Histochemical Studies on the Rumen Digestion of Rice Straw Cell Wall and on the Chemical Determination of Its Non Nutritive Residue

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Histochemical Studies on the Rumen Digestion of Rice Straw Cell Wall and on the Chemical Determination of Its Non Nutritive Residue

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Summary

Relationships between the distribution of cell wall constituents of rice straw internode and the rumen digestion of the cell wall were histochemically studied with the nylon bag technique, and the non-nutritive part of the cell wall was characterized. The material was also treated with the three methods prescribed for the determination of crude fiber, neutral detergent fiber and acid detergent fiber, and each residue was histochemically compared with that of the rumen digestion to evaluate its suitability for the estimation of non-nutritive cell wall of roughages.

The cell wall of parenchyma, which was not lignified, was readily digested in the rumen, while those of epidermis and mechanical tissues, in which silica and lignin were deposited respectively, were digested only a little, and hindered the invasion of microbes into the inner tissues.

The procedure for crude fiber determination was concluded to be unsuitable for the estimation of non-nutritive cell wall because of the decrease of polysaccharides and lignin in mechanical tissues. Neutral detergent fiber maintained lignin and polysaccharides in the digestible as well as in the indigestible parts of the cell wall. With acid detergent fiber determination, most of the lignin and polysaccharides in the indigestible part were maintained, and the polysaccharides in the digestible tissues were mostly dissolved, suggesting this procedure to be relatively suitable for the chemical estimation of non-nutritive cell wall.

In ruminants, cellulose and hemicellulose in plant cell wall are partially digestible, while lignin and silica are almost totally indigestible. In the Weende system for the proximate feed analysis, cellulose is mostly retained in the "crude fiber" (C. Fib.), whereas a considerable part of the lignin is dissolved with the same determination procedure and is regarded as "nitrogen free extract" (1). Therefore C. Fib. does not always represent the non-nutritive part of the cell wall in roughages. For a more reasonable characterization of the non-nutritive

substances in roughages, Van Soest (2) proposed a system to classify the plant constituents using detergents. Many studies on the nutritive value and the digestion procedures of roughages have been done by chemical analyses of finely ground plant materials. However, as shown by the results of analyses, roughages are not homogeneous mixtures of chemical substances, but are composed of several plant tissues varying in structural characteristics, which appear to have a certain influence on the digestion in the rumen (3, 4).

The purpose of this study is to use histochemical techniques, 1) to demonstrate the influence of structural characteristics on the rumen digestion of plant tissues, and 2) to compare some chemical procedures for the determination of the non-nutritive residue of roughages.

Materials and Methods

1. *Plant Materials*

Since it is better to have as few histochemical variations as possible in the materials, the top internodes of rice straw from the same field were used.

2. *Histochemical Identification of Cell Wall Constituents*

The dried material was immersed in water, and the air in the sample was removed *in vacuo*. The moistened material was sliced in transections (200 μ) using a hand microtome. The following histochemical methods were used for the identification of cell wall constituents:

Polysaccharides:	Periodic acid- Schiff's reaction (**PAS**) (5)
	Zinc-chlor-iodide reaction (**I**) (5)
Lignin:	Acid-phloroglucin reaction (**P**) (6)
	Mäule's reaction (**M**) (7)
	Schiff's reaction (**S**) (5)
	I
	Toluidine blue staining (**T**) (8)
Silica:	Spodogram
Lipids:	Sudan III staining

To **PAS**, homopolysaccharides are positive and some heteropolysaccharides are negative (9). Since aldehyde groups, mainly those of lignin, are positive to the Schiff's reagent (7), products of this reaction will be involved in the final stain of **PAS**. With **I**, strong electrolyte ions disrupt the hydrogen bonds which are essential for the maintenance of the molecular structures of cellulose and hemicellulose, resulting in the appearance of a blue color indicating iodine accumulation in the enlarged space. In the presence of lignin, the color will not be blue but yellow (5). **P**, **M** and **S** indicate guaiacol-, syringyl- and aldehyde-groups, respectively in the lignin (7). The existence of lignin was determined by con-

sidering the results of **I** and **T** in addition to these three reactions. **T** gives a green color to lignin and polyphenols (8). Silica was detected in spodogram, for which the section of the sample with a few drops of gelatin solution on a slide glass was incinerated in a muffle furnace at 500°C. The resulting spodogram was treated with conc. hydrochloric acid and mounted in glycerol.

3. *Studies on the Rumen Digestion*

Nylon cloth bags (9×2.4 cm, ca. 150-mesh), each containing approximately 0.2 g of the material (1 cm in length, bisected longitudinally) were placed in the rumen *via* fistula of adult sheep, that were fed 300 g grass hay and 150 g concentrate twice daily in the morning and evening. The bags were placed in the rumen shortly before the morning feed, and were removed after various periods of time. For morphological and histochemical observations, the residue in the bag was fixed with a neutral formalin buffer solution, and 200 μ sections for **P**, **M** and **S**, and 10 μ paraffin sections for other examinations were prepared from the middle part of the fixed residue. The microorganisms attached to the plant tissues were stained with hematoxylin. Dry matter of the residue was determined after washing the material with water on a 100-mesh metal sieve.

4. *Histochemical Studies on* C. Fib., *Neutral Detergent Fiber* (NDF) *and Acid Detergent Fiber* (ADF)

The 200 μ sections prepared as described in 2 were treated with the three methods prescribed for C. Fib., NDF and ADF determination (10), and each residue was histochemically examined.

Results

1. *Distribution of Cell Wall Constituents*

A tissue construction model of the experimental transection described below is illustrated in Fig. 1.

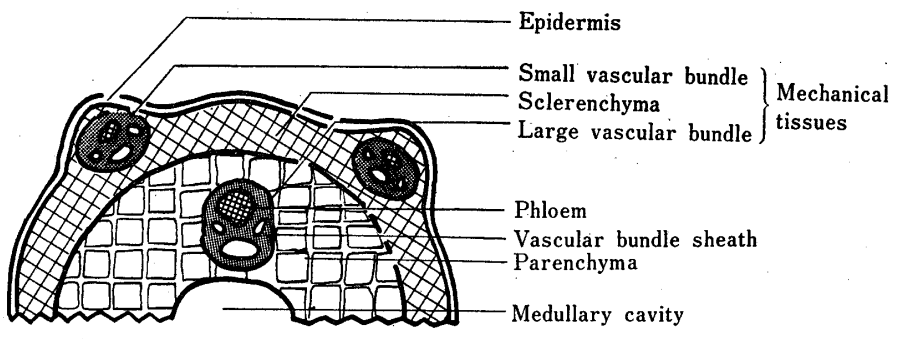

FIG. 1. Tissue construction model of transection of rice straw internode.

In all tissues the cell wall was strongly positive to **PAS** (Fig. 8). With **S**, the sclerenchyma under the epidermis, the vascular bundle sheath and vascular bundle except for the phloem, i.e. the so called mechanical tissues, were positive, but this reaction was much weaker than **PAS**. Therefore the greater part of the **PAS**-positive substances would be the polysaccharides, and they were present in the cell wall of all tissues.

With **I** (Fig. 3), the parenchyma showed a blue color, and the mechanical tissues were yellow despite the presence of polysaccharides. **P** and **M** (Fig. 9) were positive in the mechanical tissues, in which **T** also developed a green color. These results indicated that the lignin was localized in the mechanical tissues.

The spodogram (Fig. 10) showed that silica was deposited mainly in the epidermis. Lipids were not detected by Sudan III.

2. *Rumen Digestion*

2–1. *Morphological Changes*

The materials in the nylon bag showed the following morphological changes in the rumen with the lapse of time:

After 6 hr (Fig. 4): The parenchyma, which originally shrank (Fig. 3), moistened and swelled, however no disintegration of tissues was observed. Microbial colonies appeared in the medullary cavity side of the parenchyma.

After 12 hr: Disintegration of parenchyma started from the medullary cavity side.

After 24 hr (Fig. 5): The greater part of the parenchyma disappeared, and only the mechanical tissues remained intact. The phloem was partly disintegrated.

After 48 hr (Fig. 11): The morphological figure was not so different from that of the 24 hr.

After 96 hr (Fig. 6): The mechanical tissues neighbouring the parenchyma were partly disintegrated, and the phloem mostly disappeared.

After 144 hr (Fig. 7): The tissues which still remained were epidermis, sclerenchyma neighbouring the epidermis, small vascular bundles except for phloem, and the internal part and sheath of the large vascular bundle. Disintegration of tissues from the epidermis side could not be observed.

2–2. *Histochemical Changes*

Of all the tissues examined, the intensity of **PAS** scarcely changed so long as they remained in the form of visible residues in the bag (Fig. 4, 6). The area, colored yellow with **I**, decreased gradually (Fig. 3 vs. 5 vs. 7); i.e. the intact mechanical tissues maintained a yellow color, while the color of the disintegrating parts changed to blue. Apparent changes were not observed in the **P**, **M** (Fig. 11), **S** and **T**.

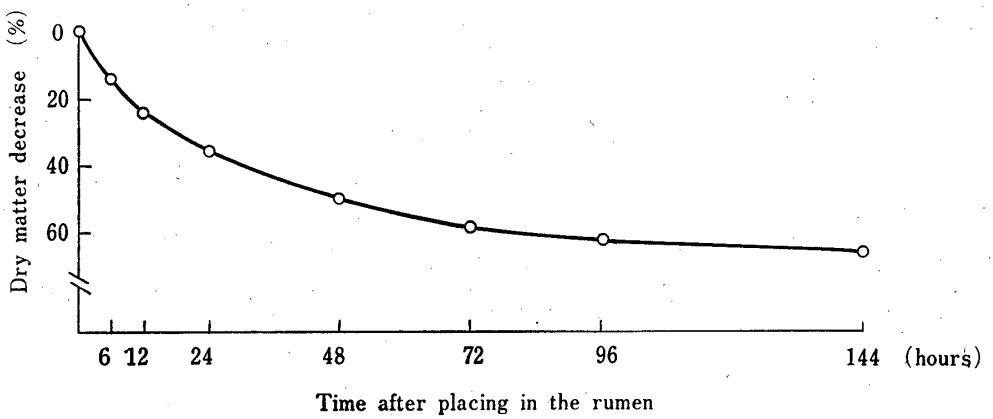

FIG. 2. Dry matter decrease of top internode of rice straw in the rumen (Nylon bag technique).

2–3. *Decrease of Dry Matter*

As shown in Fig. 2, the dry matter of residues in the bags decreased rapidly during the first 48 hr, then gradually till 96th hr. Thereafter the values remained nearly constant.

3. *Histochemical Changes by Procedures for Chemical Determination of Non Nutritive Cell Wall*

3–1. C. Fib. *Determination*

The intensity of the **PAS** in all tissues and that of **P, M** and **S** in the mechanical tissues were reduced remarkably (Fig. 12, 13). **I** stained mechanical tissues, as well as the parenchyma, blue. The green color of **T** disappeared. By sulfuric acid treatment, these histochemical changes were induced only to a limitted extent. The greater part of those changes were brought about by sodium hydroxide treatment.

3–2. NDF *Determination*

PAS was not influenced (Fig. 14), neither were **M** (Fig. 15), **I** or **T**, whereas **P** and **S** were weakened.

3–3. ADF *Determination*

PAS was not affected in the mechanical tissues, but changed to negative in the parenchyma (Fig. 16). Little changes were observed in **P, M** (Fig. 17), **S, I** and **T.**

Discussion

1. *Relationship between Distribution of Cell Wall Constituents and Rumen Digestion*

It was shown that the rumen digestion of cell wall constituents was affected by the deposition of lignin and silica in tissues and by the steric disposition of various tissues. The cell wall of parenchyma, which was not lignified, was rapidly

disintegrated, while that of the mechanical tissues was lignified and hardly decomposed even after a long time in the rumen, and little histochemical change of the latter was observed. Phloem was disintegrated less quickly than parenchyma, although both tissues were not lignified. The explanation for this difference may be that the lignified tissues enclosing phloem hinder the microbial invasion. The fact that no disintegration took place from the epidermis side can be attributed to silicification in the epidermis. Both the small vascular bundle surrounded with sclerenchyma and the sclerenchyma neighbouring the epidermis disintegrated very slowly or remained intact. This indicates that the disintegration of mechanical tissues was also affected by their steric disposition.

No attempt was made to observe the fate of the fragments which had been liberated from the original tissues. However, the decrease of dry matter even after the disappearance of parenchyma (at 24th hr) (Fig. 2, 5) suggests that the liberated materials were further chemically digested. Mechanical tissues, in contrast, were separated from each other to some extent, but chemical digestion of the tissues seemed to be small, because the dry matter decreased very slightly after 96 hr.

It is known that the digestible parts of cell wall constituents were mostly utilized in the rumen (11), and that most of the plant tissues excreated in ruminant feces were derived from the sclerenchyma, the vascular bundle and the epidermis (12). In this experiment, the residues after 24 hr rumen digestion mainly consisted of lignified mechanical tissues, of which no appreciable changes of the lignin and polysaccharides were observed until 144 hr except in the case of **I**. Therefore the residues after 24 hr rumen digestion were considered to correspond to the non-nutritive part of cell wall.

2. *Chemical Determination Procedure of Non Nutritive Cell Wall*

The treatment for C. Fib. determination remarkably decreased the lignin and polysaccharides in the mechanical tissues as well as in the parenchyma. Therefore, this treatment is concluded to be unsuitable for the chemical estimation of non-nutritive cell wall.

In the case of NDF, it was assumed that with this procedure the bulk of lignin and polysaccharides in the digestible as well as in the indigestible tissues was maintained (13).

In ADF determination, polysaccharides and lignin in mechanical tissues were not substantially attacked. In parenchyma, **PAS**-positive polysaccharides disappeared, but the cell wall was still apparent and stained blue with **I**. Consequently, the residue of ADF determination procedure was considered to be in principle similar to the indigestible part of the material, though the removal of polysaccharides from the parenchyma was not complete. The results of present study suggest that ADF is a comparatively suitable criterion for the non-nutritive cell wall on the basis of chemical determination.

Acknowledgement

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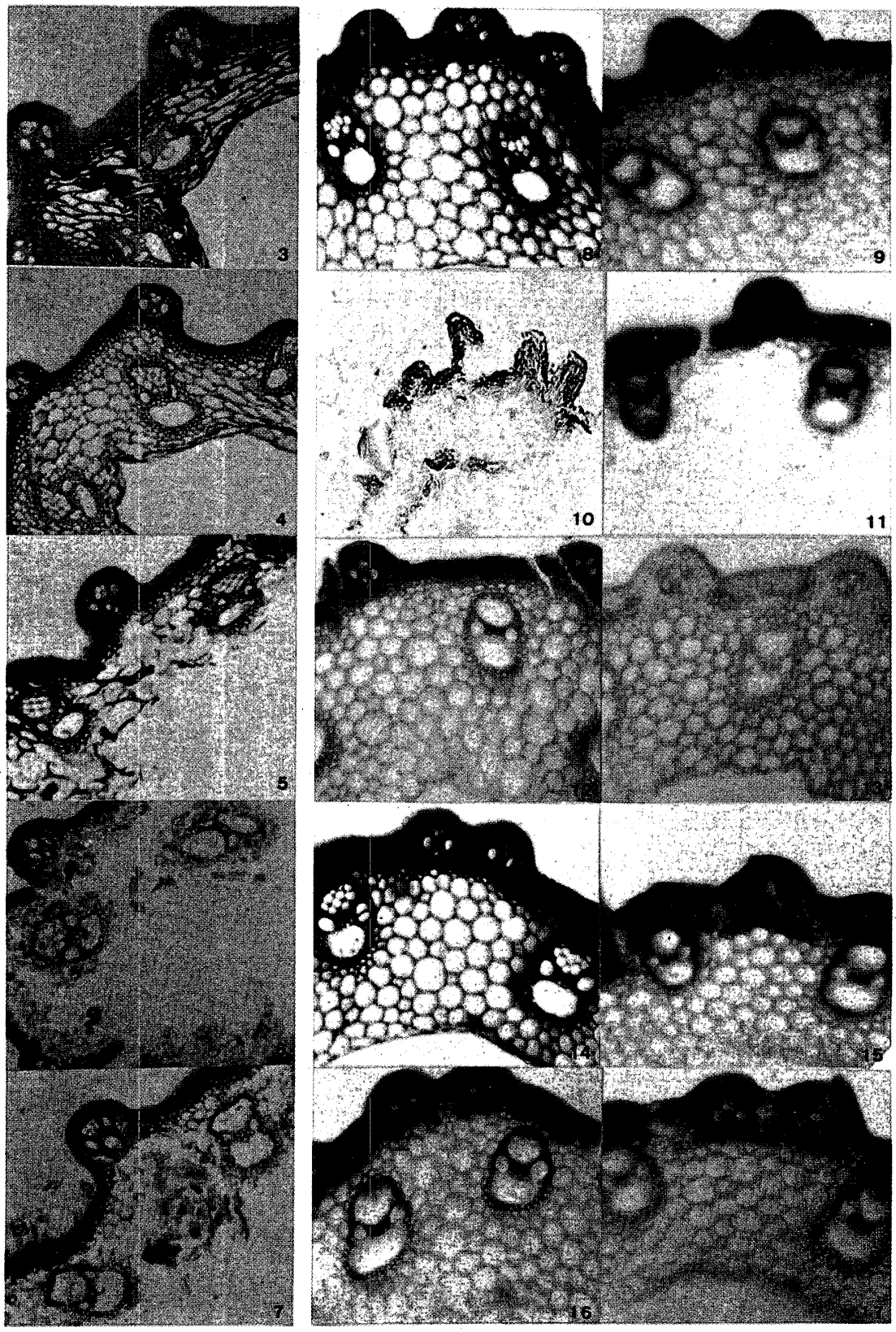
Plate 1

Explanation of Figures

Fig. 3. Transection (10 μ) of original material (top internode of rice straw) stained with zinc-chlor-iodide reaction.
Fig. 4. 6 hr after placing in the rumen, PAS reaction, 10 μ.
Fig. 5. 24 hr after placing in the rumen, Zinc-chlor-iodide reaction, 10 μ.
Fig. 6. 96 hr after placing in the rumen, PAS reaction, 10 μ.
Fig. 7. 144 hr after placing in the rumen, Zinc-chlor-iodide reaction, 10 μ.
Fig. 8. Original material, PAS reaction, 200 μ.
Fig. 9. Original material, Mäule's reaction, 200 μ.
Fig. 10. Spodogram of original material, 200 μ.
Fig. 11. 48 hr after placing in the rumen, Mäule's reaction, 200 μ.
Fig. 12. Residue of the crude fiber determination procedure, PAS reaction, 200 μ.
Fig. 13. Crude fiber, Mäule's reaction, 200 μ.
Fig. 14. Neutral detergent fiber, PAS reaction, 200 μ.
Fig. 15. Neutral detergent fiber, Mäule's reaction, 200 μ.
Fig. 16. Acid detergent fiber, PAS reaction, 200 μ.
Fig. 17. Acid detergent fiber, Mäule's reaction, 200 μ.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