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RUMEN DIGESTION OF WHEAT STARCH AS OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

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Finally, heartfelt gratitude is extended to my dearest Colette whose love, encouragement and perserverance motivates this husband's efforts to achieve.

Introduction and Literature Review

Scanning electron microscopy (SEM), although relatively new, is rapidly being exploited as an analytical tool by which small, complex structures, processes and other phenomena may be observed and recorded.

Design and operation of SEMs as well as methods of specimen preparing have been extensively documented. Contemporary work on instrument development is toward refinement and perfection of the available system rather than any change in the basic structure (Hearle, 1972).

The high-quality pictures for SEM, are valuable tools for structural identification and clarification of innate as well as biological specimens. But few researchers have considered the added advantages to be had by utilizing the concept of stereoviewing in SEM study. The procedure for obtaining stereo pictures and subsequently observing the photographed specimen in 3-D detail is aptly described by Howell (1975). Simple observation of the design and relative location of structures is enhanced when viewed in this simulation of natural conditions.

SEM is currently the method of choice for studying cereal grain starch. Observations have been made on naturally occurring granules in the split kernel. The effect of various isolation or purification techniques and various types of structural alterations or destruction of the intact granules. Pomeranz and Sacks (1972) described the structure of oats,

barley and buckwheat starches, while Hoseney et al. (1973) studied milo. Hall and Sayre (1970) and Aranyi and Hawrylewig (1969) studied a variety of cereal starches, including wheat.

Kerr (1950) found that wheat starch granules were either large, lenticular bodies, 20 to 25 μ in diameter or small spheres with a size range of 2 to 10 μ. Our observations, however, concur with MacMasters (1963); no clear distinction in particle size, with a full range of intermediates between the largest and smallest. An equatorial groove has been observed in larger starch granules (Evers, 1969) and this may be an area of preferential enzyme attack.

Starch granules are composed of molecules of amylose and amylopectin in varying ratios. Badenhuizen (1959) reported the presence of concentric rings comprised of amylose and amylopectin. Gallant (1972) offered the possibility that the concentric layers may differ in their respective amylose:amylopectin rations which may explain the difference in rate of hydrolysis of alternating layers. Whistler and Young (1960) and McConnell et al. (1958) followed deposition of C^{14} in starch granules and found that, although amylose is more readily formed than amylopectin, conversion of the former to the latter seldom occurs. Thus, the concentric rings observed on hydrolysis may indicate crystalline regions with a high degree of hydrogen bonding alternating with loosely packed, amorphorus regions which are more readily hydrolyzed. The radial digestion patterns in wheat starch granule observed by Gallant (1972) may be the result of single linear (amylose branches of amylopectin)

molecules, directed radially through the granule forming hydrogen bonds with the crystalline structures in the succeeding shells (Pomeranz, 1971) which are then quickly hydrolyzed, allowing continued enzyme hydrolysis in the concentric layers. The presence of readily hydrolyzable amorphous areas along those radially directed molecules tends to support this hypothesis.

Enzymatic hydrolysis of cereal starch is primarily a function of α-amylase in both the ruminant and nonruminant. This enzyme along with α-amylase is present in the wheat kernel itself and is activated upon sprouting. It is secreted by various bacteria, yeast and fungi, is present in pancreatic secretion of animals and in the saliva of non-ruminants. Amylase is an endoenzyme attacking the α-1,4 linkages within the molecule. Chemically, α-1:4 glucan-4-glucano-hydrolase (α-amylase) hydrolyses the α-1,4 glucosidic bonds between the glucose units, yielding first, random oligosaccharide and dextrin units, and finally maltose and glucose (Meyer, 1960). Amylopectin is also attacked at α-1,4 linkages but its α-1,6 linkage is impervious to α-amylase and degradation will result in the production of highly branched limit dextrins containing the α-1,6 bonds (Lehninger, 1970).

Many workers have observed, via SEM, the activity of α-amylase on cereal starches. Evers (1969) observed wheat starch hydrolysis by wheat α-amylase. Gallant et al. (1972) observed purified enzyme attack on several types of purified starch including wheat. Dronzeck et al. (1972) and Pompipom (1975) have observed amylase activity in sprouted wheat as

well as in other grains. Davis and Harbers (1974) and Harbers and Davis (1974) have reported extensively on the hydrolysis of milo starch by both ruminants and non-ruminants as observed by SEM. Researchers have noted the previously mentioned concentric rings and Evers (1969) was the first to report SEM observation of the equatorial groove on the larger granules and the preferential attack at the groove by amylases.

Observation of glucoamylase activity by SEM has been reported by Evers et al. (1971) and Smith and Lineback (1975). Sandstedt and Bechord (1946) observed amylolytic activity and presented substantiating photographs of enzyme activity, Whelan (1960) reported that α-1,4 linkages near α-1,6 branch linkages were not hydrolyzed, and Robyt and French (1967) proposed a multiple attack hypothesis of amylase action.

Wheat endosperm is composed of three cell layers: peripheral, prismatic and central cells as shown for hard red winter wheat by Bradebury et al. (1956). The peripheral cells lie next to the aleurone layer of the pericarp. The prismatic cells lie next to the aleurone layer of the pericarp. The prismatic cells lie inside the peripheral cells and are elongated toward the crease from the back of the kernel to the middle of the cheeks in the frontal areas. The central cells lie inward from the prismatic cells and extend from them to the crease.

The starch granules in the cells are embedded in a protein matrix. The largest concentration of starch seems to be toward the inside of the endosperm. This starch is logically accompanied

by more functional matrix protein. Storage protein granules are found toward the outside of the endosperm. They represent the majority of the endosperm protein (Hinton, 1947; Morris et al., 1946). This relative percentage of the total protein increases as the grain ripens (Jennings and Morton, 1963).

Starch comprises 64-74% of the volume of the starch endosperm while protein comprises from 8.3-13% as determined by proximate analysis (Fraser and Holmes, 1959). Starch has been reported to comprise from 54-72% of the dry weight of the entire kernel (Shellenberger et al., 1966). Amylose content of the starch ranges from 23.4 to 27.6% (Medcalf and Gilles, 1965).

Traditionally, wheat has had a much greater use as human food rather than as a feed source for animals. The 25% of the kernel not composed of starch is widely fed to animals but the starch remains cheifly a human food and is priced much higher than other grain energy sources.

Various cattle feeding trials using rations containing 0-100% wheat have been conducted and reported (Brethour and Duitsman, 1966, 1971a, 1971b, 1971c). In general, feed efficiency is increased when wheat is fed but feed intake, and consequently, gain is restricted. Wheat reduced intake by 16% and gain by 10% when fed as the only grain in a finishing ration. When limited to 50% of the ration intake dropped 5% and gain was not affected. Reconstituting wheat did not seem to improve gains (Brethour and Duitsman, 1971).

Low concentrate rations for yearling steers with 50% wheat in the grain component of the ration decreased intake 12% but did not alter efficiency although gain dropped. Carcass quality was the same for the wheat ration as it was for the all milo ration (Brethour and Duitsman, 1966). Because wheat has approximately 3.5% more crude protein than corn it can replace supplemental cotton seed meal protein at the rate of 1 lb. wheat to 1/8 lb. of CSM (Ely and Duitsman, 1967). No change in efficiency or gain was observed when wheat protein was substituted for all the CSM protein again as long as wheat does not comprise over 50% of the grain in the ration (Brethour and Duitsman, 1971).

In several feeding trials hard red winter wheat varieties were shown to be superior to soft winter varieties when considering rate of gain although nitrogen retention seemed to be higher for the soft varieties. No varietal difference was exhibited for the wheat tested. The hard varieties in this test were Scout and Golden-50 while the soft were Gaines and Stadler. When these varieties, with the exception of Stadler were fed in finishing rations as 100% of the grain it was observed that more steers foundered on the rations containing the hard wheats than did those ingesting soft wheat. Scout and Golden-50 were not shown to differ in performance from Gaines when fed in high-silage backgrounding rations (Brethour and Duitsman, 1971). Wheat also results in increased gains when substituted for milo in Starea (Brethour and Duitsman, 1971).

Metabolically, the highly fermentable wheat starch will result in a lower ruminal pH and greater VFA concentrations with a relative increase in butyric acid (Oltjen, 1971). Wheats with weak gluten properties were found to give better feedlot performance and nitrogen retention than those with strong gluten properties.

Varnee et al. (1975) compared wheat to corn in both in vivo and in vitro tests. Scout 66 wheat was found to support the highest lactate levels in both systems. Dry matter disappearance in the in vitro system was greatest for Gage, the wheat which supported the lowest lactate and, therefore, the highest pH of those wheats tested. In all cases, wheats produced higher lactate levels than corn. The harder wheats support higher lactate levels because they are more susceptible to damage during processing. The hard wheat starch granules are more susceptible to enzymatic attack by α- and β- amylases (Williams, 1967).

Prasad et al (1975) found that processing wheat increased starch availability but tended to decrease protein digestibility while increasing the nitrogen retained. The processing of wheat is essentially unnecessary as rumen lactate level tends to be high.

Overall, in high concentrate rations, wheat can be substituted pound per pound for corn, barley, or steam flaked milo but its tendency to cause acidosis requires somewhat more management and it seems to be most acceptable when fed with another grain. Again, type-variety seem to affect the

feeding value. Brethour (1970) and Lofgreen (1970) reported that NE values for wheat were slightly superior to processed milo and equivalent to barley while being slightly lower than corn. As stated these data support the NEm and NEg values reported by Lofgreen and Garrett (1968).

Other feeding trials have shown that wheat is an acceptable substitute for corn and milo when fed in swine rations although some lysine supplementation has been found to be required. Energy content seems to be somewhat lower than corn. The main concerns when considering wheat utilization are the type and varietal differences which have been observed to cause significant performance differences (Oldfield, 1970; Luce, 1970).

Lennon et al. (1972) have found that Blueboy, a high lysine variety of wheat, is of similar feeding value to corn in swine finishing rations. Pigs in this trial had the same gains although the ones on the wheat ration were more efficient.

Wheat compared favorably to milo when supplemented with soybean meal but was inferior when substituted on either a weight or isonitrogenous basis in swine rations. An increase in daily gain was observed when wheat was supplemented with lysine. Efficiencies of wheat and milo were found to be essentially the same (Luce et al., 1972). Wheat protein concentrate was an acceptable protein source when fed at levels up to 60% of the total protein in swine finishing rations (Allee et al., 1972).

The purpose of this study was to continue the logical sequence of research of cereal starches by investigating the activity of rumen microbiota on the starch granules present in the endosperm of the wheat kernel. These microbiota secrete the α-amylase enzyme which in turn hydrolyzes the α-1,4 linkages bonding the glucose units together in the amylose and straight chain regions of amylopectin to dextrins and maltose units.

Hard red winter wheats were chosen for this study as they are the primary wheats of the region. The five most abundant hard red winter wheat varieties on a percentage-of-acres-planted basis are, in order, Scout, Eagle, Triumph, Centurk, and Parker (Anonymous, 1975). These five wheats were subjected to both in vivo and in vitro digestion. Digestion of the starch granule was observed by scanning electron microscopy. The intent of SEM observations was to characterize hydrolysis patterns of the α-amylase on wheat starch. Any differences in hydrolytic patterns between varieties were also sought during the observation. The basis for the latter observation was either the confirmation or the disproval of the hypothesis that starches of different wheat varieties were not structurally different and thus could not account for the differences in digestibility of various varieties of wheat as is shown to be the case in wheat feeding trials.

Materials and Methods

Scout 66, GH-45 Eagle, HP35-Triumph 64, Centurk and CF18-Parker were obtained from the Kansas Crop Improvement Association headquarters at Kansas State University.

Initial Studies

Kernels of each variety were frozen in liquid nitrogen, fractured with razor blade and mounted on aluminum stubs with Delco No. 93 colloidal silver. Upon drying of the colloid, samples were coated with 150 Å of gold palladium and observed with and Etec Autoscan scanning electron microscope at an accelerating voltage of 20 Kv. The scanned image was photographed on Polaroid PN 55 film. Other samples were prepared in an identical manner although they were not subjected to the liquid nitrogen prior to fracturing. As opposed to the fracturing of individual granules of wheat by hand, samples were ground in a Wiley mill equiped with 0.5 mm screen. Representative particles of the grinding process were prepared for SEM observation.

Porcine α-amylase activity

Half kernels of the give wheat varieties were subjected to hydrolysis by 10 ml. twice-crystallized porcine pancreatic α-amylase (alpha - 1,4 glucan, 4 glucanohydrolase) 3.2.1.1.[1/] which had been diluted to approximately 20 I.U./ml. activity in a potassium phosphate with NaOH buffer which itself was diluted 24:1. The buffer contained .006 M NaCl as an enzyme activator. The pH of each system was 6.9. All samples were

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[1/] Worthington Biochemical Corporation, Freehold, N.J.

incubated 1 hour at 37° C in a Dubanoff metabolic shaker. After digestion the half kernels were gently washed in distilled water, dried and mounted for SEM observation.

Ground wheat samples (50 mg) were exposed to porcine α-amylase. Conditions were the same except that the standard phosphate buffer was used (Sorenson, 1909). Digestion was allowed to proceed 8 hours. The samples were then centrifuged at 42,000 x g for 5 minutes, washed, recentrifuged, collected, dried and mounted for SEM observations.

Coarsely ground samples were fed to individual 100 kg hogs three hours prior to slaughter. Samples were recovered from the gastrointestinal tracts, washed in distilled water and then mounted for SEM observation.

Rumen α-amylase activity

Kernels of each variety were split with a razor blade, then placed in individual nylon bags. The bags were weighed to assure proper depth in the rumen. The bags were suspended in the rumen of ruminally fistulated Hereford steers for 12 and 24 hours. The steers were being fed a low quality corn silage ration. Samples were retrieved from the rumen, washed several times in distilled water, allowed to air dry and were mounted for SEM observation. Other kernel halves were wrapped in six layers of cheese cloth before being placed in the nylon bags, otherwise the procedure for these kernels was identical to the former with 12 and 24 hour exposures to the *in vivo* digestion.

Wheat kernel halves were mounted on stubs with colloidal silver which was then allowed to dry. These stubs were then exposed to undiluted rumen fluid in polyethylene centrifuge tubes placed in a Dubanoff metabolic shaker. Digestion was allowed to proceed for 4 hours. At the end of digestion the kernels, with the exception of Scout, were washed twice in distilled H_2O, dried and viewed under the SEM.

Wheat kernels were ground in a Wiley mill equiped with a .5 mm mesh steel screen. One half gram samples of each variety were subjected to in vitro rumen digestion according to the method described by Tilley and Terry (1963). 30 ml. of McDougall's buffer was added to 50 ml. polyethylene centrifuge tubes. The buffer was saturated with CO_2 and the pH was adjusted to 6.8 with 6N HCL. The buffer temperature was adjusted 39°C in a Dubanoff metabolic shaker. The grain samples were then added and the system innoculated with 10 ml. fresh rumen fluid collected from a fistulated Angus steer receiving a high milo, alfalfa, starea ration. The fluid was drawn from the rumen by a syringe via a fistula tube to which a weighted screen was attached in the rumen. The tubes were capped with rubber stoppers to which a section of rubber tubing was attached. The tube was slit with a razor blade giving an approximately 1 cm opening for CO_2 gas escape. The system was flushed with CO_2 to drive off any residual O_2. The in vitro rumen fermentation systems were then incubated at 39°C in the metabolic shaker. pH was monitored hourly. Digestions were stopped after 8 hours or when pH dropped to 5.5. The rumen fluid-buffer mixture was drawn off and portions of the

samples were mounted on aluminum stubs by double stick tape. Other sample portions were washed twice in distilled H_2O and mounted in the same manner. Still other samples were subjected to pepsin digestion via a pepsin - HCl solution as described by Tilley and Terry (1963). These samples were then mounted with double stick tape, coated and viewed with the SEM.

Ground wheat samples were subjected to both HCl and lactic acid digestion in order to characterize acid hydrolysis of the starch so that it could be distinguished from amylase activity. The ground wheat samples were exposed to 1N HCl for 2 hours. 10 ml. acid was added to each .5 g sample in polyurethane centrifuge tubes which were incubated at 39°C in a metabolic shaker. A similar procedure was followed for lactic acid; digestion was allowed to proceed six hours.

Stereotechnique

Actual SEM observations of specimens involved the use of stereo-technique as well as conventional SEM photography. When stereo photographs were required for additional clarification of structural detail or starch granule damage the specimen was focused and the first picture was taken in the usual manner. Before taking the second picture the stage was rotated approximately 7°. The picture was then taken. No other parameters were changed; focus, magnification, voltage, etc. settings remained the same.

Depending on the direction of rotation the individual pictures were designated either stereo right or stereo left. The pictures were then placed under a stereo-viewer which

directs the image from the left picture to the left eye of the viewer and that image from the right picture to the right eye of the viewer.

Normal three-dimensional vision depends on the incident light rays from a particular object striking each eye at a slightly different angle. The visual image perceived is the result of one eye viewing the object at a slightly different angle than the other. This parallax results in the brain "seeing" a three-dimensional object, therefore, the observer viewing specimen photographs with the aid of the stereoviewer perceives the photographed specimen in dimensions closely resembling natural conditions.

Slime

Of concern throughout the rumen digestion trials was the slime produced in the rumen. The slime, or froth, was not as much a problem with the lowly fermentable milo stover ration received by the Hereford steer. The grain-alfalfa ration being fed to the Angus steer did, however, result in appreciable slime formation and subsequent coating of the starch sample. Hindrance of starch hydrolysis observation was often a problem although washing the samples in distilled H_2O usually seemed to remove enough of the slime for these SEM observations. Should such an amount of slime be present so as to interfere with SEM observation, the sample could be washed in ethanol so that the slime could be percipitated and removed (Guiterrez, 1961). The biochemical nature of the slime is uncertain but appears to be a function of soluble polysaccharide. Nucleic

acids and nucleoproteins are also likely associated with slime production and subsequent bloat (Gutierrez et al., 1961; Bartley et al., 1974). High voltage transmission electron microscopy may serve to better characterize the biochemical aspects of the slime.

Results and Discussion

Initial observations via SEM reveal that satisfactory fracturing of the kernels was achieved by cutting with a razor blade as opposed to freezing in nitrogen prior to fracture. Acceptable grain fracture was also obtained by grinding in the Wiley mill.

SEM observation at low magnification (20X) reveals typical anatomical detail. In the endosperm peripheral cells are flattened against the aleurone layer; prismatic cells extend from the peripheral cells to the center of the cheeks at the front of the kernel and to the crease from the back; and central cells extend from the sides of the crease to the center of the cheeks of the kernel (Fig. 1).

Observation of the varieties at 800X (Fig. 2-6) reveals starch granules embedded in a protein matrix within the cells. The large lenticular A granules comprise the majority of the starch. Smaller B granules are also present although the hardness of the wheat has resulted in B granules being popped out of the protein matrix upon fracturing, leaving "holes" in the matrix. Many intermediately sized granules are also present in all varieties. Many granules retain matrix protein covers. Equatorial grooves may be observed in the larger

granules particularly in Eagle (Fig. 3). Cell walls are clearly shown in Triumph and Centurk (Fig. 4 & 5).

Starch digestion by purified α-amylase proceeded as expected with pin-point hydrolysis resulting in many, small, concentric holes in the granule. Preferential attack occurred along the equatorial groove of the granules. All hydrolytic patterns seem to agree with those previously reported. The attack initially seems to proceed along radial lines as reported by Gallant (1972). This radial hydrolysis serves to express the enzyme to all the concentric starch layers of the granule. The enzyme expresses these layers by preferentially digesting the amorphorous layer prior to the crystalline layer of the granule.

In vivo starch digestion by butcher hogs, in which kernels were recovered from the stomach and were thus exposed to only salivary amylase, resulted in no significantly different hydrolytic pattern. Observation of a larger (1.7 mm) area of the digested kernel particle revealed rapid removal of starch cell contents but little digestion of cell walls in the stomach of the pig (Fig. 7). This pattern of digestion results from the highly soluble matrix protein of the wheat kernel being rapidly digested by the proteolytic enzymes in the stomach. The starch granule is no longer held bound in the cell and is quickly washed away. The hemicellulose composition of the cell wall prevents its rapid digestion by the monogastric, particularly prior to the small intestinal enzymes.

Rumen *in vivo* wheat starch digestion posed the same problem as did the above *in vivo* porcine digestion. The extremely soluble matrix protein is digested at such a rate that the starch granule is removed from the kernel before α-amylase attack occurs. Subsequent SEM observation of split kernels placed in the rumen 12 hours in nylon bags resulted in no observable starch damage. SEM observation of the entire split kernel did reveal preferential starch digestion in the cheek area of the kernel (Fig. 8). Several factors are responsible for this specificity of attack. The primary reason is the thickness of the cell walls in this area. Cell walls tend to be thickest near the crease. Thinner walls are observed at the back of the kernel, but the thinnest cell wall area is in the central cheek region (Wolf *et al.*, 1952). The higher relative percentage of hemicellulose in the thinner walls would also account for the walls susceptibility to rumen microbiotal attack.

Furthemore, the starch granule concentration is greater near the crease. This necessarily dictates the presence of more matrix protein around these granules. Thus, although there is more protein at the outer portion of the kernel, it is of the granular storage type (Pomeranz, 1971). The lesser amount of starch and matrix protein allows digestion to proceed much more rapidly in this area. The result is total removal of cheek area endosperm while other portions of endosperm remain.

Half kernels which were mounted on aluminum stubs and then digested in incubated rumen fluid for four hours displayed the same type of attack as described above. Pinpoint hydrolysis of the starch granules were observed as well as surface digestion which formed short grooves or canals on the surface of the granule.

Ground wheat samples subjected to the Tilley and Terry method *in vitro* digestion were hydrolyzed in a similar matter regardless of the time of digestion or the source of rumen fluid. Digestion patterns were the same whether digestion was allowed to proceed only 2 hours or as long as 12 hours. Rumen fluid from a fistulated Hereford steer being fed a milo stover ration resulted in significantly less digestion per unit time than did that fluid obtained from a fistulated Angus steer receiving a milo and alfalfa ration. The digestion patterns of the starches did not vary from animal to animal.

In the five varieties of wheat considered the hydrolytic patterns were all similar. The pinpoint hydrolysis observed by purified porcine α-amylase attack was present with digestion proceeding initially in a radial direction and then along the concentric rings with amorphorous areas being digested more rapidly than the crystalline regions. The equatorial groove was attacked preferentially. Granules of all sizes were similarly attacked (Fig. 9 & 10).

Contrary to the above digestion, a second form of starch granule digestion was sometimes observed in all *in vitro*

systems for the full range of granule sizes. The digestion appears as canals on the surface of the granules. These canals, approximately .5μ diameter and 2-3μ long, are observed to proceed in a random direction terminating in a pinpoint hydrolysis point (Fig. 11). Stereo-observation seems to reveal that rather than radially digested holes the hydrolysis proceeds angularily into the granule (Fig. 12). The hydrolysis may proceed radially in inner areas of the granule. The activity of another microbial entity secreting a chemically unique form of α-amylase must be considered a definite possibility. This form may be secreted as the microbe moves across the surface of the granule with pinhole hydrolysis commencing at the site at which the microbe comes to rest. It is also possible that areas vulnerable to attack exist only in isolated locations. In this scheme, the canals would represent areas of limited vulnerability proceeding to an area susceptible to radial attack. Differences in the patterns of attack may also be the result of the differing concentrations of α-amylase. The amylase free in the rumen media would be diluted such that it could attach only most vulnerable points while that secreted from a bacteria residing on the starch granules may be concentrated such that it is capable of hydrolyzing all areas of the granules. Subsequent dilution of the enzyme would account for the relatively short length of the canals.

The possibility of lactic or hydrochloric acid being responsible for this apparent digestion was disproved by SEM

observation of samples of the varieties tested after they had been exposed to hydrochloric acid. This treatment resulted in no observable starch damage nor was starch damage observed in particles exposed to lactic acid digestion.

Conclusion

This study indicates that there is no significant difference in the hydrolytic patterns of α-amylase among the five wheat varieties tested. Any differences in feeding values of the wheats may now be attributed to those parts of the kernel other than the starchy endosperm starch. The availability of this starch to microbiotal attack (the rate of hydrolysis) may indeed, however, affect the relative feeding value of various wheats.

The hydrolytic patterns observed show several types of granule attack by microbiota. The pinhole hydrolysis directed radially through the granule is evidenced as is the preferential attack along the equatorial groove. This attack is identical to that found in purified starch-amylase systems.

A second type of attack observed results in surface canals being hewn from the granule prior to pinhole attack. A different type of α-amylase may cause the effect, or the variances in concentration of amylase as it is secreted as opposed to that free in solution, may result in differences in attack of starch granules.

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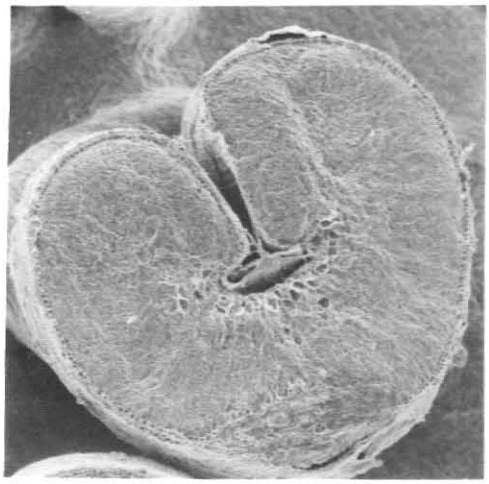

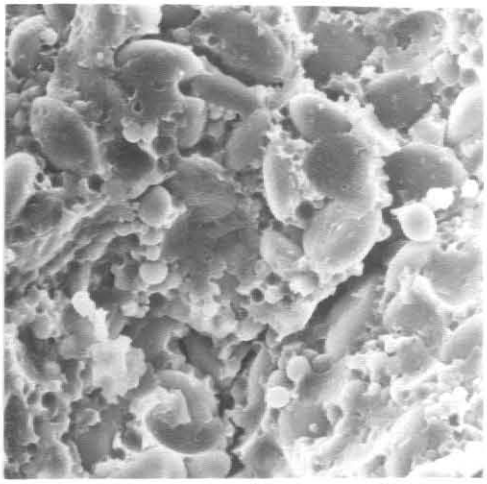

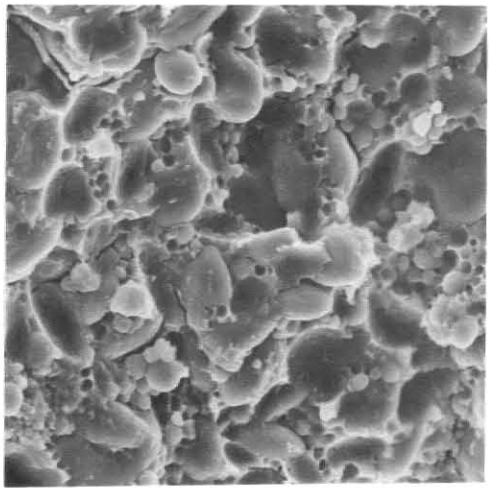

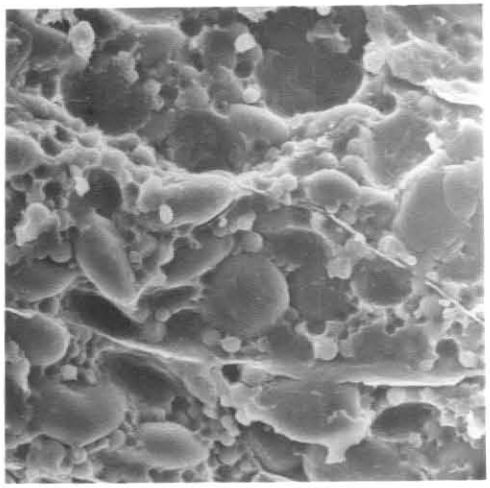

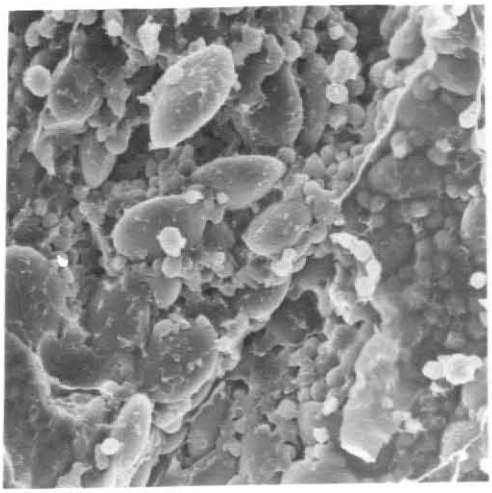

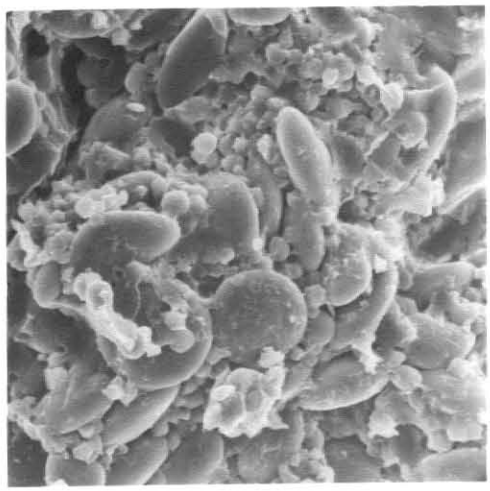

Fig. 1 Scanning electron photomicrograph of a split wheat kernel (Centurk) Pericarp, aleurone and starchy endosperm visible (30x).

Fig. 2 Scanning electron photomicrograph of starchy endosperm of wheat (Scout 66) (800x).

Fig. 3 Scanning electron photomicrograph of starchy endosperm of wheat (Eagle, GH-45) (800x).

Fig. 4 Scanning electron photomicrograph of starchy endosperm of wheat (Triumph 64, HP 35) (800x).

Fig. 5 Scanning electron photomicrograph of starchy endosperm of wheat (Centurk) (800x).

Fig. 6 Scanning electron photomicrograph of starchy endosperm of wheat (Parker, CF-18) (800x).

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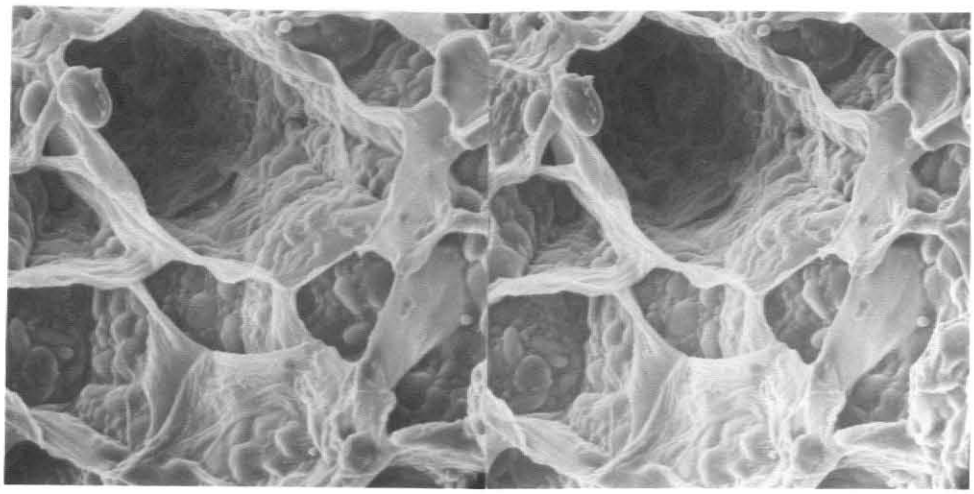
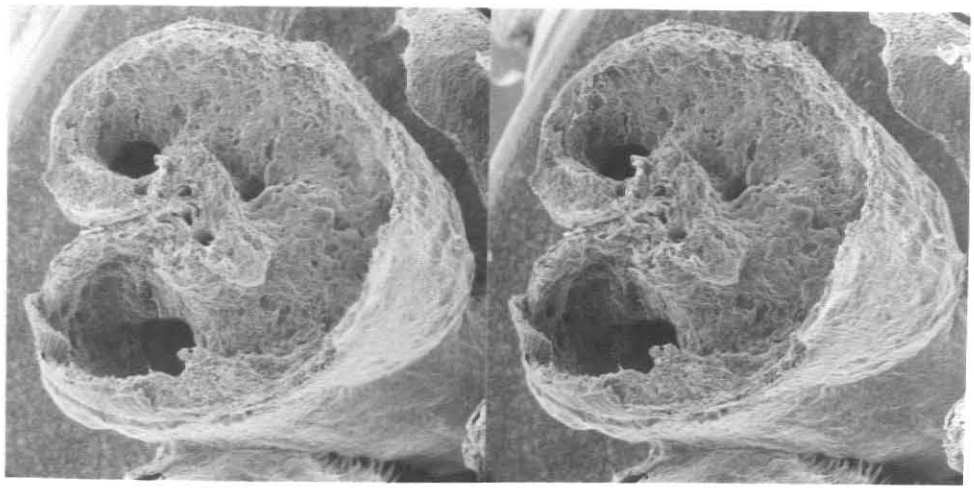




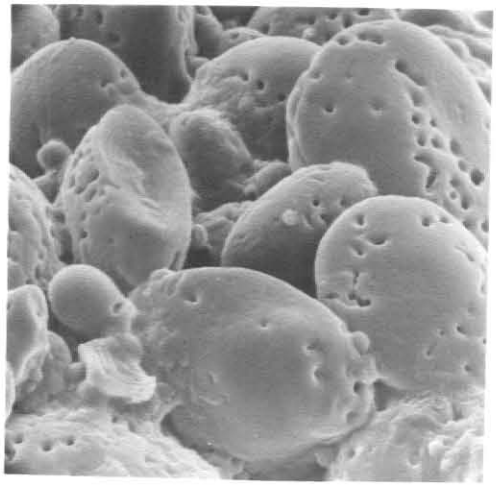
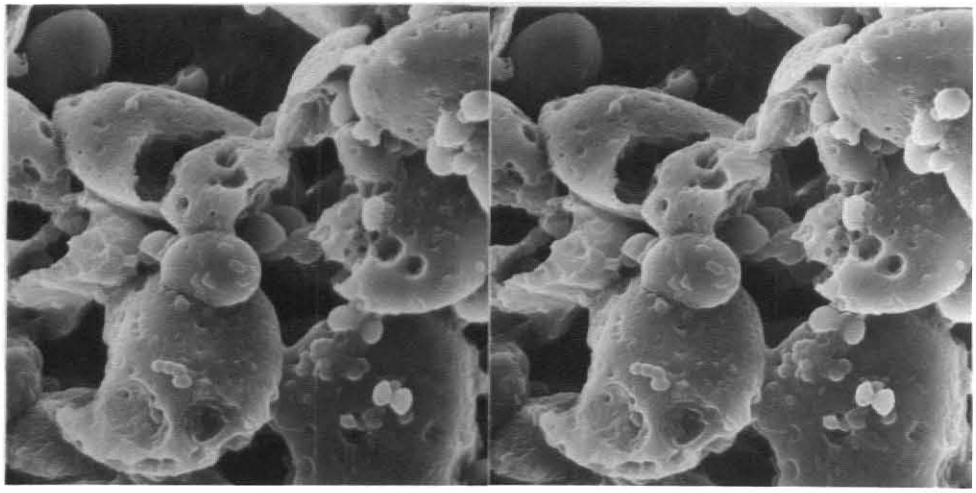


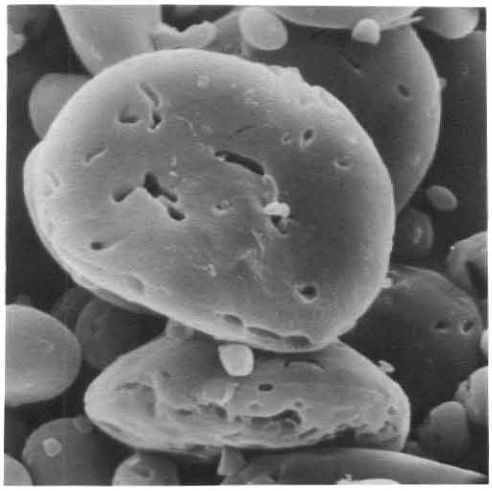
Fig. 7 Scanning electron stereo-photomicrograph of starchy endosperm starch (Centurk) following 3 hour porcine *in vivo* digestion (400x).

Fig. 8 Scanning electron stereo-photomicrograph of a split wheat kernel (Parker) following 12 hr. suspension in fistulated steer (30x).

Fig. 9 Scanning electron photomicrograph of starchy endosperm starch (Scout) following 8 hr. exposure to *in vitro* rumen fermentation (2200x).

Reduction Factor .75





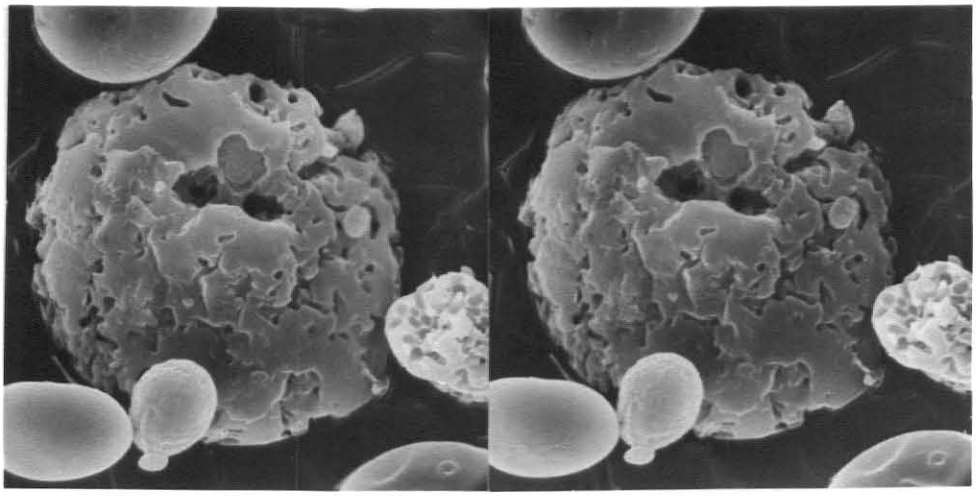


Fig. 10 Scanning electron stereo-photomicrograph of starchy endosperm starch (Centurk) following 8 hr. exposure to in vitro rumen fermentation (1700x).

Fig. 11 Scanning electron photomicrograph of starchy endosperm starch (Centurk) following 8 hr. exposure to in vitro rumen fermentation (2700x).

Fig. 12 Scanning electron stereo-photomicrograph of starchy endosperm starch (Scout) following 8 hr. exposure to in vitro rumen fermentation. (2700x).

Reduction Factor .75

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RUMEN DIGESTION OF WHEAT STARCH AS OBSERVED BY SCANNING ELECTRON MICROSCOPY

by

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B.A., St. Mary of the Plains College, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Scanning electron microscopy (SEM) was used to characterize the starch, and its digestion by α-amylase in the endosperm of five selected varieties of hard red winter wheat: Scout, Eagle, Triumph, Centurk and Parker.

Split and ground kernels were observed via SEM prior to and following hydrolysis with purified α-amylase, *in vivo* swine digestion and *in vivo* and *in vitro* rumen digestion.

Modes of enzymatic attack were observed to determine similarities and differences of starch granule digestion among the wheat varieties.

Purified amylase and porcine digestion affected wheat starch similar to milo (Davis and Harbers, 1974). Rumen microbes attacked the cheek area of the starchy endosperm preferentially due to its relatively smaller matrix protein content. They carried out the same type of point hydrolysis of the starch granule as pure α-amylase, but in addition, formed grooves on the granule surface about .5 μ wide and about 2 to 3 μ long. The grooves are thought to be the result of a high enzyme concentration in the area of the secreting microbe. Structural organization of the amylose and amylopectins at the starch granule surface, as well as chemically different amyloses, may be responsible for the particular patterns of attack.

Starch granules from the varieties studied tend to have similar hydrolytic patterns; suggesting that differences in the relative utilization potentials of various wheats lie in areas other than the endosperm starch.