Gamma Irradiation of Hulless Barley: Effect on Grain Composition, β-Glucans and Starch[1]

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ABSTRACT

Scout hulless barley was gamma irradiated to 10 Mrad (100 kGy) with cobalt-60. Irradiation had no major, apparent effect on grain composition, except for starch and β-glucans. Total nitrogen, nonprotein nitrogen, and amino acid composition of nonirradiated and irradiated barleys were identical. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of the two barleys were also identical. However, protein bands corresponding to 45 and 94 kDa showed reduced staining. Irradiated barley completely lost viscoamylogram properties and had reduced endosperm cell wall fluorescence but higher (75%) soluble β-glucans. Irradiated barley starch contained lower molecular weight amylose and amylopectin compared to the nonirradiated barley starch. However, the two starches showed similar endothermic properties (differential scanning calorimetry) and gelatinization temperatures. The starch granules appeared to be normal externally but were fractured internally. This was particularly evident during the later stages of gelatinization. Irradiation increased the susceptibility of barley starch to α- and β-amylase hydrolysis. Irradiated barley had lower in vivo dry matter (2.6%), starch (1.0%), and protein (5.2%) digestibilities as determined by mouse-feeding experiments.

Barley contains 2–10% by weight nonstarchy polysaccharides, (1→3)(1→4)-β-D-glucans (β-glucans), distributed largely in the endosperm cell walls. The deleterious effects of β-glucans in malting and feed barleys have been described elsewhere (Thomas and Pyler 1986, Classen et al 1985). In feed barley, the deleterious effects can be completely removed by treating the grain with exogenous preparations of β-glucanases or by gamma irradiation. When hulless barley irradiated to 10 Mrad (100 kGy) was fed to chicks, it was equal or superior to wheat in performance as measured by weight gain, fat, and starch absorptions (Classen et al 1985). Gamma irradiation has also been used to improve the nutritional quality of rye where pentosans, rather than β-glucans, cause problems in chicks similar to those encountered in barley (Patel et al 1980, Campbell et al 1983).

Gamma rays, like X-rays, have short wavelengths and are capable of hydrolyzing chemical bonds, thereby cleaving molecules into small fragments that may be either electrically charged ions or uncharged free radicals. The degree of cleavage is largely proportional to dose, which may vary considerably depending upon the treatment objective. Low doses (1–10 kGy) are generally used to prevent maturation of fruits and vegetables and destruction of food-borne pathogens, whereas high doses (30–50 kGy), are employed for the destruction of bacterial spores. There is no recommended dose for the disruption of chemical bonds (Urbain 1984). An extensive literature survey on the effect of gamma irradiation on various feeds and foods has been compiled by McManus (1982) and the Council for Agricultural Science and Technology (1986).

The beneficial effect of gamma irradiation on hulless barley for poultry feed has been reported previously (Classen et al 1985). Gamma-irradiated barley had greatly reduced viscosity, which indicated cleavage of β-glucans and possibly of starch. The present study was conducted to determine the extent of cleavage of the polysaccharides, as well as to examine the effect of gamma irradiation on barley composition in general.

MATERIALS AND METHODS

Materials

Scout (two-rowed) hulless barley, grown locally at the University of Saskatchewan experimental plots and obtained from B. Rossnagel of the Department of Crop Science and Plant Ecology, was divided into two lots. One lot was sent to Atomic Energy of Canada Ltd., Kanata, ON, for irradiation with cobalt-60 to a minimum dose of 10 Mrad (100 kGy) at a rate of 2.9 Mrad/hr.

The nonirradiated and irradiated barley samples were ground in a Udy cyclone mill to pass a 0.5-mm screen, and the meals were stored at 5° C.

α-Amylase, type X-A, from *Aspergillus oryzae*; β-amylase, type 11-B, from barley; porcine stomach mucosa pepsin, 2X crystallized; bovine pancreas trypsin, 2X crystallized; bovine pancreas α-chymotrypsin, 3X crystallized; and porcine peptidase, grade III, were purchased from the Sigma Chemical Co. (St. Louis, MO). Lichenase and β-glucosidase were obtained from Biocon U.S. Ltd. (Lexington, KY). All other chemicals and reagents were of analytical reagent grade.

Methods

Starch was isolated from the grain as described by Adkins and Greenwood (1966), except that the slurry was successively screened through 210- and 52-μm screens and deproteinized by the addition of 0.2% NaOH. Three times as much starch was recovered from the nonirradiated barley as from the irradiated barley.

Moisture, total nitrogen, and ether extract were determined by AOAC official methods (1984). A 70% ethanol extract of the meal was used to determine nonprotein nitrogen (micro-Kjeldahl) and soluble sugars; the latter were estimated by the method of Dubois et al (1956) with raffinose as a standard. Starch was determined by the method of Chiang and Johnson (1977), and free fatty acids by the method of Lowry and Tinsley (1976). Soluble and total β-glucans were determined by methods described previously (Bhatty 1987). Amino acid composition was determined on a Beckman 121 C amino acid analyzer after hydrolyzing the meal samples at 110° C with an excess of 5.7*M* HCl for 22 hr.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of nonirradiated and irradiated barley meal proteins and of hordeins extracted from the meal with 55% (v/v) aqueous isopropanol containing 2% (v/v) 2-mercaptoethanol at room temperature (23° C) and at 60° C was performed as described by Heisel et al (1986), except that the gels were stained in 15% trichloroacetic acid containing 0.07% Coomassie Brilliant Blue R-250.

Viscoamylograms of nonirradiated and irradiated barley meal were determined on a 10% slurry (pH 5.5) with a Brabender Viscoamylograph, using a 700 cm·g sensitivity cartridge. The heating and cooling rates were 1.5° C/min.

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Starch hydrolysis by both α- and β-amylases was measured by determining the sugars liberated by the dinitrosalicylic acid procedure (Bernfeld 1955) with maltose as a standard.

Starch granule gelatinization temperatures were measured with a Mettler FP52 hot-stage coupled to a Zeiss photomicroscope. The temperature increase was 2° C/min over the range 48–70° C. Gelatinization temperatures were recorded when approximately 10, 50, and 90% of the starch was gelatinized. Photographs of starch granules were taken at the start of the temperature operating range (48° C) and then at each degree from 50 to 70° C, using Kodacolor VR 400 film.

Gel-Permeation Chromatography

Starch. Starch granules (approximately 30 mg) were suspended in 1.0 ml of 90% dimethyl sulfoxide (DMSO)-6*M* urea solution and flushed with nitrogen. The suspension was heated at 120° C for 16 hr, and the soluble starch precipitated with two volumes of ethanol. The procedure was repeated for the irradiated sample, but this was not necessary for the nonirradiated sample because it was more soluble. The precipitated starch was taken up in 0.1*M* NaCl, heated, and diluted to 10 ml with the solvent. Clear solutions were obtained for both the starch samples, but that of the nonirradiated barley starch sample was always slightly turbid.

Starch solution (25 μl) was applied to a Fractogel TSK65F (BDH Chemical Canada Ltd.) column (160 × 0.9 cm) maintained at 60° C and eluted with 0.1*M* NaCl at 20 ml/hr. The column effluent was continuously monitored with orcinol-sulfuric acid reagent as described previously (LaBerge et al 1973). Column recoveries varied from 92 to 97%. The void volume of the column was determined using amylopectin eluted under identical conditions. Glucose was used to measure the total elution volume of the column.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) studies on isolated starch granules were carried out as described by Biliaderis et al (1985), using 20 and 40% starch slurries.

β-Glucan

The meal samples were boiled for 5 min with 80% ethanol in a ratio of 1:20. The meal was then extracted at 65° C with 5.0 ml of 0.02*M* sodium acetate buffer (pH 5.5), and the extract volume was adjusted to 7.5 ml with the acetate buffer. An aliquot of the extract was used to determine soluble β-glucan by the procedure of McCleary and Glennie-Holmes (1985); the same procedure was also used for the determination of total β-glucans. An aliquot of the extract (0.5 ml) was applied to a Fractogel TSK65F (88 × 1.6 cm) column and eluted at room temperature at 20 ml/hr with the acetate buffer. Fractions (2.0 ml) were collected in tubes containing 0.5 ml of the following mixture: 50 μl of lichenase, 50 μl of β-glucosidase, and 25.0 ml of the acetate buffer. The glucose liberated was measured with the glucose-hexokinase reagent (Boehringer-Mannheim Gluco-quant kit). β-Glucan recoveries were always greater than 90%.

Microscopy

For light microscopy, barley kernels were fixed in glutaraldehyde, dehydrated, and embedded in glycol methacrylate (Feder and O'Brien 1968). Sections (2 μm) were cut with glass knives from the midsection of the grain on an LKB 2218 Historange microtome and mounted on glass slides (Morrison and Dushnicky 1982). The sections were stained with Calcofluor white M2R (Feder and O'Brien 1968) and examined under a Wild Leitz Orthoplan fluorescence microscope. Photographs were taken on Kodacolor VR 400 and Ektachrome 400 films.

Kernels were prepared for scanning electron microscopy and photographed as described previously (MacGregor et al 1983).

TABLE I
Chemical Composition of Nonirradiated and Irradiated Hulless Barley

Component	Nonirradiated	Irradiated[a]
Moisture, %	9.1 ± 0.1	8.5 ± 0.0*
Nitrogen, %	2.6 ± 0.0	2.5 ± 0.0
Nonprotein nitrogen, mg/g	2.6 ± 0.0	2.5 ± 0.0
Ether extract, %	2.2 ± 0.0	2.1 ± 0.0
Free fatty acids, mg/g	3.3 ± 0.2	4.2 ± 0.2
Soluble sugars, %	4.3 ± 0.1	4.3 ± 0.1
Soluble β-glucan, %	1.6 ± 0.1	2.8 ± 0.0**
Total β-glucan, %	4.4 ± 0.2	4.5 ± 0.3
Starch, %	71.4 ± 0.2	73.1 ± 0.5*

[a] Means in the same row are significantly different at $P < 0.05$ (*), $P < 0.01$ (**).

Apparent Digestibilities

The diet for the determination of apparent dry matter, protein, and starch digestibilities was formulated as described earlier (Bhatty and Whitaker 1987). Each diet was fed ad libitum for seven days to nine female mice housed three per cage (three replicates); the fecal collection period was the last four days. Dry matter, total nitrogen, and starch contents of the fecal material were determined according to procedures described above.

RESULTS AND DISCUSSION

Composition

Table I shows the composition of the nonirradiated and irradiated barley. Irradiation has a small drying effect, probably due to temperature rise. The composition of the two barley samples was significantly different only for free fatty acids, soluble β-glucans, and starch contents. The total β-glucan content of the two samples was identical. A higher (75%) soluble β-glucan content, determined by two different procedures (McCleary and Glennie-Holmes 1985, Bhatty 1987), of the irradiated sample suggested that the starch and the nonstarchy polysaccharide was cleaved to smaller fragments, but not eliminated. The irradiated barley completely lost swelling properties (Fig. 1), suggesting that starch as well as β-glucans had been sufficiently degraded by irradiation. The higher starch content of irradiated barley was likely due to improved dispersal of meal in water during starch extraction and gelatinization. Identical soluble sugar contents of the nonirradiated and irradiated barley suggested that little or no breakdown of starch or other polysaccharides to ethanol-soluble sugars occurred.

Effects of Irradiation

Apparently, gamma irradiation had no major effect on barley proteins. The nonirradiated and irradiated barleys had similar total nitrogen, nonprotein nitrogen (Table I), and amino acid composition (Table II), which was nearly identical for the individual as well as the total amino acids. Similarly, the SDS-PAGE patterns of the two barleys and of hordein extracted under different conditions seemed identical, although the high molecular

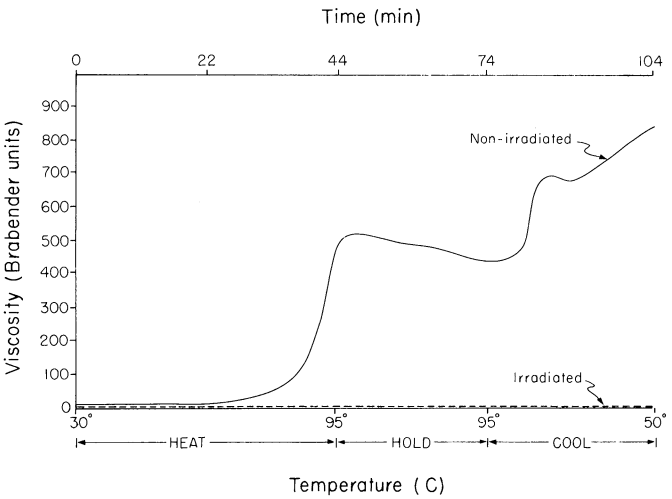

Fig. 1. The viscoamylogram properties of a 10% slurry of nonirradiated and irradiated barley meal.

weight bands in the irradiated samples (tracks 3, 5, and 7) corresponding to 45 and 94 kDa were less intensely stained (Fig. 2). This may suggest some degradation of these bands, because the amount of protein applied to each set was identical. The SDS-PAGE patterns clearly identify (tracks 4 and 5, 6 and 7) D, C, and B hordeins with an apparent molecular weight range of about 35 to 100 kDa, in agreement with those reported for many cultivars of Canadian and American barleys (Heisel et al 1986, Marchylo 1987). Thus, the data suggest that the effect of gamma irradiation in barley was selective, and that the protein composition of the grain was not greatly affected.

Few studies have been reported in the literature on gamma irradiation of barley proteins. A number of studies on irradiation of wheat proteins (gluten) have been reported. Kennedy (1965) reported small losses (<10%) in leucine, isoleucine, and methionine content of gluten irradiated at 5 Mrad; methionine was affected most by the radiation treatment. Doguchi (1969) reported an 8% reduction in cystine but no change in methionine content of wheat irradiated at 10 Mrad. Irradiation reduced both the gliadin and glutenin proteins, and a dose greater than 3 Mrad seemed to cause change in molecular configuration of the gliadin fraction. In another study (Srinivas et al 1972), wheat irradiated at 1 Mrad revealed no differences in its amino acid composition or that of the gluten fraction. However, there was a small increase in the free amino acid content of the irradiated sample; free isoleucine, tyrosine, valine, and alanine were particularly noticeable. Thus, the effect of gamma irradiation on wheat protein seems variable and inconsistent. There may be many reasons for these differences. The wheat proteins, particularly the glutenin fraction, are stabilized by inter- and intramolecular-SH bonds, which are susceptible to irradiation (Lee 1962). However, other mechanisms may also be involved in structural alterations of gliadin proteins on irradiation. Free amino acids and isolated protein may be more susceptible to irradiation than protein-bound amino acids and intact proteins. Protein degradation increases in dilute solutions, as shown by the effect of X-rays on the viscosity of gluten (Lloyd et al 1957). Furthermore, on irradiation protein and peptides may cleave not at the peptide bond but at side chains, giving rise to hydrocarbons such as *n*-alkanes, benzene, and toluene (McManus 1982), which were not measured in any of the above studies.

β-Glucans and Starch

Figure 3 shows micrographs of Calcofluor-stained cross sections of nonirradiated and irradiated barley kernels. In the nonirradiated barley (Fig. 3a and b), the endosperm cell walls gave a bright fluorescence showing marked affinity of the stain for the β-glucosyl bonds of the cell wall polysaccharides. The subaleurone layer (mono layer) cells seemed smaller but fluoresced just as intensely as the larger endosperm cells. The clearly visible tricellular aleurone layer fluoresced weakly, indicating that some β-glucans were present in this tissue as well, although this may also be partly due to autofluorescence of ferulic acid (Fulcher et al 1972). In the irradiated barley (Fig. 3c and d), the fluorescence was greatly reduced in the endosperm cell walls, confirming degradation of the β-glucans. At the highest magnification (400×, Fig. 3d), the fluorescence of the irradiated barley was barely visible, unlike that of the nonirradiated barley (Fig. 3b). The fluorescent pattern suggested that in Scout barley, and presumably in other barleys, β-glucans are uniformly distributed in the endosperm cell walls, although barley mutants having uniformly thick cell walls (high β-glucans) or thin cell walls (low β-glucans) have been reported (Aastrup 1983). Such a distribution of β-glucans in barley is different from that in oats, where the highest concentration may be immediately below the monocellular aleurone layer. In Hinoats (high-protein oats) some of the subaleurone cells were five times thicker than the midendosperm cell walls (Fulcher and Wood 1983).

The reduction in molecular weight of β-glucans on irradiation was confirmed by gel permeation chromatography (Fig. 4). The method used for the determination of β-glucans in the effluent fractions was specific (McCleary and Glennie-Holmes 1985), and there was little chance of glucose contamination from barley starch or pentosans. Under the chromatographic conditions used, the recovery of β-glucans was always greater than 90%. Figure 4 shows that β-glucans of nonirradiated barley eluted in a broad peak, suggesting a mixture of various components. In the case of irradiated barley, the elution of β-glucans was considerably retarded, suggesting a reduced molecular weight of the polysaccharide. No attempt was made to estimate the molecular weight reduction of the polysaccharide, which would have been difficult without the availability of appropriate molecular weight standards. The complete loss of swelling properties (Fig. 1), reduced fluorescence (Fig. 3), and a retarded elution profile of β-glucans (Fig. 4) suggested a random cleavage of the glucosyl bonds, probably in the interior of the polymer caused by the penetrating power of gamma rays, producing lower molecular weight fragments. These fragments seemed to have coeluted during gel-permeation chromatography. Lack of increase in the ethanol-soluble sugars of irradiated barley (Table I) confirmed that small molecular weight products were not present in irradiated barley.

The starch yield from irradiated barley was only about one-third of that from nonirradiated barley. Irradiated barley starch was more soluble, giving a clear solution in DMSO-urea, whereas clear

TABLE II
Amino Acid Composition of Nonirradiated and Irradiated Scout Hulless Barley (dry basis)

Amino Acid	Nonirradiated (mol %)	Irradiated (mol %)
Lysine	3.3 ± 0.2	3.2 ± 0.2
Histidine	2.2 ± 0.1	2.1 ± 0.2
Arginine	4.0 ± 0.0	3.9 ± 0.1
Aspartic acid	6.4 ± 0.1	6.0 ± 0.1
Threonine	4.3 ± 0.1	4.2 ± 0.0
Serine	6.2 ± 0.1	6.2 ± 0.1
Glutamic acid	28.6 ± 0.2	28.5 ± 0.3
Proline	15.2 ± 0.1	15.1 ± 0.2
Glycine	7.3 ± 0.1	7.2 ± 0.1
Alanine	6.3 ± 0.1	6.4 ± 0.0
Valine	6.1 ± 0.1	6.1 ± 0.1
Methionine	1.4 ± 0.1	1.6 ± 0.0
Isoleucine	3.7 ± 0.1	3.9 ± 0.2
Leucine	7.9 ± 0.1	7.9 ± 0.1
Tyrosine	2.3 ± 0.2	2.3 ± 0.0
Phenylalanine	4.9 ± 0.2	4.9 ± 0.3
Total	110.1 ± 1.9	109.5 ± 2.2

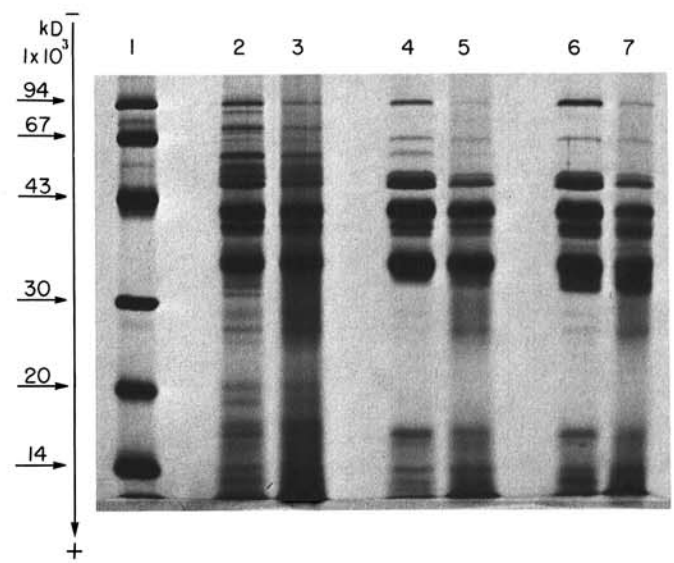

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of nonirradiated and irradiated barley meal proteins (tracks **2** and **3**, respectively), hordein fraction extracted at room temperature (tracks **4** and **5**, respectively), and at 60° C (tracks **6** and **7**, respectively). Track **1**, low molecular weight standard.

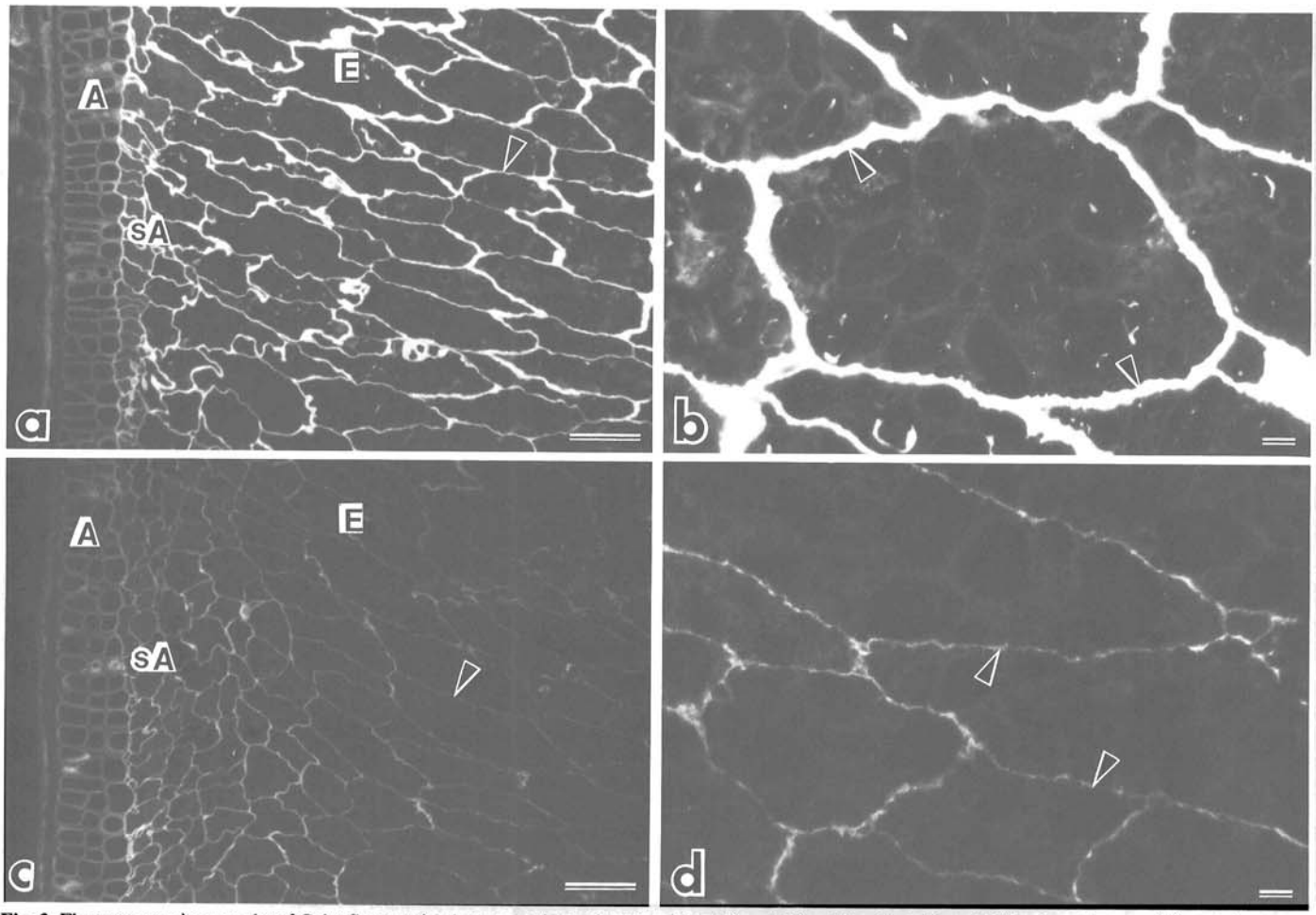

Fig. 3. Fluorescent micrographs of Calcofluor-stained cross sections of nonirradiated (**a** and **b**) and irradiated (**c** and **d**) barley: aleurone (**A**), subaleurone (**sA**), endosperm (**E**), cell walls (**arrows**). In a and c, bar = 100 μm, magnification 100; in b and d, bar = 10 μm, magnification 400.

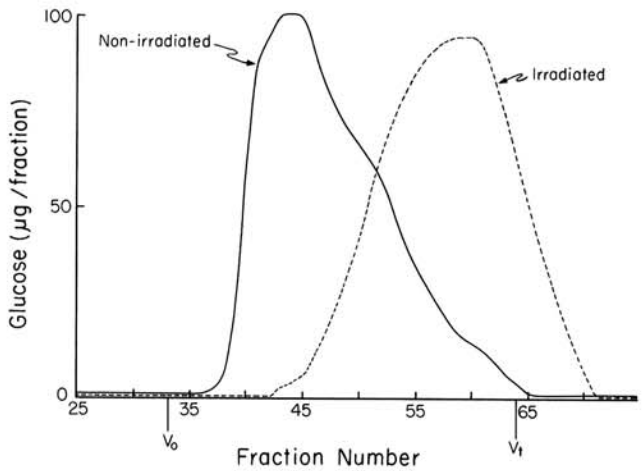

Fig. 4. Elution profile of nonirradiated and irradiated barley β-glucan on gel-permeation chromatography. Vo = void volume; Vt = total elution volume of the column.

solutions were not obtained with starch from nonirradiated barley. The increased solubility probably led to loss of starch during its isolation from the irradiated barley. During gel-permeation chromatography, nonirradiated barley starch resolved into two peaks (Fig. 5). The major peak, eluted at the void volume, probably consisted of amylopectin, whereas the other polydispersed peak would be mainly amylose (Yeh et al 1981). A slight depression at Vt was most likely due to traces of DMSO present in the sample. In contrast to nonirradiated barley starch, irradiated barley starch gave only a single broad peak that was eluted after the amylose peak in nonirradiated barley starch. The peak at the void volume, corresponding to amylopectin, completely disappeared, suggesting that the amylopectin had been degraded to lower molecular weight fragments that coeluted with the partially degraded amylose component. These results suggested that gamma irradiation had broken some covalent bonds in the starch granule in much the same way these bonds were broken within β-glucan molecules.

The structural alterations observed in the irradiated barley starch on gel permeation chromatography made it more susceptible to both α- and β-amylase hydrolysis; the increase in the reducing sugar was much greater for α-amylase hydrolysis (Fig. 6). The increase in β-amylase hydrolysis may partly be due to contamination of the β-amylase with a small amount of α-amylase. Extensive studies of the effect of gamma irradiation on cereal carbohydrates (in situ or isolated) have been reported in the literature (Ananthaswamy et al 1970, Dauphin and Saint-Lebe 1977). These studies report many dose-related effects of gamma irradiation on starch, some of which were observed in the present study, such as increase in water solubility, reduction in chain length as shown by decreased iodine affinity, reduction in viscosity, susceptibility to amylolysis with different reaction rates for amylose and amylopectin, increase in acidity, and the appearance of many radiolytic products. These changes are the result of random cleavages of the glucosyl bonds rather than a systematic rupture of the polysaccharide.

Starch Granule Structure

The effect of gamma irradiation on the starch granule was investigated using scanning and light microscopy. Figure 7 shows longitudinal sections of the endosperm and starch granule of nonirradiated (Fig. 7a and b) and irradiated (Fig. 7c and d) barleys, respectively. In both the barleys, the grain structure appeared

normal and similar, showing scutellar epithelium, endosperm, cell walls, and starch granules embedded in the protein matrix. No irradiation damage of the structure seemed to have occurred. Similarly, the structure of the starch granules from the nonirradiated and irradiated barleys showed no apparent damage. Both samples contained the bimodal distribution of granules varying in size from 10–15 μm (large) to 2–5 μm (small) in diameter. The granule size, shape, and distribution appeared typical of normal barley starch.

In another experiment, gelatinization temperatures of starch granules from nonirradiated and irradiated barley were determined, and average results from several replicates are shown in Table III. Granules were judged to have gelatinized when they had swollen irreversibly and had lost their birefringence. Granules from nonirradiated barley started to swell and lose birefringence at about 60° C. As the temperature increased, both the extent of swelling and the number of swollen granules increased. The process was 90% complete at 63.1° C. Granules from irradiated barley had a slightly lower gelatinization temperature.

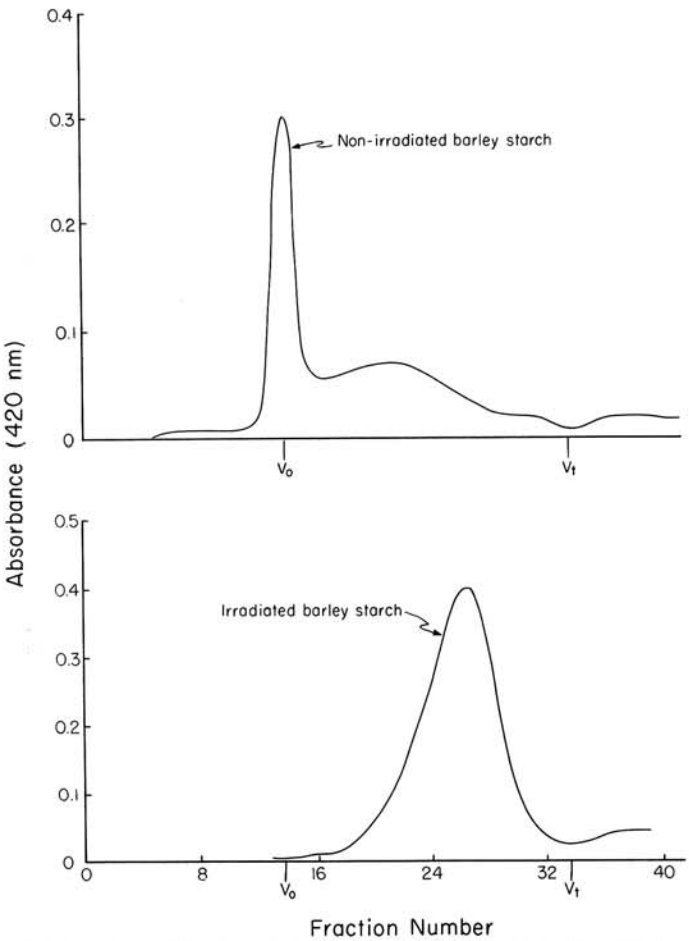

Fig. 5. Elution profile of starch, isolated from nonirradiated and irradiated barley, on gel-permeation chromatography. Vo = void volume; Vt = total elution volume of the column.

Endothermic properties of the two starches, determined by differential scanning calorimetry, were investigated at two concentrations, 20 and 40% (w/w). Only at the higher starch concentration was melting of the amylose-lipid complex observed (Table IV). No significant differences were observed between nonirradiated and irradiated samples for onset and transition temperatures of gelatinization, although values for the irradiated starch were always lower than those for the nonirradiated sample. Heats of transition of both gelatinization and melting of the amylose-lipid complex were also similar for the two starch samples. These results are in good agreement with the gelatinization temperature data reported in Table III.

Light micrographs of the starches were taken at regular intervals during the gelatinization process. At 48° C (Fig. 8a and c), no swelling or loss of birefringence was detected in either sample, and there were no apparent physical differences between the two starch samples. When the granules started to swell, however, there was an obvious difference in the way in which the two sets of granules behaved. Granules from the nonirradiated barley (Fig. 8b) increased significantly in volume as the temperature was raised above 60° C. However, the granules maintained their outline and integrity—they did not break apart. When granules from irradiated barley started to swell, internal fissures appeared in the granule interior, and in many cases these extended to the granule surface. The granules appeared to break apart (Fig. 8d). Breakage of covalent bonds in the starch granules by gamma radiation appeared to weaken granule structure, but this only became apparent when the granules started to gelatinize. Until that time, hydrogen bonding within the granule was, presumably, strong enough to maintain integrity.

TABLE III
Gelatinization Temperatures of Starch Granules from Nonirradiated and Irradiated Barley

	% Granules Gelatinized		
Barley	10% (°C)	50% (°C)	90% (°C)
Nonirradiated	60.1	61.5	63.1
Irradiated	58.4	60.1	62.2

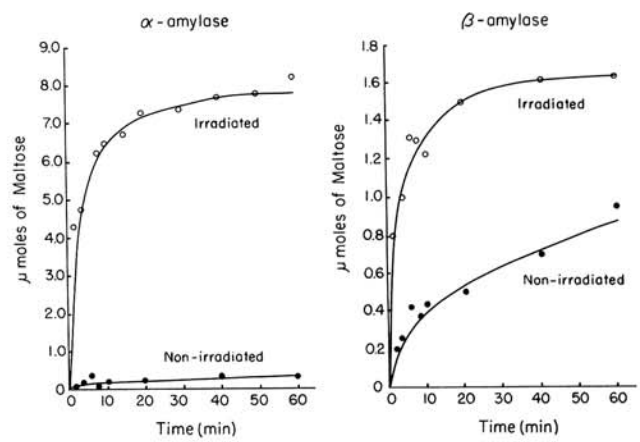

Fig. 6. Hydrolysis of nonirradiated and irradiated barley starch by α- and β-amylases.

TABLE IV
Differential Scanning Calorimetry Characteristics of Starch Granules from Nonirradiated and Irradiated Barley

		Gelatinization[a]			Melting of Amylose-Lipid Complex[a]		
Sample	Concentration (w/w)	To (°C)	Tp (°C)	ΔH (J/g)	To (°C)	Tp (°C)	ΔH (J/g)
Nonirradiated	20%	61.09	64.06	9.85	...	...	...
Irradiated	20%	60.25	63.61	9.98	...	...	...
Nonirradiated	40%	61.49	64.05	9.14	91.91	103.90	2.38
Irradiated	40%	60.22	63.27	9.45	92.52	106.57	2.69

[a]To, Tp, and ΔH refer to onset temperature, endothermic transition temperature, and heat of transition, respectively.

When hydrogen bonding within and between starch components was weakened by the influx of water, granular structure began to break down. Even if gamma radiation disrupted some hydrogen bonds, new bonds could be formed readily, whereas new covalent bonds were not likely to be formed to replace those that had been broken by irradiation.

Barley Digestibility

Protein digestibility of nonirradiated and irradiated barleys was first determined using an in vitro procedure (Hsu et al 1977); casein was included for comparison. Figure 9 shows the larger drop in pH (hence more digestible) for irradiated barley compared with nonirradiated barley over the entire assay time of 10 min. The *t* test showed the difference in pH between nonirradiated and irradiated barleys to be marginally significant ($P < 0.05$) for a 10-min assay.

The multienzyme in vitro assay procedure of Hsu et al (1977) does not always reveal differences in protein digestibility because the enzyme mixture excludes pepsin which, being nonspecific, hydrolyzes peptide bonds more extensively than do the other proteases. In a previous study (Bhatty and Whitaker 1987), pepsin digestibility alone was positively correlated with in vivo digestibility of barley protein. However, in the present study, pepsin digestibility of nonirradiated and irradiated barleys was identical. The in vitro protein digestibility data of nonirradiated and irradiated barley thus seemed contradictory and inconclusive. To obtain additional evidence, digestibility of nonirradiated and irradiated barley was determined in vivo. Data in Table V show that dry matter, protein, and starch digestibilities of irradiated barley, though not greatly reduced, were, however, significantly lower than those of nonirradiated barley, the differences being 1.0, 2.6, and 5.2% for starch, dry matter, and protein digestibilities respectively. Earlier studies (Metta and Johnson 1959, Kennedy 1965) reported some loss in the nutritive value of corn and wheat proteins on irradiation. In the case of gluten proteins, the loss in nutritive quality was entirely due to a decrease in methionine (Kennedy 1965). However, in the present study no loss of methionine or any other essential amino acids occurred on irradiation (Table II). Irradiation can denature native proteins by breaking hydrogen bonds and other linkages involved in protein structure. The reasons for the lower protein digestibility of irradiated barley, similar to one obtained in corn at a dose of 9.3 Mrad by Metta and Johnson (1959), are not clear. There is the possibility that some degradation of hordein proteins occurred as suggested by reduced staining of some of the protein bands (Fig. 2). It is not known if this degradation was responsible for the reduced protein digestibility of irradiated barley.

The dry matter, starch, and protein digestibility data in Table V

TABLE V
Apparent Dry Matter, Protein, and Starch Digestibilities of Nonirradiated and Irradiated Hulless Barley Determined by Mouse Feeding (dry basis)

	Apparent Digestibility, %	
Component	**Nonirradiated**	**Irradiated[a]**
Dry matter	85.3 ± 0.1	83.1 ± 0.0*
Protein	77.5 ± 0.4	73.7 ± 0.3**
Starch	98.1 ± 0.1	97.1 ± 0.1**

[a]Means in the same row are significantly different at $P < 0.05$ (*), $P < 0.01$ (**).

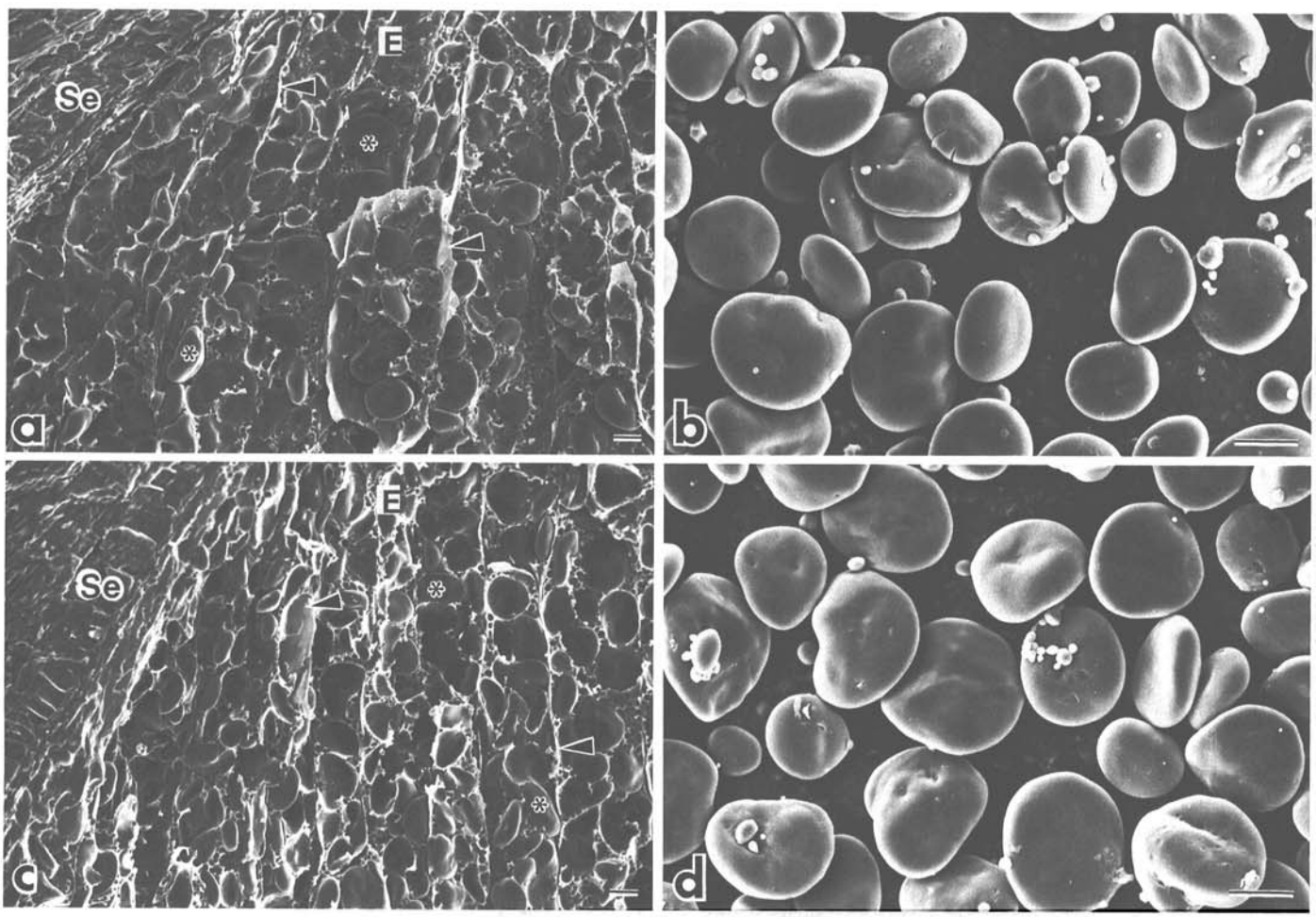

Fig. 7. Scanning electron micrographs of longitudinal section of **a,** nonirradiated barley grain, and **b,** starch; **c,** longitudinal section of irradiated barley grain, and **d,** starch. Scutellar epithelium (**Se**), endosperm (**E**), cell wall (**arrows**), and starch granule (*). Bars = 10 μm.

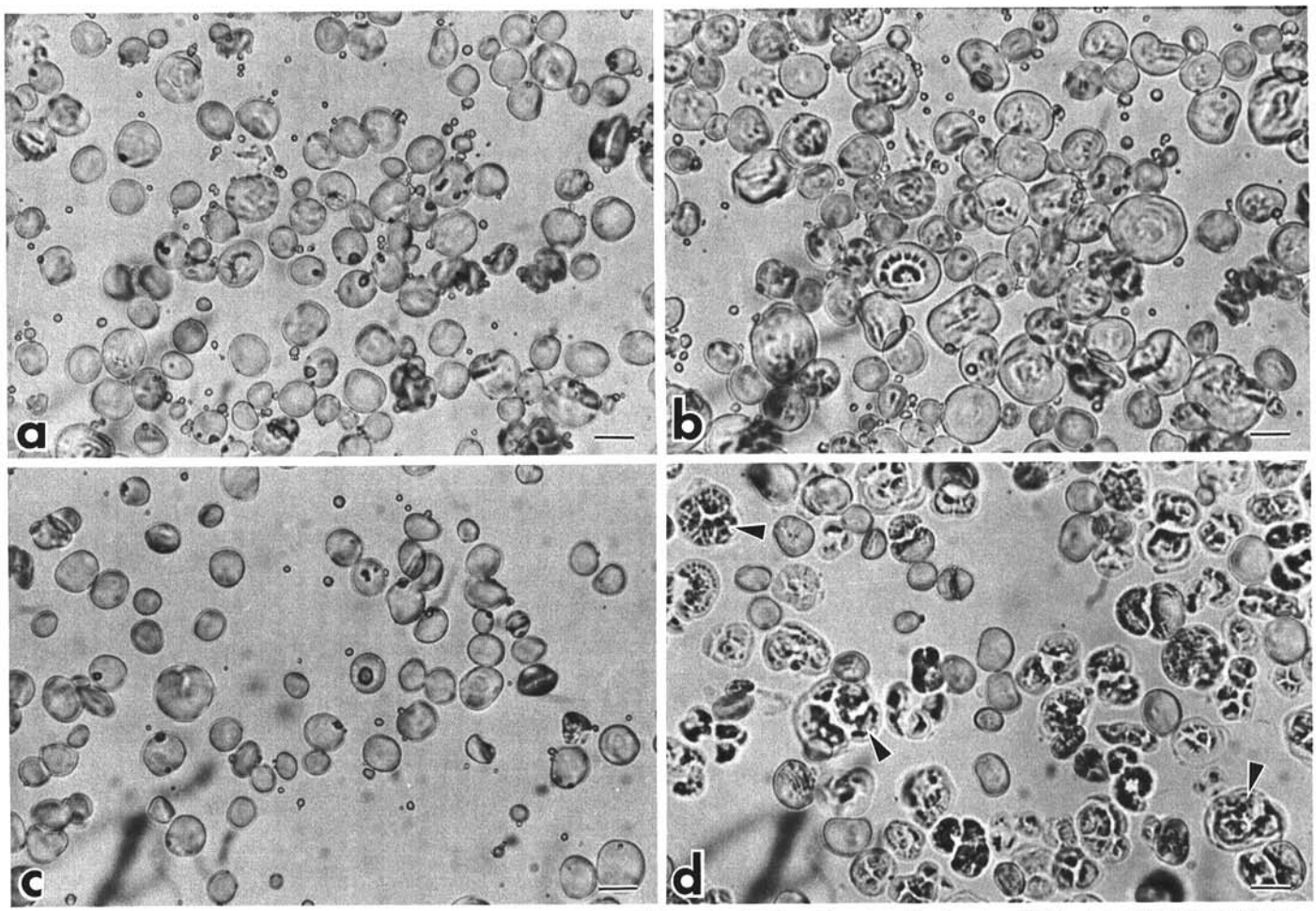

Fig. 8. Light micrographs of starch granules of nonirradiated barley at **a**, 48° C and **b**, 62° C; and irradiated barley at **c**, 48° C and **d**, 62° C. Bars = 20 μm. **Arrows** indicate internally fractured starch granules.

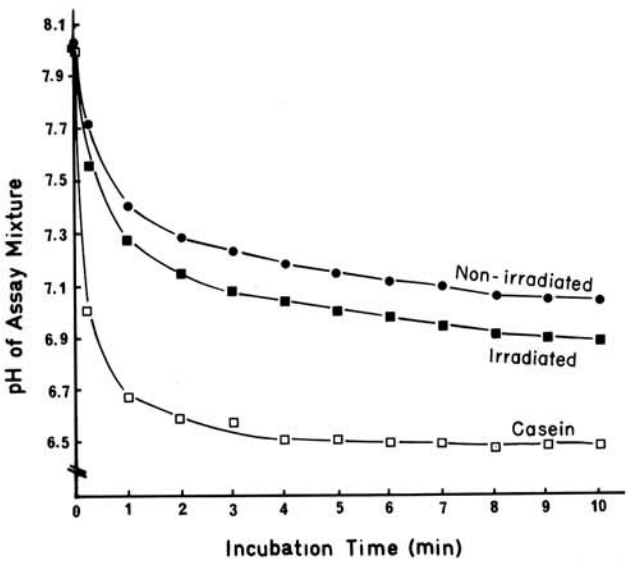

Fig. 9. Apparent in vitro protein digestibility of nonirradiated and irradiated barley determined by the multienzyme technique of Hsu et al (1969). Casein was used for comparison.

do not necessarily conflict with improvements reported earlier in chick weight gain, fat, and starch digestibilities on irradiation (10 Mrad) of hulless barley (Classen et al 1985). Only the starch digestibility data in the two studies are directly comparable. Starch digestibility decreased by 1.0% in the present study. This difference in starch digestibility was probably accentuated by the use of different animal species in the two studies.

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