability. Those might show the disolution of starchy grain by the gastric mucin invasion.

(d) Stainability of the scutellum.

The stainability of the epithelium and parenchyma of the scutellum indicates the changes from red to dark red, light blue or blue coloring in both non-damaged and damaged cut-surface. Accordingly, this might be taken as the transformation of the fine structure to the rough structure in addition to the mucous invasion.

5. Modus of the invasion of bacteria and fungus into the corn-grains in the ruminal fluids.

It is well-known that in the living body the breakdown of starch to sugars is a form of fermentation. In this meaning the alterations of modus of bacterial invasion into the clover-leaves in the ruminal fluids are of interest to the authors.

Table 5. Invasion modus of microorganisms into corn-grains in the ruminal fluids.

			Progress of time after putting corn-grains into fistula									
			Intercellular multiplication					Intracellular multiplication				
			0 hr.	4~8 hrs.	12~16 hrs.	20~24 hrs.	36 hrs.	0 hr.	4~8 hrs.	12~16 hrs.	20~24 hrs.	36 hrs.
Non-damaged cut-surface cortex to interiors	Seed-coat	Cuticular layer	–	Bac. (s)	Bac. (s)	Bac. (s)	Bac. (s)	–	–	–	–	–
		Crushed nucellar cells	–	–	–	–	–	–	–	–	–	–
	Aleuron layer		–	–	–	–	–	–	–	–	–	–
	Starchy layer		–	–	Bac.	Bac.	Bac.	–	–	–	Bac.	Bac.
	Scuthellum	Epithelium	–	–	–	–	–	–	–	–	–	–
		Parenchyma	–	–	Bac.	Bac.	Bac.	–	–	–	–	Bac.
Damaged cut-surface in artificial wounds	Seed-coat	Cuticular layer	–	Bac.	Bac.	Bac.	Bac. Protoz.	–	–	–	–	–
		Crushed nucellar cells	–	Bac.	Bac.	Bac.	Bac.	–	–	–	–	Bac.
	Aleuron layer		–	–	Bac. Fungus	Bac. Fungus	Bac. Fungus	–	–	–	–	Bac.
	Starchy layer		–	Bac. Fungus	Bac. Fungus	Bac. Fungus	Bac. Fungus	–	Bac. Fungus	Bac. Fungus	Bac. Fungus	Bac. Fungus
	Scutellum	Epithelium	–	Bac. Fungus	Bac. Fungus	Bac. Fungus	Bac. Fungus	–	–	–	Bac. Fungus	Bac. Fungus
		Parenchyma	–	Bac. Fungus	Bac. Fungus	Bac. Fungus	Bac. Fungus	–	–	–	Bac. Fungus	Bac. Fungus

In the previous report, there were found the modus of bacterial penetration into the clover-leaves by the following changes: 1) adhesion to the cell surface of the papillary process, 2) penetration into the cell membrane of the papillary process with the destruction of the cell membrane and protrusion of the cytoplasmic process, 3) proliferation of bacteria within the swollen intercellular spaces between the papillary process and collenchyma and parenchyma cells, 4) Bacteria around the parenchymatous cells, 5) penetration through the pits of the cell membranes, 6) nodule formation as if an abscess within the intercellular spaces and soft cells in the animal diseased tissuses, 7) bacterial multiplication within the cells with the destructed plastids, 8) bacillus at first, and then bacillus and coccus, and finally coccus, 9) bacterial colonies consisted of one kind of microorganisms, and mixed types such as bacillus and coccus.

The results in the present paper are shown in Table 5. The penetration of microorganismus into the corn-grains were shown to be the following:

a) adhesion: Bacterial attachment to the surface of the cuticular layer in the seed-coat appeared on and after the time of four to 36 hours in both the non-damaged surface and the damaged cut-surface.

b) penetration and proliferation within the enlarged cellular spaces between the cuticular layer and nucellar cells in the damaged cut-surface on and after the time of four to 36 hours.

c) aggregation of sporangiospore of fungi in the upper surface of the wound at the time of four to eight hours.

d) no microorganisms in the aleuron layer of the non-damaged surface for four to 36 hours.

e) Proliferation of the fungi in the damaged cut-surface of the starchy layer, invasion of vegetative hypha within the intercellular spaces, and the formation of the hyphal branches at the time of 12 to 36 hours.

f) Proliferation of bacterium and fungi in the damaged cut-surface of the scutellum.

g) No fungi in the aleuron layer, starchy layer, and scutellum under the non-damaged surface.

h) Intracellular bacterial multiplication in the starchy layer (for 20 to 36 hours), and parenchyma (for 36 hours) under the non-damaged surface. On the contrary the intracellular bacterial multiplication in the crushed nucellar cells (for 20 to 36 hours), aleuron layer (for 36 hours), starchy layer (for 4 to 36 hours), epithelium of scutellum (for 20 to 36 hours) and parenchyma of scutellum (for 20 to 36 hours) in the damaged cut-surface.

i) Intercellular invasion of fungi in the starchy layer (for 4 to 36 hours), epithelium of scutellum (at 20 to 36 hours) and parenchyma of scutellum (for 20 to 36 hours) in the damaged cut-surface.

Discussion and Summary

Histochemical studies on the feed tissues such as the corn-grains put in the artificial fistula of the goat has been investigated. In a meaning this investigation seemed to be a study on the decomposition of the plant tissues by the digestion of domestic animals. The present study described the histopathological postmortem alterarions; histochemical alterations such as cuticular substances in the seed-coat; pectic substances in the cell membranes of the aleuron- and starchy- layers, and scutellum; fats in the nucellar cells, aleuron layer, and scutellum; protein in the aleuron layer and scutellum; nuclei acids in various cells except in the seed-coat; and starch within the starchy endosperms: lipophanerosis in the decomposed starch grains; modus of bacterial invasion into the corn-grains; and biological mechanisms of the sporangiospore. vegetable hypha and hyphal branches in the cut-surface of the corn.

The results of the investigation summarized as the relationships between the histopathological, histochemical and microbiological alteration of the corn-grains in the ruminal fluids are shown in Table 6 and 7.

To summarize the results shown in Table 6 and 7 it seemed to be quite clear that the relations between the three alterations were associated with each other. The results of the investigation are summarized as follows:

1) Histopathologically following the progress of time after putting the corn-grains in the ruminal fistula revealed the following alterations:

In the corn grains after four to eight hours:

On the non-damaged cut-surface: swelling and desquamation of the seed-coat, mucous invasion to the seed-coat, swelling of the aleuron layers, slight vacuolization in the epithelium and parenchymatous soft cells of the scutellum. On the damaged cut surface: the same as the above-described in addition to the nuclear hydrops in the aleuron layer.

In the corn grains after 12 to 16 hours:

On the non-damaged cut-surface: swelling of the seed-coat, the aleuron layers and starchy layer, the epithelium and parenchyma of the scutellum; desquamation of the seed-coat; cytoplasmic vacuolization; mucous invasion to the seed-coat. On the damaged cut-surface: the same as the above-described in addition to the mucous invasion to the aleuron-layer, starchy layer, the epithelium and parenchyma of the scutellum; the nuclear hydrops in the aleuron-layer; decomposition of starch grain; vacuolization of the epithelium and parenchyma of the scutellum.

In the corn-grains after 20 to 24 hours:

On the non-damaged cut-surface: swelling and desquamation of the seed-coat, remarkable swelling in the aleuron and starchy layers; swelling in the epithelium and parenchyma of the scutellum; mucous invasion to the seed-coat, aleuron layer, starchy layer, and the epithelium and parenchyma of the

Table 6. Relationships between histopathological, histochemical and microbiological findings of corn grains in the rumen, especially on the non-damaged cut-surface.

Structure \ Periods / Methods		Progress of time after throwing corn-grains into fistula				
		0	4~8 hrs.	12~16 hrs.	20~24 hrs.	30 hrs.
Seed-coat (cuticular layer, nucellar cells)	Histopatho.	–	Swelling, desquamation mucous invasion	Swelling, desquamation, mucous invasion	Swelling, desquamation, mucous invasion	Desquamation, erosion.
	Histochem.	–	Proteinic immersion by gastric juice	Protein invasion by gastric juice, decrease of fat	Protein invasion by gastric juice, decrease of fat	Protein invasion in gastric juice, decrease of fat.
	Bacteriol.	–	Slight adhesion of bacteria	Slightly bacteria	Slightly bacteria	Slightly bacteria
Aleuron layer	Histopatho.	–	Swelling	Swelling	Remarkably swelling	Remarkably swelling
	Histochem.	–	Decrease of RNA	Disappearance of RNA, increase of protein by mucous depolymerization of DNA	Disappearance of RNA, protein-increase by mucin, depolymerized DNA, decrease of cellulose.	Disappearance of RNA protein-increase by gastric mucin, depolymerization of DNA, decrease of cellulose.
	Bacteriol.	–	–	–	–	–
Starchy layer	Histopatho.	–	–	Swelling, deformation	Swelling, isolation, cavity formation	Swelling, cavity-formation
	Histochem.	–	Protein increase by gastric mucin	Protein increase by gastric mucin, depolymerized DNA, decrease of starch.	Protein increase by gastric mucin, depolymer. DNA, decrease of starch	Protein increase by gastric mucin, depolym. DNA, decrease of starch
	Bacteriol.	–	–	Bacteria	Bacteria	Bacteria
Epithelium of scutellum	Histopatho.	–	Slight vacuolization	Swelling, vacuolization	Swelling, vacuolization	Swelling, vacuolization
	Histochem.	–	Protein increase by gastric mucin	Decrease of RNA, protein increase by gastric mucin	Disappearance of RNA, protein decrease, decrease of fat	Disappearanice of RNA, protein decrease decrease of fat.
	Bacteriol.	–	–	Bacteria	Bacteria	Bacteria
Parenchyma of scutellum	Histopatho.	–	Vacuolization	Swelling vacuolization	Swelling, vacuolization	Swelling vacuolization
	Histochem.	–	Protein increase by gastric mucin	Decrease of RNA, protein increase by gastric mucin	Disappearance of RNA, protein decrease, decrease of fat	Disappearance of RNA, protein decrease, decrease of fat.
	Bacteriol.	–	–	Bacteria	Bacteria	Bacteria

Table 7. Relationships between histopathological, histochemical and microbiological findings of corn-grains in the rumen, especially on the alterations of damaged wounds in the corn.

Structure \ Periods / Methods	Progress of time after throwing corns into fistula				
	hr. 0	4~8 hrs.	12~16 hrs.	20~24 hrs.	36 hrs.
Seed-coat (cuticular layer and nucellar cells) — Histopatho.	–	Swelling, desquamation, mucous invasion	Swelling, desquamation, mucous invasion	Swelling, desquamation, mucous invasion	Desquamation, erosion
Seed-coat (cuticular layer and nucellar cells) — Histochem.	–	Protein by gastric mucin.	Protein by gastric mucin, decrease of fat	Protein by gastric mucin decrease of fat	Protein by gastric mucin, decrease of fat
Seed-coat (cuticular layer and nucellar cells) — Microbiol.	–	–	Bacteria	Bacteria	Bacteria, protozoa
Aleuron layer — Histopatho.	–	Swelling, mucous invasion, nuclear hydrops	Swelling, mucous invasion, nuclear hydrops	Swelling, mucous invasion, nuclear hydrops	Swelling, destruction mucous invasion
Aleuron layer — Histochem.	–	Decrease of protein, RNA, fat and DNA.	Decrease of protein, disappearance of RNA, fat and DNA	Decrease of protein, disappearance of RNA, fat and DNA	Decrease of protein, disappearance of RNA, fat and DNA
Aleuron layer — Microbiol.	–	–	Bacteria, fungus	Bacteria, fungus	Bacteria, fungus
Starchy layer — Histopatho.	–	Swelling, mucous invasion desquamation	Swelling, hydrops, decomposition, depolymerisation of starch grain.	Destruction, isolation, solution of starch grains.	Destruction, isolation, solution of starch grains, cavity formation.
Starchy layer — Histochem.	–	Protein by gastric mucin, decrease of DNA and starch	Protein by gastric mucin, disappearance of DNA and starch	Protein by gastric mucin, disappearance of DNA, starch, slight lipophanerosis.	Protein by gastric mucin, disappearance of DNA, starch, slight lipophanerosis
Starchy layer — Microbiol.	–	Bacteria, fungus	Bacteria, fungus	Bacteria, fungus	Bacteria, fungus.
Epithelium of scutellum — Histopatho.	–	Slight vacuolization	Swelling, vacuolization	Swelling, vacuolization	Swelling, vacuolization
Epithelium of scutellum — Histochem.	–	Protein increase by gastric mucin.	Decrease of RNA, protein increase by gastric mucin	Disappearance of RNA, increase of protein decrease of fat	Disappearance of RNA, decrease of protein and fat.
Epithelium of scutellum — Microbiol.	–	Bacteria	Bacteria, fungus	Bacteria, fungus	Bacteria, fungus
Parenchyma of scutellum — Histopatho.	–	Slight vacuolization	Swelling, vacuolization	Swelling, isolation, decomposition	Isolation, decomposition
Parenchyma of scutellum — Histochem.	–	Protein increase by gastric mucin	Decrease of RNA, increase of protein by gastric mucin	Disappearance of RNA, decrease of fat, increase of protein by gastric mucin	Disappearance of RNA, decrease of protein and fat.
Parenchyma of scutellum — Microbiol.	–	Bacteria	Bacteria, fungus	Bacteria, fungus	Bacteria, fungus

scutellum; isolation and cavity formation in the starchy layer.

Oh the damaged cut-surface: the same as the above-described in addition to the nuclear hydrops in the aleuron-layer, destruction, and resolution of starch grain, isolation and decomposition of the parenchyma of the scutellum.

In the corn grains after 36 hours:

On the non-damaged cut-surface: desquamation and erosion of the seed-coat; remarkable swelling and cavity formation in the aleuron layer and starchy layer; swelling and vacuolization in the epithelium and parenchyma of the scutellum; mucous invasion to the seed-coat, aleuron layer, and starchy layer.

On the damaged cut-surface; same as the above-described in addition to the destruction of the aleuron layer, starchy layer and the parenchyma of the scutellum; decomposition of the parenchyma of the scutellum.

2) Histochemically following the progress of time after putting the corn-grains in the ruminal fistula revealed the following alterations:

In the corn-grains after four to eight hours:

On the non-damaged cut-surface: proteinic immersion in the seed-coat, starchy layer, and scutellum, decrease of RNA in the aleuron layer.

On the damaged cut-surface: same as the above-mentioned in addition to the protein invasion to the starchy and aleuron layer, and parenchyma of scutellum, decrease of DNA, fat in the aleuron layer, starchy layer.

In the corn-grain after 12–16 hours:

On the non-damaged cut-surface: protein invasion to seed-coat, aleuron layer and starchy layer, epithelium and parenchyma of the scutellum; decrease of fat in the seed-coat; depolymerisation of DNA in the aleuron layer, starchy layer; decrease and disappearance of DNA in the aleuron layer, starchy layer; decrease of RNA in the epithelium and parenchyma of the scutellum; decrease of starch grain.

On the damaged cut-surface: decrease of fat in the aleuron layer; decrease of RNA; disappearance of starch.

On the damaged surface: same as the above-mentioned.

In the corn-grain after 20 to 24 hours:

On the non-damaged cut-surface: proteinic invasion to the seed-coat and aleuron layer, the starchy layer, the epithelium of the scutellum; decrease of fat in the seed-coat and the epithelium and parenchyma of the scutellum; disappearance of RNA in the aleuron layer, scutellum-epithelium and scutellum-parenchyma; depolymerization of DNA in the aleuron layer; decrease of cellulose in the aleuron layer and decrease of starch in the starchy layer.

On the damaged cut-surface: the same as the above-mentioned in addition to the disappearance of fat in the aleuron layer and the scutellum-epithelium and scutellum-parenchyma, lipophanerosis in the starchy layer, disappearance of starch in the starchy layer; decrease of protein in the aleuron layer, starchy

layer, the epithelium and parenchyma of the scutellum.

3) Bacteriologically following the progress of time after throwing the corn-grains into the ruminal fluids there were found the following multiplications:

In the corn-grains after four to eight hours;

On the non-damaged cut-surface: slight adhesion of bacteria in the seed-coat.

On the damaged cut-surface: the same as the above-described in addition to the bacteria and fungus in the starchy layer; bacteria in the epithelium and parenchyma of the scutellum.

In the corn-grains afetr 12-16 hours:

On the non-damaged cut-surface: bacteria in the seed-coat, starchy layer, and scutellum.

On the damaged cut-surface: the same as the above-mentioned in addition to the bacteria aleuron layer, and scutellum; fungus in the aleuron layer, starchy layer and scutellum.

In the corn-grains after 20 to 24 hours:

On the non-damaged cut-surface: bacteria in the seed-coat, starchy layer, and scutellum.

On the damaged cut-surface: the same as the above-mentioned in addition to the bacteria in the aleuron layer; the fungus in the aleuron layer, starchy layer, and the scutellum.

In the corn-grains after 36 hours:

On the non-damaged cut-surface: bacteria in the seed-coat, starchy layer, and scutellum.

On the damaged cut surface: the same as the above-mentioned in addition to the bacteria in the aleuron layer; fungus in the seed-coat, aleuron layer, starchy layer, and the scutellum.

The relationships between pathological, histochemical and bacteriological alterations of the corn-grains in the ruminal fluids are shown in Table 6 and 7.

To summarize the results shown in Table, it seemed to be quite clear that the relations between the three alterations were associated with each other.

1) The non-damaged corn-grain after four to eight hours in the ruminal fluids showed slightly the alteration such as the swelling and desquamation of the seed-coat by the mucous invasion, and adhesion of bacterium on the seed-coat, proteinic immersion by gastric juice, and RNA decrease in the swollen aleuron cells.

2) The non-damaged corn-grain after 12 to 16 hours in the ruminal fluids showed the alterations such as desquamation of the swollen seed-coat decreased fat, mucous invasion contained bacillus by gastric juice, swollen aleuron layer disappeared RNA and increased protein by the mucous invasion of gastric juice, swollen starchy layer decreased starch and contained depolymer of DNA by the mucous invasion of gastric juice and bacillus; swollen scutellum decreased RNA

and increased protein, and contained the cytoplasmic vacuolization by the mucous invasion of gastric juice.

3) The non-damaged corn-grain after 20 to 40 hours in the ruminal fluids showed the alterations such as desquamation of swollen seed-coat decreased fat by the mucous invasion of the gastric juice and bacteria; swollen aleuron layer disappeared RNA and cellulose, and contained depolymer of DNA by the mucous invasion of the gastric juice and bacteria; isolated swollen starchy layer decreased starch and contained depolymer of DNA by the mucous invasion of the gastric juice and bacteria; swollen, vacuolized scutellum disappeared RNA, protein and fat by the mucous invasion of the gastric juice and bacteria.

4) The non-damaged corn-grain after 30 hours in the ruminal fluids showed the alterations such as desquamation and erosion in the seed-coat decreased fat by the mucous invasion with bacillus; swollen aleuron layer decreased RNA and fat, increased protein by the mucous invasion with bacillus, swollen starchy layer decreased fat and contained protein and depolymerized DNA by the bacillus-contained mucous invasion; swollen scutellum disappeared RNA, protein and fat by the bacillus-containing mucous invasion.

5) The alterations in the non-damaged corn-grains in the ruminal fluids were weaker than that in the damaged cut-surface of the corn-grains because of the easy penetration of gastric mucin into the interiors and the participation of fungus multiplication in the cut-surface, and the growth of the sporangium and the hyphe-formation.

6) The penetration and multiplication of the microorganisms into and around the cells were the following: Bacterial attachment to the surface of the cuticular layer in the seed-cort for four to 36 hours, penetration and proliferation within the enlarged cellular spaces between the cuticular layer and nucellar cells in the damaged cut-surface for four to 36 hours, aggregation of the sporangiospore of the fungi in the upper surface of the wound for four to eitht hours, proliferation of the fungi in the damaged cut-surface of the starchy layer, invasion of vegetative hypha within the intercellular spaces, formation of the hyphal branches at 12 to 36 hours, proliferation of bacterium and fungi in the damaged cut-surface of the scutellum, intracellular bacterial multiplication in the starchy layer for 20 to 36 hours and parenchyma on 36 hours under the non-damaged surface, on the contrary the intracellular bacterial multiplication in the crushed nucellar cells for 20 to 36 hours, aleuron layer for 36 hours, starchy layer for four to 36 hours, epithelium of the scutellum for 20 to 36 hours, and parenchymatous scutellum for 20 to 36 hours in the damaged cut surface, intercellular invasion of fungi in the starchy layer for 24 to 36 hours, scutellar epithelium for 20 to 36 hours and scutellar parenchyma for 20 to 36 hours in the damaged cut-surface.

No one has ever found the presence and significance of fungi in the corn-grains in the ruminal fluids. It seemed to be an important role for the decomposition

of the cellular substances and isolation of the cells.

In the present study it was described that the onset of autolytic phenomena with necrobiosis, coagulation, and necrosis, and degeneration of the various cells such as decrease of protein and starch, lipophanerosis and fat-decrease, nuclear changes and the multiplication of microorganisms, especially fungus, in the intercellular spaces and in the cells of the corn-grains used as the feed in the rumen, from the side of histochemistry.

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Plate 1

Explanation of Figures

Fig. 1. Polysaccharides within the cells of the parenchyma and epithelium of the scutellum and starch layers. This is a section of boiled corn-seed stained with McManus's PAS-staining. Photograph shows the parenchymatous scutellum (P), epithelial scutellum (E) and starchy layer (S) from the upper side to the lower side. There are shown the pectin and cellulose in the cell wall of the various parts, and starch in the starchy layer. Microphoto. ×100.

Fig. 2. Polysaccharides within the cells of the cuticular layer (C), nucellar cells (N), aleuron layer (A) and starchy layer (S). This is a section of boiled corn-seed stained with McManus's PAS-staining. Photograph shows the cuticular cells (black), nucellar cells (space under the cuticular layer), aleuron layer and starchy layer from the upper side to the lower side. There are also shown the cutinic substance in the cuticular cells and nucellar cells, and pectin in the cell wall of the aleuron- and starchy-layers, and starch in the aleuron- and starchy-layers. Microphoto. ×100.

Fig. 3. Fats within the cells of the parenchyma and epithelium of the scutellum, and staining. Photograph shows the parenchymatous scutellum, epithelial scutellum and starchy layer from the upper side to the lower side. There is also shown a large amount of fat within the parenchymatous scutellum. Microphoto. ×100.

Fig. 4. Fats within the cells of the cuticular layer, nucellar cells, aleuron layer and starchy layer. This is a section of boiled corn-seed stained with Sudan III-staining. Photograph shows the parenchymatous scutellum, epithelial scutellum and starchy layer from the upper side to the lower side. There is shown a moderate amount of fat in the crusted nucellar cells and a large amount of fat in the aleuron layer. Microphoto. ×100.

Fig. 5. Protein within the cells of the parenchyma and epithelium of the scutellum, and starchy layers. This is a section of boiled corn-seed stained with DUJN's acrolein-SCHIFF reaction. Photograph shows the parenchymatous scutellum, epithelial scutellum, and starchy layer from the upper side to the lower side. There is shown a large amount of protein in the parenchyma and epithelium of the scutellum and small quantity of protein in the starchy layers. Microphoto. ×100.

Fig. 6. Protein within the cells of the aleuron layer and starchy layer. This is a section of boiled corn-seed stained with DUJN's acrolein SCHIFF reaction. Photograph shows the parenchymatous scutellum, epithelial scutellum and starchy layer from the upper side to the lower side. There is shown a large amount of protein in the aleuron-layer and a small quantity of protein in the starchy layer. Microphoto. ×100.

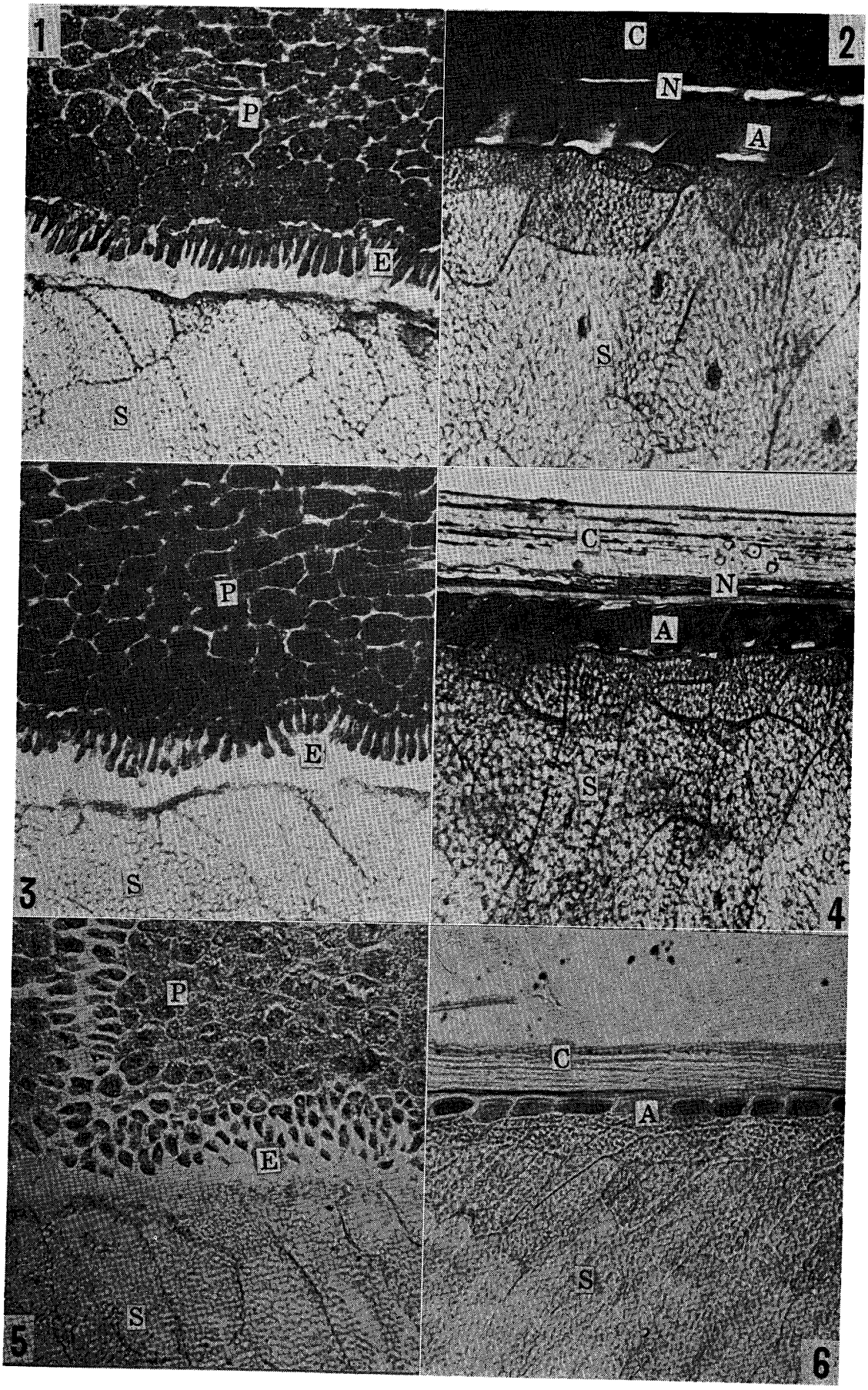
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Plate 2

Explanation of Figures

Fig. 7. Cut-surface in the seed-coat, parenchymatous scutellum of the corn-seed put into the ruminal fluid for four hours. There are shown the dissociation of the parenchyma, and decomposition, destruction of the ones in the damaged cut-surface ; and swelling of the cuticular layer and mucin invasion to the seed coat. Microphoto. ×100, stained with PAS.

Fig. 8. Cut-surface in the seed-coat, nucellar cells, aleuron layer and starchy layer of the corn-seed put into the ruminal fluid for 12 hours. There are shown the swelling of the cuticular layer, nucellar cells, aleuron layer and starchy layer and invasion of gastric mucin into these cells (H. blackish stained parts), and fungus-multiplication in the orifice (F), bacillar multiplication in the starchy layer (B). This blackish stained part was the PAS-positive portion. Mucin invasion between the cuticular layers indicated the lamellar band stained with PAS. Microphoto. ×40, stained with PAS.

Fig. 9. Cut-surface in the seed-coat, parenchymatous scutellum, epithelial scutellum and starchy layer of the corn-seed put into the ruminal fluid for 16 hours. There are indicated the isolation of scutellum, fungus-multiplication (F) and mucous invasion to the scutellum and starchy layer(M). Microphoto. ×40, stained with PAS.

Fig. 10. Destruction soft cells (D) and bacterial multiplication (B) and mucin invasion (M) in the starchy layer.

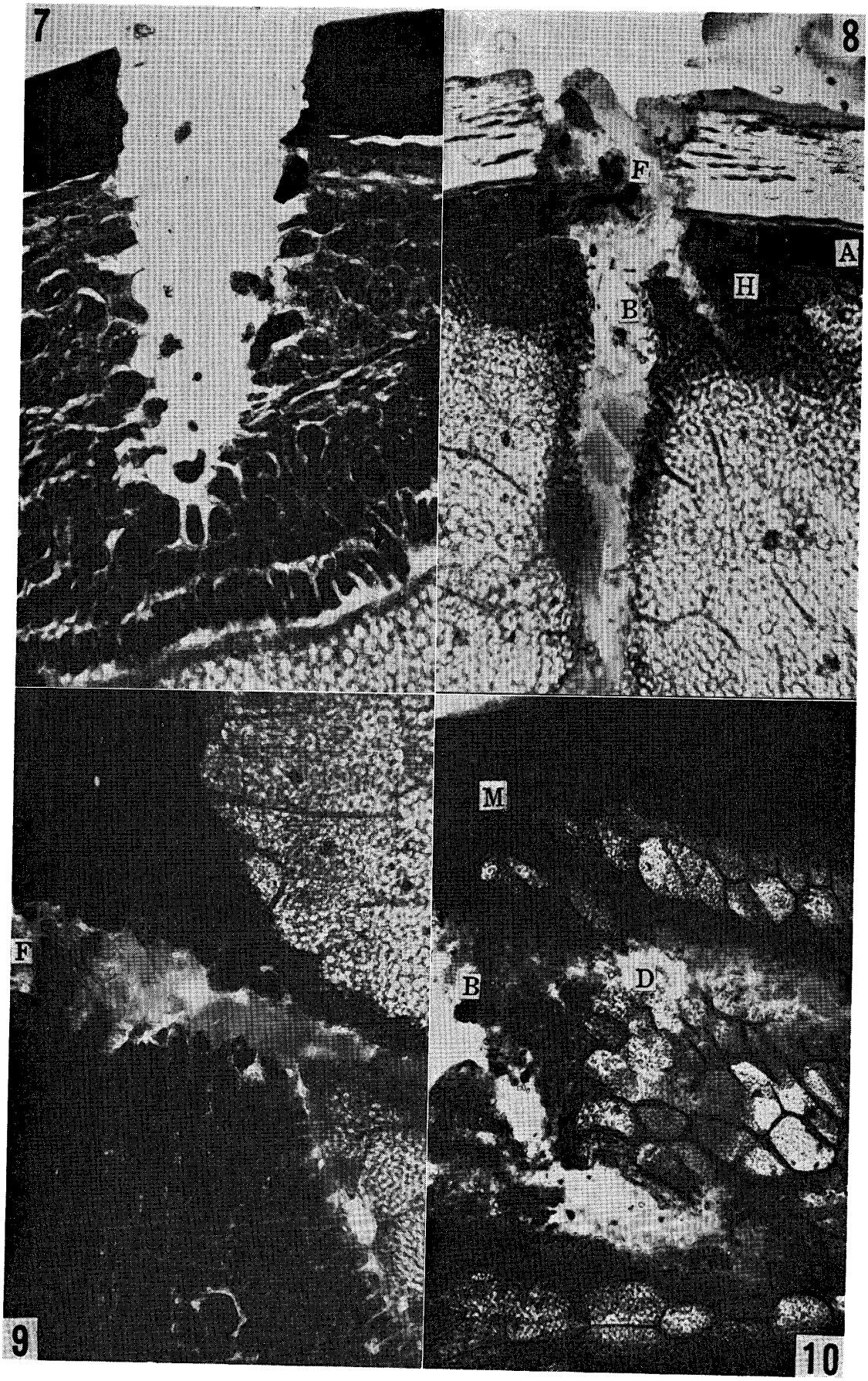
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Plate 3

Explanation of Figures

Fig. 11. Fungus (F) penetrated between the intercellular spaces of the starchy layer and bacteria (B) multiplicated around the starch grains. This is the cut-surface of the corn-seed put into the ruminal fluid for 20 hours. Microphoto. ×200, stained with PAS.

Fig. 12. Starch grain decomposed with bacillus. There are indicated bacillus around the starchy grain (B) and within the starchy grain (I). This is the cut-surface of the corn-seed put into the ruminal fluid for 24 hours. Microphoto. ×400, stained with PAS.

Fig. 13. Vacuolization and bacillus invasion in the parenchymatous scutellum. There are indicated the vacuolization (V) and cavity formation (C) within the parenchyma cells, and fragmentation (F) and bacterial multiplication (B) in the parenchymatous scutellum of the corn put into the ruminal fluid for 24 hours. Microphoto. ×200, stained with PAS.

Fig. 14. Decomposition of the cells (D) and bacterial invasion (B) into the cells of the starchy layer. This is the cut-surface of the corn-seed put into the ruminal fluid for 24 hours. Microphoto. ×200, stained with PAS.

Fig. 15. Bacterial multiplication (B) and mucin invasion (H) in the intercellular spaces of the starchy layer. This is the cut-surface of the corn-seed put into the ruminal fluid for 24 hours. Microphoto. ×200, stained with PAS.

Fig. 16. Roset-formation (R) in the scutellum and the deposition and of the parenchymatous scutellum and bacterial invasion to the starchy layer. This is the cut-surface of the corn-seed put into the ruminal fluids for 20 hours. Microphoto. ×100, stained with PAS.

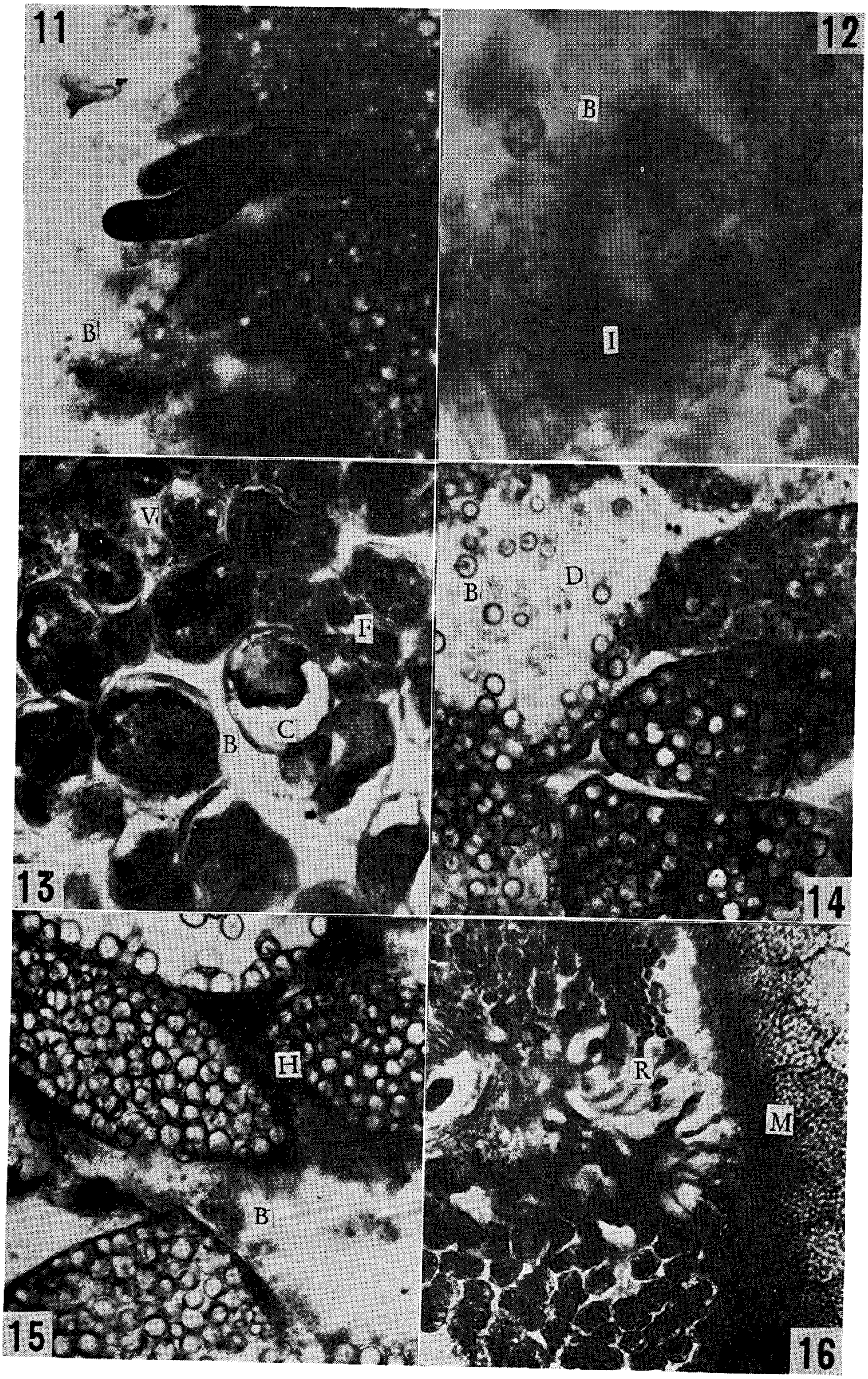
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Plate 4

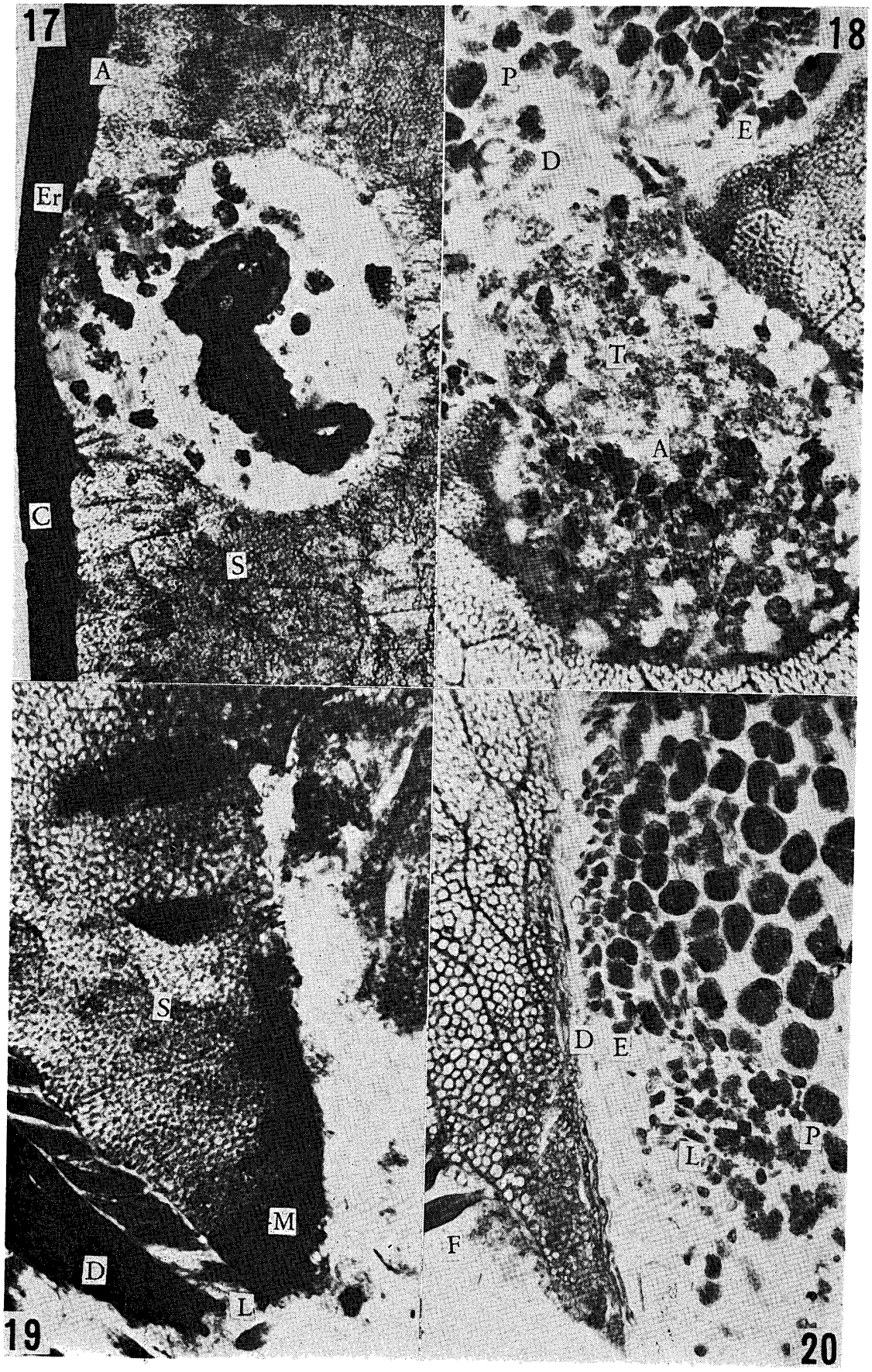
Explanation of Figures

Fig. 17. Cavity formation based on the necrosis of the cuticular layer, nucellar cells, aleuron layer and starchy layer leading to the cut-surface. There are indicated the wavy erosion (ER) in the cuticular layer (C), disappearance of the aleuron layer (A) and starchy layer (S), the polysaccharide-containing fragment of the external coat and endosperms in the cavity, and mucin invasion into the cavity with bacillus. This is a section of the corn-seed put into the ruminal fluid for 36 hours. Microphoto. ×100, stained with PAS.

Fig. 18. Decomposition of the parenchymatous scutellum and starchy layer, appearance of fat droplets-masses in the scutellum, and abscess formation in the starchy layer. There are shown the decomposition of the parenchymatous scutellum (D) and epithelial scutellum (E) and starchy layer (S), abscess formation (A) in the starchy layer, thrusting (T) of the parencymatous scutellum into the starchy layer. This is a section of the corn-seed put into the ruminal fluid for 36 hours. Microphoto. ×100, stained with Sudan III-staining.

Fig. 19. Alterations in the cut-surface containing the cuticular-, aleuron- and starchy layers. There are shown the desquamation of the cuticular layer (D), destruction and isolation of the aleuron cells (L), mucous invasion in the starchy layer and in the intercellular spaces of the starchy layer (M), and solution of starchy grain in the wound (S). This is a section of the corn-seed put into the ruminal fluid for 36 hours. Microphoto. ×200, stained with PAS.

Fig. 20. Decomposition of the starchy layer and the parenchymatous scutellum and epithelial scutellum, and fat-droplets masses in the damaged scutellum, and fungus-multiplication in the damaged starchy layer. There are found the isolation and decomposition of the epithelial scutellum (E) and parenchymatous scutellum (P), fat droplet-masses (L), decomposition of starchy grains with bacteria (D), and fungus-multiplication on the damaged cut-surface of the starchy layer (F). This is a section of the corn-seed put into the ruminal fluid for 36 hours. Microphoto. ×200, stained with Sudan III-staining.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