NATURE OF PROTEINS IN TRITICALE AND ITS PARENTAL SPECIES

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ABSTRACT

Protein characteristics and composition of one line of hexaploid Triticale (6A190), its durum wheat (T. durum cv. Stewart) and rye (S. cereale cv. Prolific) parents, and one variety of hard red spring wheat with high breadmaking quality (T. aestivum cv. Manitou) were compared to determine the inheritance of proteins in the interspecific hybrid from its parents and the reason for the lack of breadmaking quality as compared with hexaploid bread wheats. Solubility characteristics and amino acid composition showed that the proteins of Triticale are intermediate in these properties between those of its parent species. Gel filtration was used to separate solubility fractions into albumins, globulins, gliadins and glutenins. Quantitative differences of these proteins among the four cereal species were determined. Disc electrophoretic patterns of these proteins showed that proteins of Triticale are directly inherited from its parents. Differences in protein composition between the hard red spring wheat and the other species which might be related to breadmaking quality were observed.

PREFACE

Although considerable progress has been made on the detailed analysis of the nature of the proteins of bread wheats, it is still not possible to relate their structure, insofar as it has been resolved, to breadmaking quality. In the case of bread wheats, quality is the ability of the flour to produce a loaf of bread of high volume, uniform grain, silky texture, and bright, white color. Both qualitative and quantitative factors interplay to produce the optimum bread quality by a particular baking procedure. As yet, however, it is not possible to say which endosperm proteins are essential for breadmaking quality and which are not.

The present study was undertaken with two main objectives. The first was to obtain additional, new information on the structure of bread wheat proteins and those of related species that might be relevant to breadmaking quality. A closely related question that has been receiving considerable attention in recent years concerns the inheritance of this quality. This was envisaged as the second objective of the present study.

The classical approach to studies of the nature of breadmaking quality and its inheritance has been to examine the proteins of the same type of wheats, i.e. bread wheats, which differed quite widely in quality. Little work has been done on genetically related species belonging to different genera.

Modern genetic techniques have produced materials such as the monosomics, substitution lines, ditelocentrics, and synthetic species obtained from addition or subtraction of genome(s). Sufficient quantities of many of these are now available for quality and chemical

analyses. Application of modern techniques of protein chemistry, such as gel electrophoresis, to analyse protein composition of these materials might lead to the location of chromosomes, and eventually genes, which control the synthesis of the proteins that are essential for this quality.

In the present investigation, the approach to the study of the inheritance of proteins was at the genome level. The species adopted for the investigation are a hexaploid Triticale, its tetraploid (durum wheat) and diploid (rye) parents, and one high quality variety of Canadian hard red spring wheat. The choice of material was to a certain extent dictated by what was available at the time the project was initiated.

A somewhat different from the customary format has been adopted for this thesis. Part I of the thesis deals with the general review of pertinent published papers. The description of materials and experimental procedures, results, discussion, and contributions to knowledge are presented in Parts II and III. Part II presents the portion of the investigation, written in the form of scientific papers, which can be taken directly from the thesis and submitted for publication. This section comprises three separate papers written in the editorial style of the Canadian Journal of Plant Science. Part III includes useful observations and results that could not be included in the scientific papers but are normally recorded in a thesis. This section also includes suggestions for further work and a list of contributions to knowledge. The main advantage of this format is that it will eliminate the time lapse between the preparation of the thesis and the preparation of the scientific papers resulting from the investigation.

TABLE OF CONTENTS

PART ONE

LITERATURE REVIEW

LITERATURE REVIEW

It is now well established that the breadmaking quality of a flour depends primarily on its proteins. The desirable visco-elastic properties of bread doughs can be directly attributed to analogous properties of the flour proteins, especially those of gluten. The literature on wheat flour proteins that is pertinent to this thesis will be reviewed in three sections. Section I will discuss the accumulated information on their fundamental physical and chemical properties; section II will deal with the inter-relationships between pertinent properties of the proteins and the breadmaking quality of flour; and the final section will review studies on the inheritance of proteins in wheat endosperm.

I. Physical and Chemical Properties of Wheat Flour Proteins

The first recorded separation of gluten from wheat flour was made by Beccari (2) in 1745. However, it was not until the turn of the present century that a detailed study of wheat proteins was reported by Osborne (1). The proteins were classified into four groups according to their solubility:

Albumin (leucosin) - soluble in water and coagulated by heat.

Globulin - soluble in dilute salt solution.

Gliadin - soluble in 70% aqueous ethanol solution.

Glutenin - soluble in dilute acid or alkaline solution.

In addition to these four classes of soluble proteins, most bread wheat flours contain small amounts of protein-containing materials that do not dissolve in any of the above solvents (3, 4). Classification based on

solubility depends strongly on the fractionation conditions. In spite of this shortcoming, the original classification of Osborne has been extremely useful in subsequent studies and is still used by cereal chemists with minor modifications. Accordingly, it seemed logical to review the literature in sections devoted to individual solubility components. This format is essentially the same as that adopted by Pomeranz (5) in his recent review article.

1. - Albumins

Proteins extracted from flour with water are mainly albumins contaminated with small amounts of gliadin, globulin, and glutenin. The gliadin and glutenin impurities can be significantly decreased by making the initial extract with dilute salt solution. This extraction procedure removes the globulins in addition to the albumins; the former can be separated as a precipitate by dialysis against distilled water (1). Early attempts to purify the albumins have shown that they are also strongly associated with flour carbohydrates (6). Results based on quantitative extractions by Pence and Elder (7), using a wide range of bread wheat flours, show that albumins form from 0.7 to 1.5% of the flour or from 6 to 11% of the protein in the flour.

The most comprehensive study of the physical and chemical properties of wheat albumins is that of Pence and Elder (7). They extracted hard wheat flour with dilute phosphate buffer, and separated the albumins from the extract by fractional precipitation with ammonium sulfate. The purified albumin obtained by this method appeared as one broad peak in the sedimentation pattern of the analytical ultracentrifuge. Electrophoresis of this preparation on filter paper showed at least six components. Chemically, it was characterized by a relatively high tryptophan

and low amide nitrogen contents as compared with other proteins of wheat flour. Its number-average molecular weight determined by osmotic pressure was about 28,000 in dilute salt solution, and 17,000 in sodium salicylate or urea solution.

Feillet and Nimmo (8) reported recently on their successful isolation and purification of the two major albumins (Albumin 13A and Albumin 13B) of wheat flour using consecutive fractionations by free boundary electrophoresis, and gel-permeation and ion-exchange chromatography. The two albumins were judged homogeneous by polyacrylamide- and starch-gel electrophoresis. Albumin 13B had a relatively high valine content but no histidine or phenylalanine compared with Albumin 13A. Both had no cysteine. The molecular weights were approximately 13,000 and 20,000 for albumin 13B and albumin 13A respectively.

2. - Globulins

The globulin fraction can be obtained as a precipitate by dialyzing a dilute salt extract of wheat flour against distilled water. Globulin content in flour protein is in the range of 5 to 8% of the total protein (9). Pence et al (7, 10) extracted crude globulin with 1M of sodium chloride solution, and partially purified it by fractional precipitation with ammonium sulfate followed by dialysis against distilled water. Three main components, alpha, gamma and delta, were detected by ultracentrifugation. Globulin prepared by these workers was characterized by relatively low tryptophan and amide-nitrogen contents compared with other flour proteins. Its arginine content was nearly double that of the albumins. Coates and Simmonds (11) in their study of the role of globulins in the breadmaking quality of flour, isolated this group of proteins by fractionating a sodium pyrophosphate extract by ion exchange chromatography

on DEAE-cellulose. They obtained one fraction that had a high arginine content.

3. - Gluten

Gluten is the proteinaceous material that remains after the solubles and starch are removed from a flour-water dough with water. The early work on gluten was reviewed by Bailey (2); later work was reviewed recently by Pence et al (12) and by Pomeranz (5). For the purpose of the present review, the literature on gluten will be considered in two parts. This section will deal with the studies on the total gluten which were made in an attempt to obtain information on its function in dough. Studies on the two major protein components of gluten, gliadin and glutenin, will be reviewed in separate sections.

Osborne (1) showed that about 80% of the flour proteins can be separated in the gluten. Proximate composition of dry gluten is 85% protein, 10% lipid, and 5% carbohydrate and minor constituents. Actual composition of a specific preparation depends on the type or grade of flour, and on the washing procedure. Purified gluten prepared by freeze drying of acetic acid dispersion of crude gluten by Lusena (13) had 90% protein, 8% lipid, and 2% other constituents.

In spite of its wide heterogeneity, gluten has been studied extensively by cereal chemists mainly because its viscoelastic properties determine the analogous properties of flour-water doughs. These are considered to be essential for breadmaking quality of flour.

Studies of the molecular properties of gluten components have been hampered by the lack of suitable solvents. Results obtained with strong dispersing solvents such as acetic (14), formic (15) and lactic acids (16), concentrated urea solution (17), and sodium salicylate

solutions (18) are very interesting, but there is some doubt regarding their intrinsic value. These studies emphasized the fact that the components of gluten interact strongly with one another.

Amino acid composition analyses of gluten (19, 20, 21) showed that it has extraordinarily high contents of glutamine and proline. These constitute almost half of the total amino acids. The content of amino acids with ionizable groups on the side chain is relatively low. Apparently, this is the main reason for the low solubility of gluten proteins in neutral salt solutions (20). The large number of side-chain amide groups interact to form hydrogen bonds which are extremely important in the structure of gluten (22). Gluten proteins also contain relatively large proportions of amino acids with nonpolar side chains. In the presence of water, these interact to form hydrophobic bonds which apparently also contribute significantly to the stability of gluten. The effects of urea and of organic solvents such as isopropanol on the solubility of gluten can be explained in terms of disruption of hydrogen and hydrophobic bonds respectively (22). These bonds appear to be extremely important in the physical properties of dough. Addition of urea to dough markedly decreased its consistency (23) while heavy water, which forms a stronger hydrogen bond than normal water, increased dough consistency (24). The effect of acetone and a number of other organic solvents on gluten consistency is similar to that of urea. This was attributed to disruption of hydrophobic bonds (23).

Flour contains approximately one micromole of cysteine and 10 micromoles of cystine per gram (25). Sulfhydryl groups are approximately equally divided between soluble and gluten proteins (26, 27). Pence and Olcott (28) observed that the viscosity of gluten dispersions decreased

markedly when its disulfides were cleaved by reducing agents. Disulfide groups are essential to the functional properties of wet gluten. Additions of small amounts of disulfide reducing chemicals completely destroyed its elasticity (29). On the other hand, sulfhydryl groups are important because of their participation in disulfide interchange reactions (30); a single sulfhydryl group can initiate the interchange of a large number of disulfide bonds.

4. - Gliadin

Gliadin is the fraction of wheat protein that has the solubility properties of the prolamin group of proteins. It can be isolated from flour by a variety of procedures. Crude gliadin can be extracted directly from flour or gluten with 70% ethanol solution. Jones et al (31) separated relatively pure gliadin from a 0.1M acetic acid solution extract of gluten. To separate the gliadin this extract was neutralized, mixed with ethanol to give 70% concentration, and centrifuged. The gliadin remained in the supernatant. Gliadin has also been purified by gel-filtration chromatography (32).

There have been numerous studies of the molecular properties of the gliadin group of proteins. Heterogeneity of gliadin was first observed by Krejci and Svedberg (33) with the analytical ultracentrifuge. Schwert et al (34) showed by free boundary electrophoresis that the gliadin group comprises at least seven components. A much better resolution of the gliadins was obtained by Jones et al (31) by the same technique using low concentrations of protein and aluminum lactate buffer solution. These workers examined dispersions of gluten in aluminum lactate buffer of pH 3.4, and found five gliadin components. The electrophoretically distinct components were subsequently isolated and identified by column chromatography using carboxy-methylcellulose (35).

The application of the starch-gel electrophoresis technique to flour proteins was reported first by Elton and Ewart (36); eight gluten components were detected by this technique. Woychik et al (37) incorporated urea into the starch-gel electrophoresis technique; they detected eight gliadin components.

There have been several physicochemical studies on the size and shape of the gliadin molecules. Molecular weight data are summarized in Table I. The table shows a wide variation in molecular weights. Actual values seem to depend on the procedures used for the preparation of the gliadin, and the method used to measure the molecular weight. Viscosimetric data suggested that the gliadin molecule is relatively compact; however, it is not spherical. Studies with the analytical ultracentrifuge, viscosity and diffusion (45) gave an axial ratio of 10.5 to 11.1. In neutral solution, gliadin tends to aggregate strongly to form much larger particles (38). This aggregation depends on ionic strength and pH (39, 47). It is much more pronounced at higher ionic strengths and higher pH. Kasarda et al (46) used electron microscopy to study this aggregation. They observed that globular alpha-gliadin aggregates reversibly to form long fibrils. The degree of reversibility of this aggregation depends on the rate of agitation of the gliadin suspension. It has been speculated that this effect might be related to development of gluten structure in doughs during mixing.

Amino acid composition data for crude gliadin and its components are compatible with its physical properties (48). Its high glutamine content explains its tendency to aggregate, probably by formation of hydrogen bonds Its relatively high proline content parallels the low helical content in its secondary structure (48, 90).

Table I. - Molecular Weights of Gliadin and Gliadin Components

Proteins	Method	Solution	Mol. wt. X 10^3	References
Gliadins	Sedimentation	Dil. HCl	17 - 35	(33)
	Sedimentation and light scattering	60% ethanol	24	(38)
	Sedimentation	6M G-HCl*in 0.1M acetic acid	46	(32)
	Osmotic pressure ORD**, viscosity sedimentation	3M urea, pH 4.7	46.6	(39)
γ-gliadin	Sedimentation	4M.G-HCl in 0.01M HCl	26	(40)
	Sedimentation	6M.G-HCl in 0.01M HCl	31	(41)
γ_1-gliadin	amino acid		16	(42)
γ_2-gliadin	amino acid		17	(42)
γ_3-gliadin	amino acid		18	(42)
β-gliadin	Sedimentation	4M G-HCl in 0.01 HCL	31 - 42	(43)
High mol.wt. gliadin	Sedimentation	6M G-HCl in 0.1M acetic acid	104	(32)
α- gliadin	Gel filtration, light scattering	0.017M Al-lactate pH 3.1	50	(44)

* G-HCl, guanidine hydrochloride

** ORD, optical rotatory dispersion

Compared with other types of protein, gliadin has a relatively high content of amino acids with nonpolar side chains - hence its solubility in organic solvents. This characteristic is probably important also in the aggregation in aqueous solution which could result from hydrophobic bonding. Gliadin has very few sulfhydryl groups; its disulfide bonds appear to be mostly of the intramolecular type (49, 50).

5. - Glutenin

The glutenin fraction of wheat proteins is usually extracted from gluten with dilute acetic acid after the gliadins are removed with 70% ethanol solution. Most of the fundamental work on wheat glutenins has been done at the Northern Regional Laboratory of the United States Department of Agriculture.

Electron micrographs of shadowed films of glutenin (51) revealed extensive agglomeration of particles which appear to be oblate ellipsoids in shape. Jones et al (43) found that glutenin is highly heterogeneous having a weight-average molecular weight of about two to three million. Glutenin also contains small molecules aggregated with the large ones. From their studies with the analytical ultracentrifuge, Taylor and Cluskey (52) concluded that glutenin has a high molecular weight, is highly heterogeneous, and comprises randomly coiled polypeptide chains. Glutenin appeared homogeneous in the moving-boundary electrophoresis (31); however this homogeneity was attributed to the extremely high molecular weight. In starch-gel electrophoresis (37) glutenins remained at the point of application.

Amino acid composition data show characteristics that are peculiar to glutenin. As in the case of gliadin, glutenin contains a relatively high amount of glutamine (20). Its proline content is also relatively high (20) and this apparently prevents the formation of helical secondary

structures. Relative to gliadin, glutenin contains a higher proportion of amino acids with ionizable side chains, and a slightly lower proportion of nonpolar amino acids (53).

The marked decrease in viscosity of gluten dispersions on the addition of reducing agents has been attributed to the reduction of disulfide bonds of glutenin which are presumed to crosslink long polypeptide chains (28, 54). Elton and Ewart (55) observed that reduction of glutenin disulfides produced a number of protein fragments which can be detected by starch-gel electrophoresis. On the basis of these observations, it has been suggested that glutenin is built up of gliadin-like units linked together by disulfide bonds. In later studies, Beckwith and co-workers (32, 56, 57) showed that some of the low molecular weight proteins produced by reduction of glutenin were similar but not identical to gliadin. At the present time, it is not known if there is a direct biosynthetic relationship between the gliadins and the glutenins.

2. Relation of Flour Proteins to Baking Quality

Protein content of a bread flour is considered to be the most significant single indicator of its baking quality. This conclusion is based on numerous studies with a large variety of flours. Positive correlations between protein content and baking quality have been obtained for all the major bread wheats (58, 59, 60). The same correlation has been obtained between gluten content and baking quality (61, 62).

Finney and Barmore (63) determined the baking quality of a large number of varieties of hard red spring and hard red winter wheat ranging in protein content from 8 to 18%. They obtained linear, positive regressions for loaf volume against protein content. The slope of the regression lines varied with the variety from 40 to 75 c.c. for each

percentage unit of protein. Similar positive regressions were obtained by Fifield et al (64) for 589 flour samples from 10 varieties of hard red spring wheat grown during four years. Results for one variety for the four years fell on the same regression line, indicating that environment did not affect the quality of the protein for breadmaking. Intervarietal differences in slope were obtained. A positive correlation between loaf volume and protein content was obtained by Bushuk et al (65) with the remix baking test for 62 samples of the variety Manitou of the 1966 crop and 66 samples of the 1967 crop. The slopes of the regressions for the two years did not differ significantly. This agrees with previous findings that protein quality is not affected by environment.

Reconstitution studies have helped to delineate the role of protein components in breadmaking quality. In this experimental approach, the effect of removal or addition of a particular component on the baking quality of a flour is examined with the baking test.

Aitken and Geddes (66) showed that baking strength, as measured by loaf volume, can be increased by addition of dry gluten to the flour. Harris and Frokjer (67) found that gluten from hard red spring wheat improved the baking quality of soft wheat flour considerably more than durum gluten. A positive effect of the soluble proteins of flour on its baking quality was obtained by Pence et al (68).

The most elegant reconstitution studies in relation to breadmaking quality are those of Finney and co-workers (16, 69, 70). These investigators have shown that the water solubles were not responsible for quality differences, although they are essential for the production of a normal loaf. The quality factor seems to be located in the gluten group of proteins. On the basis of electrophoretic mobility in starch

gel, the quality factor is similar to gliadin. Accordingly, the role of the major protein components of flour in baking quality appears to be reasonably well established.

There have been many studies of the physical and chemical properties of the proteins of flour in relation to its breadmaking quality. Viscosity measurements of dispersions in 10% sodium salicylate solution of glutens from hard red spring and durum wheats (71) indicated that the hard red spring gluten gave rise to much larger particles (molecules) than the durum gluten. Cluskey et al (72) obtained a higher intrinsic viscosity for hard wheat gluten than for soft wheat gluten. On the other hand, Matsuo and McCalla (73) did not find any significant difference in the sedimentation coefficients, diffusion constants, and molecular weights of glutens from hard red spring and durum wheats. Most of these physicochemical studies were made on dispersions of highly heterogeneous proteins so that small differences in physical properties which might affect baking quality could be easily masked under these non-ideal conditions.

After the classic work of Osborne (1) on the solubility of flour proteins, there have been numerous attempts to relate this property to breadmaking quality. The earlier hypothesis that baking quality might be related to the gliadin to glutenin ratio could not be confirmed, and was eventually abandoned (74). In 1965, Mullen and Smith (75) found that long-mixing flours had more acid insoluble and less beta gluten than short-mixing flours. More recently, Pomeranz (76) showed that flours of poor breadmaking quality contained a greater proportion of protein that was extractable with 3M urea solution. Ewart (77) reported that the quantity of protein soluble in 0.1M acetic acid solution was directly related to baking quality.

During the last 10 years, there have been many attempts to relate breadmaking quality to the qualitative composition of flour proteins determined by various types of column chromatography and/or electrophoresis. In 1948, Laws and France (78) used free boundary electrophoresis to examine glutens from flours of different baking quality. These workers did not find any significant differences in the electrophoretic patterns. In 1959, Jones et al (31) introduced the use of lactate buffer for electrophoretic studies of gluten. With this technique, Cluskey et al (72) showed that there were small significant differences in the patterns for the glutens from hard and soft wheat flours. Subsequently, other investigators (79, 80) found differences in electrophoretic patterns for glutens from various classes of wheat. Flours of good baking quality contained more of the alpha- and omega-gluten components. Pence et al (81) found that the relative amounts of individual components in the albumin group from varieties of the same class of wheat were essentially the same. However, the patterns for the albumins from hard red spring and durum flours were distinctly different.

Recent studies of flour proteins by ion-exchange chromatography (82, 83, 84) and starch-gel electrophoresis (85, 86, 87) showed that quantitative and some qualitative differences exist between varieties from different classes. However, there are no major differences in the patterns for varieties of the same class but of different baking quality. Studies with immunoelectrophoresis (97, 98) showed that there are no qualitative differences between the proteins from good and poor quality flours. Doekes (87) examined the proteins of eighty varieties and selected breeding lines by starch-gel electrophoresis. The patterns that he obtained could be classified into five main groups each comprising

a number of sub-groups; however, there was no clear-cut relation between the electrophoretic pattern and breadmaking quality.

With the advent of automatic analyzers, amino acid compositions of a large number of different flours (88,89) and glutens (19, 21, 73) have been determined. Although small differences were observed among types, varieties and grades of flour, there were no reproducible differences between similar flours but of different baking quality.

Sulfhydryl and disulfide groups of flour proteins play an extremely important role in the breadmaking quality of flour (91, 92). Sulfhydryl groups react with chemical flour improvers and facilitate the interchange of disulfide bonds that are considered essential for mechanical development of doughs (30). Accordingly there have been many attempts to correlate the cysteine or sulfhydryl, and cystine or disulfide contents of flour with baking quality.

In 1950, Wostmann (93) determined the cystine contents of 23 flours and found that the breadmaking quality increased with increase in disulfide. Sokol et al (94) found that sulfhydryl contents of low grade flours were substantially higher than in the higher grade flours milled from the same wheat. Axford et al (95) found that disulfide content varied inversely with protein content of flours milled from single varieties or mixed grists. Tsen and Anderson (96) determined sulfhydryl and disulfide contents of flours milled from hard red spring, soft and durum wheat varieties. Hard wheat flours contained more sulfhydryl and disulfide than the soft wheat flours; durum flours were similar to hard wheat flours. Rohrlich and Essner (5) determined the disulfide to sulfhydryl ratios for a series of flours milled from Canadian and German wheats. A significant positive correlation was obtained between the ratio and

loaf volume for 50 flours from German wheats. Belderok (5) found that the flours which gave disulfide to sulfhydryl ratios of 15 to 19 gave the maximum loaf volume.

In addition to differences among flours in the total contents of sulfhydryl and disulfide, there are differences in the degree of accessibility of these groups to chemical reagents under various conditions. Bushuk (99) suggested that it is the accessible sulfhydryls that play the major functional role in dough formation. Further studies by Tsen and Bushuk (100) showed that dough mixing strength as defined by the farinograph curve is inversely related to the amounts of reactive sulfhydryl and disulfide groups. On the other hand, Lee and Lai (101) have obtained a significant positive correlation between the content of masked sulfhydryls and loaf volume. The current consensus is that both sulfhydryl and disulfide groups are extremely important in the breadmaking quality of flour. However, the details of this role remain to be clarified.

III. Inheritance of Proteins in Wheat and Related Species

For the purpose of this review, the literature on the inheritance of proteins (in relation to breadmaking quality) in common wheats will be divided into three sections:

1. Inheritance studies with selected wheat varieties or crosses.
2. Effects of genome removal or addition.
3. Studies at the chromosomal level.

1. - Inheritance Studies with Selected Wheat Varieties or Crosses.

Inheritance of protein content and quality in bread wheats has, for many years, been the most important challenge to the geneticist and the cereal chemist. Early studies (102) showed that protein content of

a pure variety of wheat was determined mainly by environmental factors. In 1964, Lebscock et al (103) obtained 37 and 70% for the heritabilities of protein in F5 and F6 material respectively, when correlated with the protein content in the F3 material. For a single Gabo x Selkirk cross, Kaul and Sosulski (104) obtained heritability values ranging from 81.8% for the F3 generation to 75.7% for the F5 lines. The heritability of sedimentation value for the same cross estimated from the variances of the F2 and backcross populations was 89.8% (105).

There have been a number of attempts to determine or confirm genetic relations among wheat species on the basis of the electrophoretic patterns of their proteins (106, 107). Coulson and Sim (108) were the first to demonstrate that the pattern for one variety is not affected by environment. Similar conclusions were reached by Lee and Ronalds (109). Doekes (87) examined 80 varieties and selected lines and concluded that the electrophoretic patterns in starch-gel showed proteins are inherited and not affected by environment. Since the relationship between the protein patterns and baking quality has not been resolved so far, it is not yet possible to relate these observations to inheritance of baking quality.

2. - Effects of Genome Addition or Removal

Studies by Yong and Unrau (110) and Barber et al (111) of the proteins of the wheat-rye hybrid, Triticale, have shown 'new' protein bands not present in the patterns for the parents. In contrast, Johnson and Hall (112) reported that the starch-gel pattern for the protein extract of an octaploid Triticale (rye x common wheat) was the same as the pattern for a mixture of the extracts from the rye and wheat parents. Using the same technique, Johnson et al (113) showed that nine of the 12 albumin

bands of the hexaploid (AABBDD) sub-species were homologous with the nine albumin bands of the AABB tetraploid; the remaining three bands were attributed to the D genome. Recent work by Boyd and Lee (114) on AABBDD hexaploids obtained by adding the D genome from different accessions of Aegilops squarrosa to the extracted AABB tetraploid Canthatch, showed that the patterns for the reconstituted hexaploid contained only protein components of the parents. On the basis of these findings, it appears that the gene systems of polyploid wheats are a simple addition of the genes of the diploid progenitors; however, additional studies are required to confirm this hypothesis. Further studies are needed also to clarify the apparent discrepancy between the results of Yong and Unrau (110) and Barber et al (111) on the one hand, and those of Johnson and co-workers (112) and Boyd and Lee (114) on the other.

An apparent discrepancy exists in relation to the effect of the removal of the D genome from AABBDD hexaploids. In their first paper, Boyd and Lee (114) reported that removal of the D genome from the variety Canthatch did not affect the starch-gel pattern of the urea extracts. Later Boyd et al (115) reported that the gluten of Tetracanthatch wheat lacks at least four of the gluten proteins present in the hexaploid parent. Apparently in the original study, two samples of Canthatch were examined because of a labeling error. Similar studies by Dronzek et al (116) using a more elaborate fractionation procedure on the endosperm proteins of extracted AABB tetraploids from Prelude, Rescue, and Thatcher, showed the same patterns, for each tetraploid and its hexaploid parent. Extracts from whole wheat showed minor differences in the slow-moving bands. Varietal differences in the patterns were observed. Removal of the D genome from bread wheats, produced a marked decrease in baking quality in

three of the four varieties that have been studied so far (117, 118). For the variety Prelude, the extracted tetraploid had essentially the same quality as its hexaploid parent (117). This has been tentatively attributed to a translocation of a part of one or more of the D genome chromosomes to the chromosomes of the other two genomes.

3. - Studies at the Chromosomal Level

Studies on various aneuploid lines of wheat have produced useful information on the relation of specific chromosomes to protein components and baking quality. Welsh and Hehn (119) examined the quality of 15 monosomic lines of Kharkov MC22 using the Pelshenke and Farinograph tests. They found that chromosome 1D had the greatest influence on flour quality as measured by these tests. Later work of Kaltsikes et al (117) and Boyd and Lee (114) supports this conclusion.

Morris and co-workers (120, 121, 122, 123) used substitution lines as well as monosomics to investigate the contributions of various chromosomes to the quality in the varieties Thatcher and Cheyenne. Chromosomes 3D, 4D, 5A and 7B were found to be associated with the genetic control of protein content in Thatcher. In Cheyenne, chromosomes 4B, 5D and 7B had the greatest influence on dough properties as measured on the mixograph. These workers concluded that the substitution lines were superior to monosomic lines for studies of multigenic inheritance of factors that might be related to baking quality.

A more detailed report on the chromosomal control of wheat endosperm proteins was published recently by Shepherd (124). He used the starch-gel technique to examine urea extracts of a large series of aneuploid lines of the variety Chinese Spring. Both qualitative and quantitative chromosomal effects were observed. Nine of the seventeen major

protein bands of Chinese Spring were accounted by removal of individual chromosomes. The remaining eight are presumably controlled by multiple factors on more than one chromosome pair. Significant dosage effects were observed. The most marked quantitative deviations from the patterns for the disomic were in the patterns for the group 1 and group 6 nullitetrasomics.

The published information on the inheritance of endosperm proteins in wheat can be summarized as follows. In hybrids of common wheat, protein content and quality appear to be inherited from the parents. In a single variety, protein content is strongly dependent on environmental factors; however, the qualitative composition of the protein remains a varietal characteristic. This feature can be used to identify or classify wheat varieties. It is not known what factors control the quantitative distribution of protein components.

Studies on one common wheat-rye hybrid, extracted AABB tetraploids, and reconstituted AABBDD hexaploids suggest that polyploid wheats contain only the proteins of their diploid progenitors. However, if each of the three genomes of common wheat originated from a common diploid progenitor, as suggested by Riley (125), then all varieties of this class of wheat should show identical protein-band patterns. Significant varietal differences are known to exist. In contrast, two studies on hexaploid wheat-rye hybrids, have shown that a 'new' protein not present in the parents is produced in the hybrid. This has been attributed to an interaction of genes in the alien chromosomes.

Considerable progress has been made on the relationship between specific chromosomes and protein bands. This work has been facilitated by the tremendous achievements by cytogeneticists in the development of

monosomics, substitution lines and other aneuploids of wheat. Exact intervarietal or interspecies identity of the proteins in the different bands will require detailed information on their primary structure. Finally, the inheritance of breadmaking quality will require complete understanding of the role played in this property by individual protein components.

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NATURE OF PROTEINS IN *TRITICALE* AND ITS PARENTAL SPECIES

I. SOLUBILITY CHARACTERISTICS AND AMINO ACID COMPOSITION OF ENDOSPERM PROTEINS[1]

C. H. Chen and W. Bushuk

ABSTRACT

Solubility characteristics and amino acid composition of the endosperm proteins of one line of *Triticale*, its durum wheat and rye parent varieties, and one variety of hard red spring wheat were compared. Quantitative distribution of the soluble protein fractions and amino acid compositions showed that the proteins of *Triticale* are intermediate in these properties between analogous properties of the proteins of its durum wheat and rye parents. The major differences between the hard red spring wheat and the other three species were its lower content of water-soluble proteins and higher content of insoluble or gluten proteins. This appears to be the main reason for the superior breadmaking quality of the hard red spring wheat variety compared with the other species used in this study.

INTRODUCTION

Triticale, a synthetic cereal species is produced by combining the genomes of wheat (*Triticum*) and rye (*Secale*). Depending upon whether a tetraploid wheat (*T. turgidum* L., 2n = 4x = 28) or a hexaploid wheat (*T. aestivum* L. en Thell., 2n = 6x = 42) is used, either a hexaploid or an octaploid *Triticale* (2n = 8x = 56) is produced, respectively. Genomically the two *Triticale* types and their parents can be represented as follows:

1. *T. turgidum* + *Secale* = *Triticale* (hexaploid)

   AABB + RR = AABBRR

2. *T. aestivum* + *Secale* = *Triticale* (octaploid)

   AABBDD + RR = AABBDDRR

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Because it is an inter-species hybrid, Triticale is a useful research material for investigations of the inheritance of components from the parents which normally are widely different. In an initial study of the proteins of Triticale, Yong and Unrau (12) observed four "new" protein bands not present in either parent. These authors examined, by starch gel electrophoresis, extracts obtained with four different solvents. Barber et al. (2) detected a "new" esterase band in octaploid AABBDDRR Triticale by the same technique. On the other hand, Johnson and Hall (5) reported that the electrophoresis pattern for the extract from AABBDDEE octaploid Triticale was essentially the same as the pattern for a mixture of extracts from the two parents. Similar results were obtained by Boyd et al. (3) for other amphiploid lines.

The present study was undertaken to extend the investigation of Yong and Unrau (12). Recently developed techniques of protein chemistry were adopted to study the proteins of one line of hexaploid Triticale and its parents. In addition, Manitou, a Canadian variety of hard red spring wheat, was included in the study as the representative of high quality bread wheats. Results of the study will be presented in three parts. Part I will deal with quantitative differences in protein solubility and amino acid composition; Part II will examine the proteins of various solubility fractions from the four species by gel filtration and disc electrophoresis; and Part III will compare disc electrophoretic patterns to determine homology of proteins from the four species studied.

MATERIALS AND METHODS

Description and Preparation of Flour Samples

Seed stocks of the strain of Triticale (6A190) used were already available in the Department of Plant Science, University of Manitoba. This strain was originally produced by crossing durum wheat, T. turgidum,

cv. Stewart 63 and rye, S. cereale cv. Prolific. The four species used in this study were grown together in 1966 on large experimental plots at the University of Manitoba. The samples were milled into flour on the Buhler experimental mill by the same milling procedure after tempering overnight to 16.5% moisture. Pertinent technological data for the four samples are shown in Table 1. Standard A.A.C.C. methods (1) were used except where otherwise specified. Table 1 includes much more information than normally required to characterize the flour samples, however it is felt that the data should be recorded as it might be of interest to plant breeders and cereal technologists.

Extraction of the Proteins

The extraction procedure employed was similar to the classical protein fractionation procedure of Osborne (6), and was carried out in a cold room at 4°C. Flour samples (10 g. d.b.) were extracted with 40 ml. of 0.5M sodium chloride solution by stirring with a magnetic stirrer in a centrifuge bottle for 2 hr. Mild stirring was adopted to avoid artifacts that might be produced by high shear stirring (11). Each suspension was centrifuged for 30 min. at 1860 x g., and the supernatant was decanted. This was followed by a second similar extraction for 1 hr. The residue was extracted with 40 ml. of distilled water for 30 min. to remove residual salt. The three supernatants were combined. This extract was dialysed against cold distilled water for 48 hr. and centrifuged to separate the precipitated salt-soluble proteins; water-soluble proteins remained in the supernatant. The residue remaining after extraction with salt solution was then extracted similarly with two portions of 70% ethanol solution, and the resulting residue was further extracted with two portions of 0.05M acetic acid solution. Ethanol was removed from the combined ethanol-solution super-

Table 1. Characteristics of Experimental Materials

Genus	Triticale	S.cereale	T.turgidum	T.aestivum
Common name	--	Spring rye	Durum wheat	Hard red spring wheat
Strain or variety	6A190	Prolific	Stewart 63	Manitou
Genomic composition	AABBRR	RR	AABB	AABBDD
Grain				
Test weight, kg./l.	0.89	0.86	1.03	0.96
lb./bu.	57.2	55.4	65.9	61.3
1000 kernel weight, g.	43.4	28.6	44.9	30.6
Moisture content, %	14.0	13.0	13.7	13.5
Flour				
Yield, % (14% m.b.)	58.9	59.6	67.7	71.7
Ash content, % (14% m.b.)	0.42	0.68	0.66	0.48
Color, Kent-Jones Units (1)	4.1	15.3	1.3	0.8
Moisture content, %	14.0	13.0	13.7	14.8
Protein content, % (14% m.b.)	9.8	9.9	12.1	13.6
Sedimentation value, ml.	17.8	17.0	21.8	69.0
Peak amylograph viscosity, B.U.	40	90	45	735
Farinograph absorption, %(14% m.b.)	58.8	59.0	67.3	65.3
Farinograph D.D.T., min.	1.0	1.0	1.5	4.5
Farinograph M.T.I., B.U.	170	190	100	20

natants in a rotary evaporator. The flour soluble fractions and the final residue were freeze-dried and stored in a deep-freeze.

Determination of Protein Contents

The semi-micro Kjeldahl method was used to determine nitrogen contents of the protein extracts and the residues. Protein contents were obtained using the conversion factor 5.7.

Amino Acid Analyses

Flour samples (100 mg.) were weighed into test tubes and 5 ml. of 6N hydrochloric acid was added. The tubes were evacuated and sealed under vacuum. Hydrolysis was carried out at 120°C for 16 hr. The hydrolysate was dried over sodium hydroxide pellets in a desiccator. The residue was dissolved in 5 ml. of 0.1N hydrochloric acid and then filtered. A Technicon amino acid analyzer was used for the amino acid analyses (8).

RESULTS AND DISCUSSION

In general, the solubility distribution of proteins in the Triticale, as determined from duplicate extractions, was intermediate between those for its durum wheat and rye parents (Table 2). The only exception is the amount of insoluble residue which was slightly lower for Triticale than the value for rye which contained the lowest amount of this fraction. Durum wheat flour contains a relatively large amount of material that is soluble in 70% ethanol. From its protein content, it appears that for this particular species, the alcohol solution removes large amounts of non-protein material. The striking difference in this property between the durum wheat and Triticale or HRS wheat deserves further study. There was some variability in the total nitrogen recovery for the four species; rye and Triticale gave the lowest recoveries. This indicates a species variability in the contents of low molecular weight nitrogenous materials in the salt-soluble extract which would be lost during dialysis. The

Table 2. Solubility Fractionation of the Endosperm Proteins

	Rye	Triticale	Durum	Hard Red Spring Wheat
Water soluble fraction				
Weight, mg.	960	555	574	348
Protein content, %	40.3	53.1	28.7	53.3
Fraction of total protein, %	34.3	26.4	12.2	11.9
Salt soluble fraction				
Weight, mg.	178	102	86	107
Protein content, %	67.6	71.1	75.2	76.2
Fraction of total protein, %	10.7	6.5	4.7	5.2
Alcohol soluble fraction				
Weight, mg.	427	348	929	494
Protein content, %	50.4	78.3	60.9	89.7
Fraction of total protein, %	19.0	24.4	40.7	28.5
Acetic acid soluble fraction				
Weight, mg.	234	338	396	368
Protein content, %	45.4	57.4	63.5	70.2
Fraction of total protein, %	9.4	17.3	18.3	16.6
Residue				
Weight, mg.	7884	8175	7993	8219
Protein content, %	3.0	2.6	4.0	6.4
Fraction of total protein, %	20.6	19.0	23.2	34.0
Nitrogen recovery, %	94.0	93.6	99.1	96.2

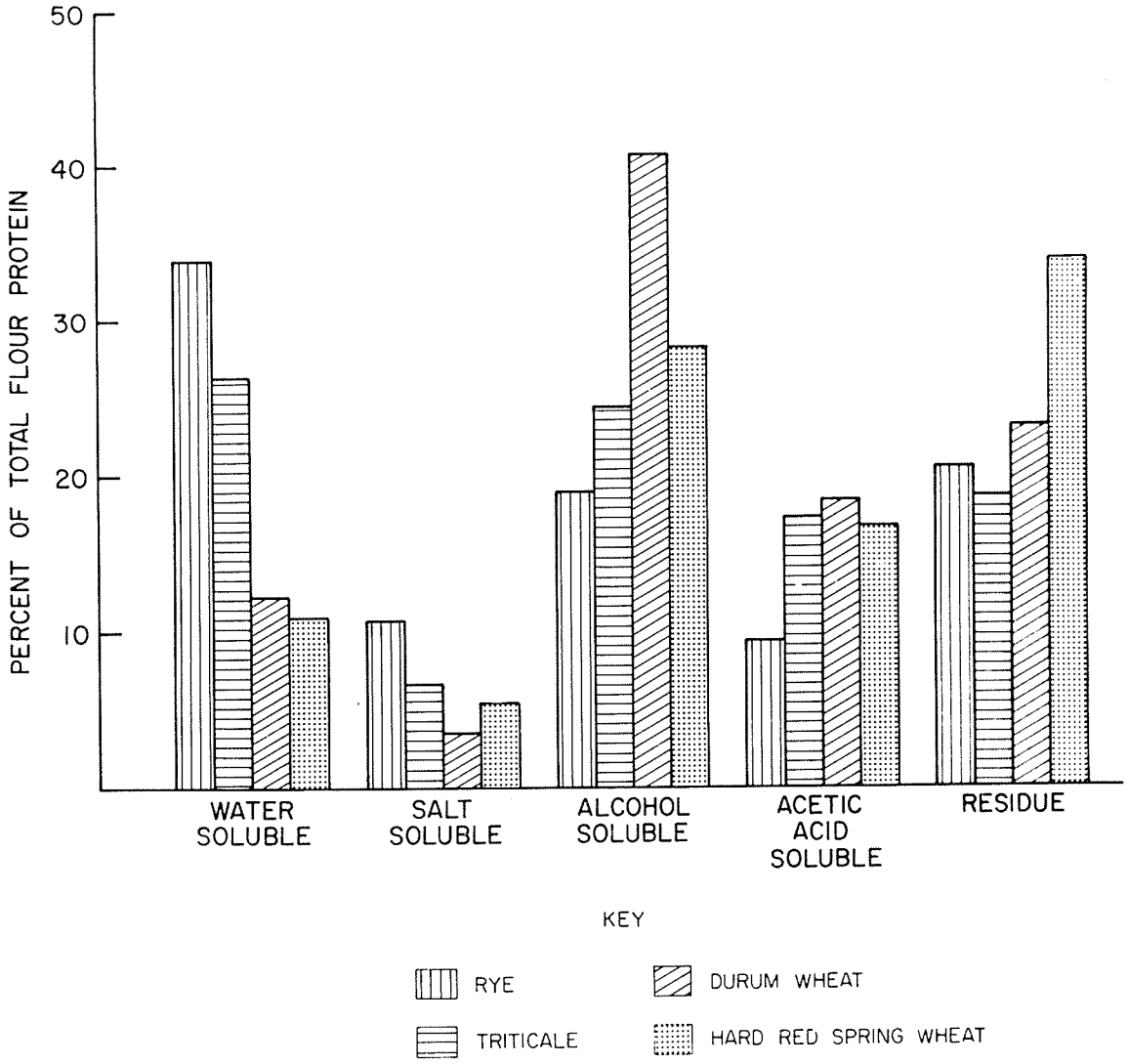
nature of the dialyzable nitrogen-containing substances was not investigated.

Protein contents of the various solubility fractions varied significantly among the four species examined. Fractions from the rye endosperm were, in general, lower in protein content. The salt-soluble fraction for durum wheat was extremely low in the protein content. HRS wheat protein fractions were highest in protein content; especially the insoluble residue. These differences indicate a wide variation in the amounts of non-protein materials extracted by various solvents and presumably reflect differences in the structure of the endosperm of the four species studied.

Rye endosperm contains relatively large quantities of water-soluble and salt-soluble proteins (Fig. 1). Durum wheat endosperm was very low in water-soluble and salt-soluble proteins but extremely high in alcohol-soluble proteins. This might be a characteristic typical of durum wheats but remains to be confirmed by further studies using a large number of varieties. The intermediate solubility distribution of Triticale proteins compared with the proteins of its rye and durum wheat parents is readily apparent from Fig. 1.

The HRS wheat variety studied was lowest in water soluble proteins, similar to Triticale in salt, alcohol and acetic acid soluble protein contents, and highest of all four species in the amount of insoluble residue. The content of insoluble residue proteins and much lower content of soluble proteins in the bread wheat relative to the other three cereal species might be typical of high quality bread wheats. This observation is in general agreement with results of Pomeranz (9) who found that the dispersibility of flour proteins in 3M urea solution was much higher for flours of poor breadmaking quality.

Fig. 1. Distribution of proteins in the five solubility fractions for the four grain species.


PERCENT OF TOTAL FLOUR PROTEIN
50
40
30
20
10
WATER SOLUBLE
SALT SOLUBLE
ALCOHOL SOLUBLE
ACETIC ACID SOLUBLE
RESIDUE
KEY
RYE
TRITICALE
DURUM WHEAT
HARD RED SPRING WHEAT

Amino Acid Composition

Comparison of the amino acid compositions of Triticale and its parental species (Table 3), shows that the content of most of the amino acids in Triticale was intermediate between those of its parents, durum wheat and rye. This is in agreement with the results on the same line of Triticale obtained by Yong and Unrau (13). The amounts of glycine, cystine, tyrosine and arginine were slightly higher in Triticale than in the parent species. The lysine content of Triticale was lower than that of rye but significantly higher than that of durum wheat. Fox and DeFontaine (4) found that the lysine content of one line of octaploid Triticale was intermediate between the values for the parent species whereas the value for leucine content was higher than that of both parent varieties. The distribution of amino acids in the HRS wheat (cv. Manitou) flour was essentially the same as that reported for the variety Selkirk (10). The recovery of amino acids of rye sample was rather low; the reason for this was not investigated.

Comparison of the amino acid composition of the HRS wheat with those of the other three species shows that the bread wheat has a higher content of glutamic acid and lower aspartic acid and lysine contents. It has been shown that water-soluble wheat flour proteins are higher in aspartic acid and lysine contents, and wheat gluten is high in glutamic acid content (7). Thus the results of amino acid analyses are compatible with the solubility characteristics of the proteins examined.

This study showed that both solubility characteristics and amino acid composition of Triticale proteins are in general intermediate between those of its parent species, durum wheat and rye. The functional properties of Triticale flour proteins should also be intermediate between those for the

Table 3. Amino Acid Compositions of Rye, Triticale, Durum Wheat and Hard Red Spring Wheat

(Micromoles amino acid per milligram nitrogen in sample.)

Amino Acid	Rye	Triticale	Durum	Manitou
Aspartic acid	2.41	2.23	1.89	1.47
Threonine	1.29	1.34	1.29	1.20
Serine	1.84	2.17	2.26	2.21
Glutamic acid	9.77	11.7	13.2	15.7
Proline	6.26	6.44	6.56	6.21
Glycine	2.40	2.70	2.54	2.73
Alanine	2.15	2.08	1.99	1.84
Valine	2.10	2.20	2.21	2.11
Cystine	0.52	0.70	0.60	0.59
Methionine*	0.68	0,79	0.78	0.65
Isoleucine	1.51	1.74	1.87	1.71
Tyrosine	0.56	0.81	0.80	0.78
Phenylalanine	1.47	1.58	1.80	2.10
Ammonia	9.41	10.0	12.5	13.6
Lysine	1.12	1.00	0.85	0.82
Histidine	0.65	0.79	0.83	0.85
Arginine	1.23	1.43	1.13	1.20
Tryptophan**				
N recovery, %	76.2	84.5	87.8	91.7

* Including methionine oxide (approximate value).

** Not analyzed.

rye and the durum wheat parents. Accordingly, it might be concluded from these observations that a hexaploid Triticale synthesized from durum wheat and rye could not have bread-making quality approaching that of a HRS wheat such as Manitou since both its parents are inferior in this respect.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Dr. F. W. Hougen in the determination of the amino acid compositions.

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NATURE OF PROTEINS IN TRITICALE AND ITS PARENTAL SPECIES.

II. GEL FILTRATION AND DISC ELECTROPHORESIS RESULTS[1]

C. H. Chen and W. Bushuk

ABSTRACT

The compositions and identity of protein components in the water, salt solution, 70% ethanol, and acetic acid solution extracts of the endosperm from one line of Triticale, its parents, and one variety of hard red spring wheat were investigated by gel-filtration and disc electrophoresis. Albumins, globulins, and gliadins, as defined by their solubility, molecular weight, and electrophoretic mobility, were obtained by gel filtration on Bio-Gel P-150. Approximate molecular weights determined from elution volumes were 1×10^4 to 3×10^4, 5×10^4 to 9×10^4, and above 1.5×10^5 for albumins and globulins, gliadins and glutenins respectively. Each gel filtration fraction contained several protein components as determined by disc electrophoresis. Quantitative distribution of fractions varied with species. For each fraction the amount for Triticale was intermediate between the amounts for the parental species. The gel-filtration spectrum for Triticale was similar to that for the hard red spring wheat. However , disc electrophoretic patterns for fractions obtained by gel filtration for the two species were quite different.

INTRODUCTION

Amino acid compositions and quantitative distributions of endosperm proteins among four solubility groups for one line of Triticale, its durum wheat and rye parents, and one variety of Canadian hard red spring wheat were reported in the previous paper (4). To establish the inheritance of proteins in the interspecific hybrid from its parents, it is necessary to show that specific protein components in the hybrid can also be detected in either of the parents. This can be done with some degree of certainty by examining a single extract of the grain (3, 10, 15). Because the number of individual protein components in cereal grain endosperm is extremely large,

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it was felt that more precise information would be obtained if the proteins were first fractionated into groups comprising smaller number of components. This experimental approach will also give information on the quantitative distribution of a particular group of similar proteins among related species. Accordingly the four solubility groups obtained as described in the first paper (4) were further fractionated by gel filtration. Each fraction obtained was examined by disc electrophoresis. Results of these experiments are reported in this paper.

MATERIALS

The four soluble fractions used in this study were prepared from flours milled from one line of Triticale (6A190), and one variety of durum wheat (T. turgidum cv. Stewart 63), rye (S. cereale cv. Prolific), and hard red spring (HRS) wheat (T. aestivum, L. em Thell cv. Manitou) as described in the previous paper (4). The proteins in these extracts will be referred to as the water-, salt-, alcohol- and acetic acid-soluble proteins. The five proteins of known molecular weight used for calibrating the gel-filtration column were obtained from Calbiochem. All chemicals used were of reagent grade unless stated otherwise.

METHODS

Gel filtration

Cross-linked polyacrylamide gel, Bio-Gel P-150, 100-200 mesh, (Bio-Rad Laboratories, Richmond, California) was used to fractionate the proteins in each of the four extracts. The chromatographic column was prepared as follows: Dry gel powder was soaked in excess distilled water (10 g. in 500 ml.) with gentle stirring to facilitate maximum swelling. It was allowed to settle and the supernatant was decanted. The swollen gel was then equilibrated with several changes of the eluant for a minimum period of 48 hours. Air bubbles were removed from the gel by stirring inside an

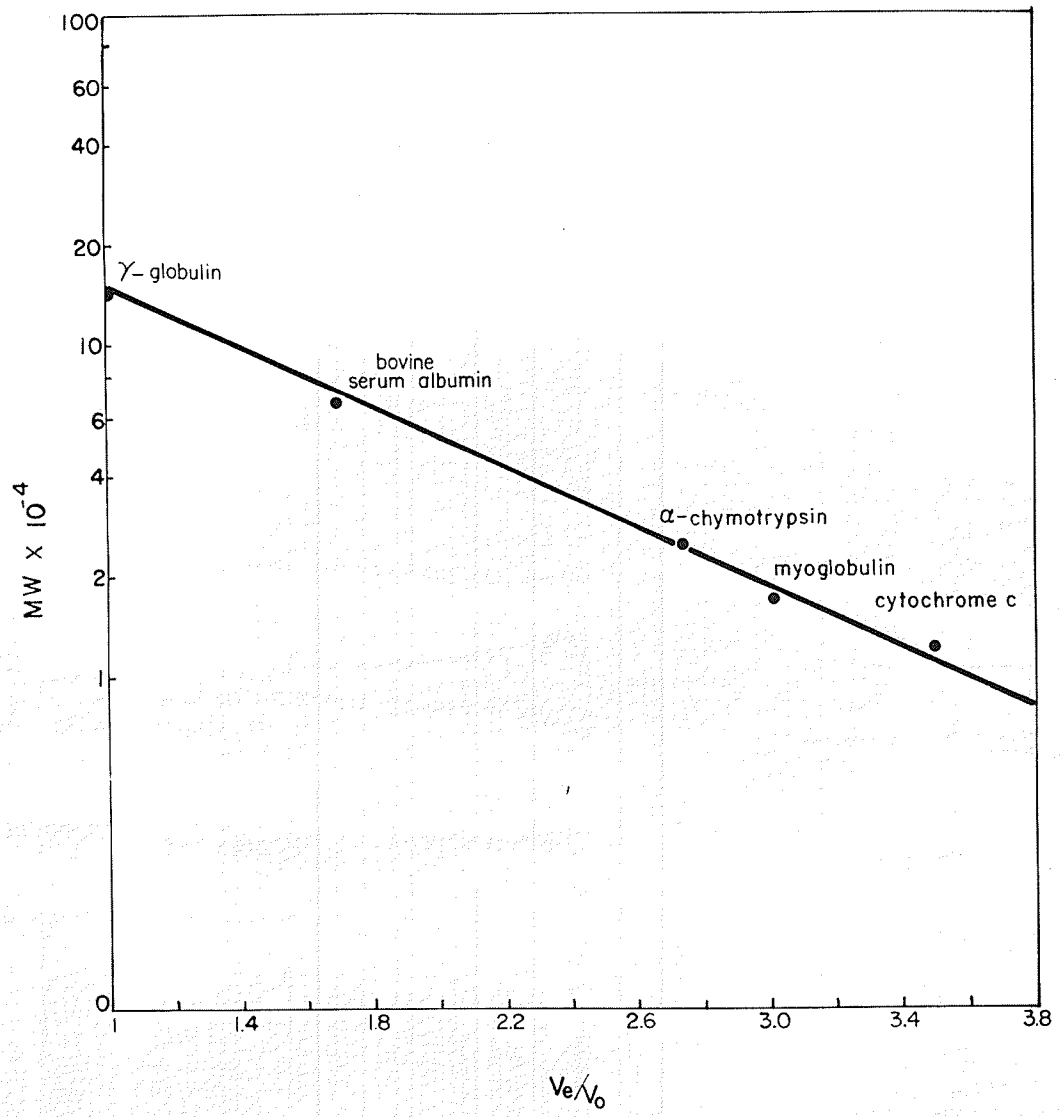
evacuated desiccator. The equilibrated gel was poured into the chromatographic column and was allowed to settle by gravity; more gel was added until the gel bed maintained a constant height. The eluant was connected to the column and the equilibration continued by elution at the desired flow rate for at least 24 hr. The final height of gel was 47 cm. in a 2 x 50 cm. column.

The protein solution for gel filtration was prepared by dissolving 50 mg. of solid material in 5 ml. of the appropriate solvent (given below). In cases where the extract did not dissolve completely, the small amount of insoluble residue was removed by centrifugation. The solution was applied to the column with an applicator (a Mariotte flask) connected to the eluant inlet of the column. Elution was monitored with an ISCO (Instrumentation Specialties Co. Inc., Lincoln, Nebr.) UV detector at 280 mμ and the eluate was collected on a time-controlled fraction collector. Recovery of protein in the gel filtration experiments was from 90 to 102% which was considered as satisfactory.

To obtain maximum solubility of wheat flour protein fractions for column chromatography, urea or some similar dissociating agent is usually added to the solvent. Accordingly, 3M urea and 2M N,N-dimethylformamide (DMF) solutions were compared as solvents for the water soluble proteins using a solution containing 0.1M sodium chloride and 0.05M acetic acid as the eluant. Much better resolution of peaks that absorbed at 280 mμ was obtained with the dimethylformamide than with urea in the solvent. Similar difficulties with urea were encountered by others working on wheat proteins (2, 11). Accordingly, N,N-dimethylformamide was adopted for preparing all solutions for gel filtration.

For estimation of molecular weights, the gel-filtration column was calibrated using proteins of known molecular weight as recommended by Whitaker (17). A linear relationship between the logarithm of molecular

Fig. 1. Calibration curve for Bio-Gel P-150 column with reference proteins dissolved in 2M DMF - 0.05M acetic acid solution: Bovine gamma-globulin - 160 x 10^3 (14); bovine serum albumin - 66.5 x 10^3 (13); alpha-chymotrypsin - 22.5 x 10^3 (16); myoglobin - 17 x 10^3 (8); and cytochrome c - 12.4 x 10^3 (12).


100
60
40
20
10
6
4
2
1
0
MW x 10^{-4}
γ- globulin
bovine
serum albumin
α-chymotrypsin
myoglobulin
cytochrome c
1
1.4
1.8
2.2
2.6
3.0
3.4
3.8
V_e/V_o

weight and the ratio of elution volume to void volume was obtained (Fig. 1).

Disc electrophoresis

Fractions obtained from gel filtration were examined by disc electrophoresis. The electrophoresis procedure used was the same as that developed by Davis (7), except that the compositions of the gel solutions were different. The compositions used are given in Table 1.

Buffer solutions for upper and lower electrolytes were made up with the following components (per liter): upper buffer (pH 4.0) -- 28.1 g. of glycine and 3.05 ml. of acetic acid; and lower buffer (pH 4.3) -- 43 ml. of acetic acid and 120 ml. of 1N potassium hydroxide solution.

The protein solution for disc electrophoresis was made up to 1% (w/v) in appropriate solvent (given below). Sucrose was added to the solution at a concentration of 15-20% to increase the density. Fifty µl. of the final solution was applied to the top of the upper gel. Electrophoretic separation was carried out for 140 min. with a constant current of 4 mA per column. Methyl green was used as marker for the buffer front. The gel was stained with 0.5% Amido Black (Buffalo Black, Allied Chemical Co., N.Y.) in 7% acetic acid solution overnight. Destaining was by electrophoresis using 7% acetic acid for upper and lower electrolytes.

Acidic pH (3.8) was used in the disc electrophoresis to permit the use of acidic solvents for solubilizing the proteins. As in the case of gel filtration, incorporation of 3M urea into the polyacrylamide gel in disc electrophoresis, did not improve the resolution of protein bands. Some protein bands were too faint to reproduce photographically. Accordingly interpretive drawings were made of the electrophoretic patterns using different density of hatching to represent band intensity. The mobility

Table 1. Compositions of Solutions for Disc Electrophoresis

	Upper gel pH 5.8			Lower gel pH 3.8		
Chemicals	per 100 ml.		Volume ratio	per 100 ml.		Volume ratio
Solution A			1			1
Acrylamide	10	g.		30	g.	
N,N-Methylene-bisacrylamide	0.8	g.		0.8	g.	
Solution B			1			2
IN Potassium hydroxide	48	ml.		24	ml.	
Acetic acid	3.6	ml.		53.2	ml.	
N,N,N,N-Tetra-methylenediamine	0.2	ml.		0.4	ml.	
Solution C			2			1
Ammonium persulfate	60	mg.		120	mg.	
Riboflavin	1	mg.		2	mg.	

for each band was expressed by its R_f value, which is the ratio of the distance travelled by the protein band to the distance travelled by the marker.

RESULTS AND DISCUSSION

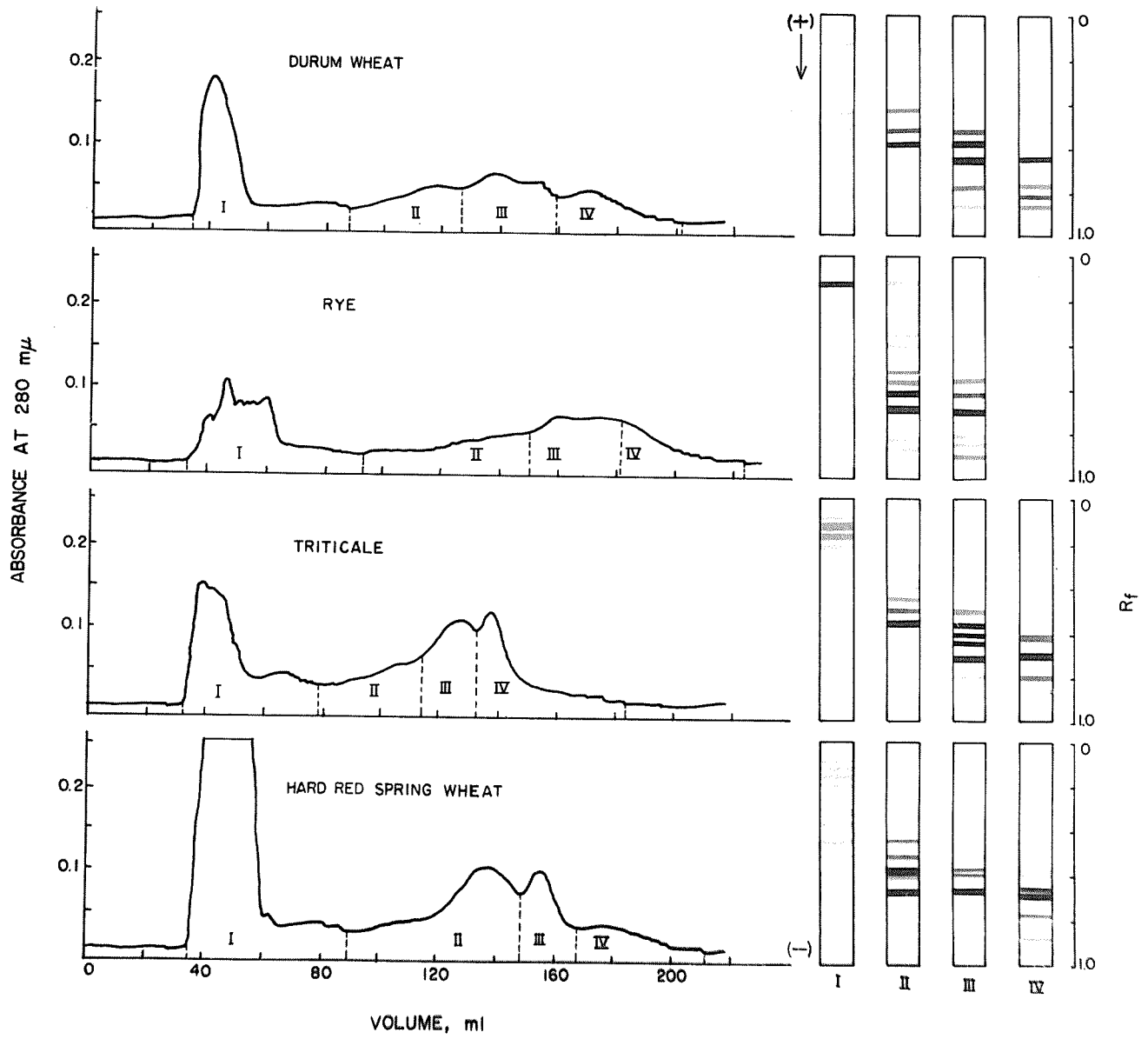
Water-Soluble Proteins

For gel filtration, weighed samples of water-solubles were dissolved in 2M DMF-0.05 acetic acid-0.1M sodium chloride solution and eluted with 0.05M acetic acid-0.1M sodium chloride solution at a flow rate of 18-19 ml. per hr. Fractions obtained were dialysed against distilled water and then freeze-dried. The freeze-dried fractions were redissolved in the same solvent for the disc electrophoresis experiments.

Figure 2 shows the gel filtration spectra and the disc electrophoresis patterns for the four species studied. Elution cuts were collected as indicated. The fraction numbers (I-IV) identify gel filtration fractions with disc electrophoresis patterns for each species. Fractions represented by the same number may or may not represent the same proteins for different species. The fractions cut for each species were made to include specific peaks and a range of molecular weight.

The water-soluble proteins of all four species were separated by gel filtration into two major groups of proteins. The first group (fraction I) contained high molecular weight proteins which appeared at exclusion limit of the column ($>$150,000) and those having molecular weights of about 80,000-90,000. When this fraction was subjected to disc electrophoresis, most of the protein did not migrate into the polyacrylamide gel and remained at the origin. A number of faint protein bands, which appear to be similar to gliadins, were detected in this fraction. The amount of the gliadin-type protein in fraction I for the rye was relatively higher than for the other

Fig. 2. Gel filtration and disc electrophoretic patterns from the water-soluble proteins from durum wheat, rye, Triticale, and HRS wheat.


DURUM WHEAT
RYE
TRITICALE
HARD RED SPRING WHEAT
ABSORBANCE AT 280 mμ
0.2
0.1
I
II
III
IV
0
40
80
120
160
200
VOLUME, ml
(+)
(−)
0
1.0
Rf

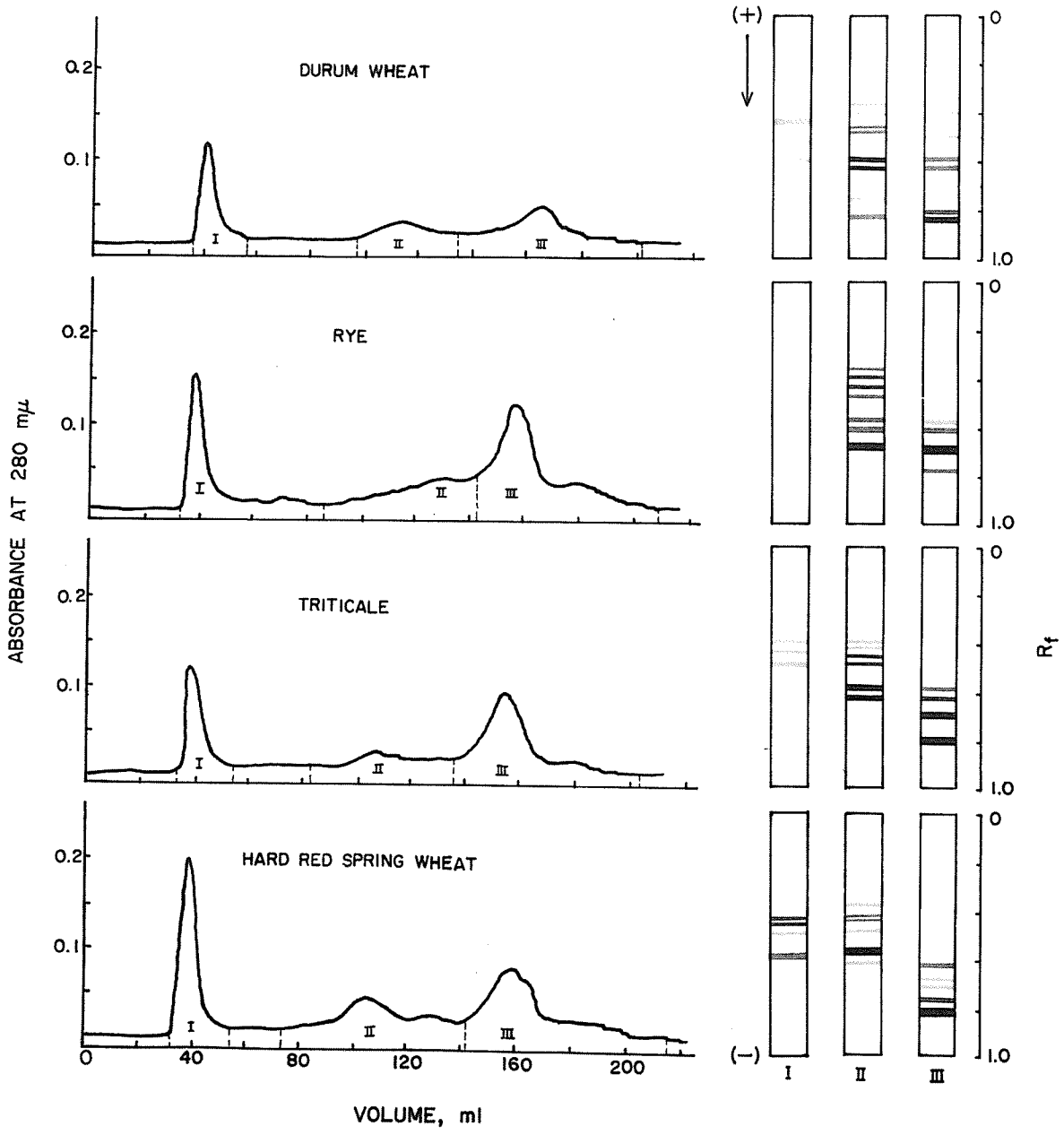
species. The HRS wheat contained the largest amount of protein in fraction I. These results for the HRS wheat are similar to those of Abbott and Johnson (1) who found by gel filtration on Sephadex G-100 that water extracts of wheat flour contained about 40% gliadin and glutenin.

The second major group of proteins of water-soluble extract were the low molecular weight fractions (II-IV). The molecular weights for these fractions ranged from 6,000 to 23,000 for the durum wheat; 6,000 to 14,000 for the rye; from 10,000 to 28,000 for the Triticale; and from 6,000 to 28,000 for the HRS wheat. Disc electrophoretic patterns for these fractions showed that each contained several distinct proteins. There was considerable overlapping between neighboring fractions. According to their solubility and molecular weight, the proteins of these fractions are considered to be albumins (1, 11). Comparison of the albumins for the four species examined showed that they are characterized by approximately the same R_f values. The albumins of the durum wheat contained two main bands and five minor bands; the rye albumins showed two major bands and seven minor bands; Triticale albumins showed four major bands and five minor bands. Fraction IV of the rye did not show any protein bands in disc electrophoresis.

Salt-Soluble Proteins

The gel filtration system used for the fractionation of the salt-soluble proteins was the same as for the water-soluble proteins. By this method, the salt-soluble proteins were separated into three fractions (I-III, Fig. 3). Fraction I appeared at exclusion limit of molecular weight ($\geq$150,000). Disc electrophoresis of this fraction showed a number of faint protein bands for all species except the rye. The nature of these minor protein components in fraction I was not investigated further although they could well be globulins since molecular weights for this group of wheat proteins as high as 200,000 have been reported (6).

Fig. 3. Gel filtration and disc electrophoretic patterns for the salt-soluble proteins from durum wheat, rye, Triticale, and HRS wheat.


DURUM WHEAT
RYE
TRITICALE
HARD RED SPRING WHEAT
ABSORBANCE AT 280 mμ
0.2
0.1
I
II
III
0
40
80
120
160
200
VOLUME, ml
(+)
(−)
0
1.0
R_f

Fraction II comprised proteins with molecular weight about 28,000 for the durum wheat, Triticale and the HRS wheat; and about 20,000 for the rye. Fraction III appeared with molecular weight about 8,000 for the four species studied. By disc electrophoresis each gel filtration fraction contained one or two major proteins, and a large number of minor components. In total, ten different globulins were detected in the salt-solution extracts from the durum and HRS wheats, and eight in the rye and Triticale extracts.

Alcohol-Soluble Proteins

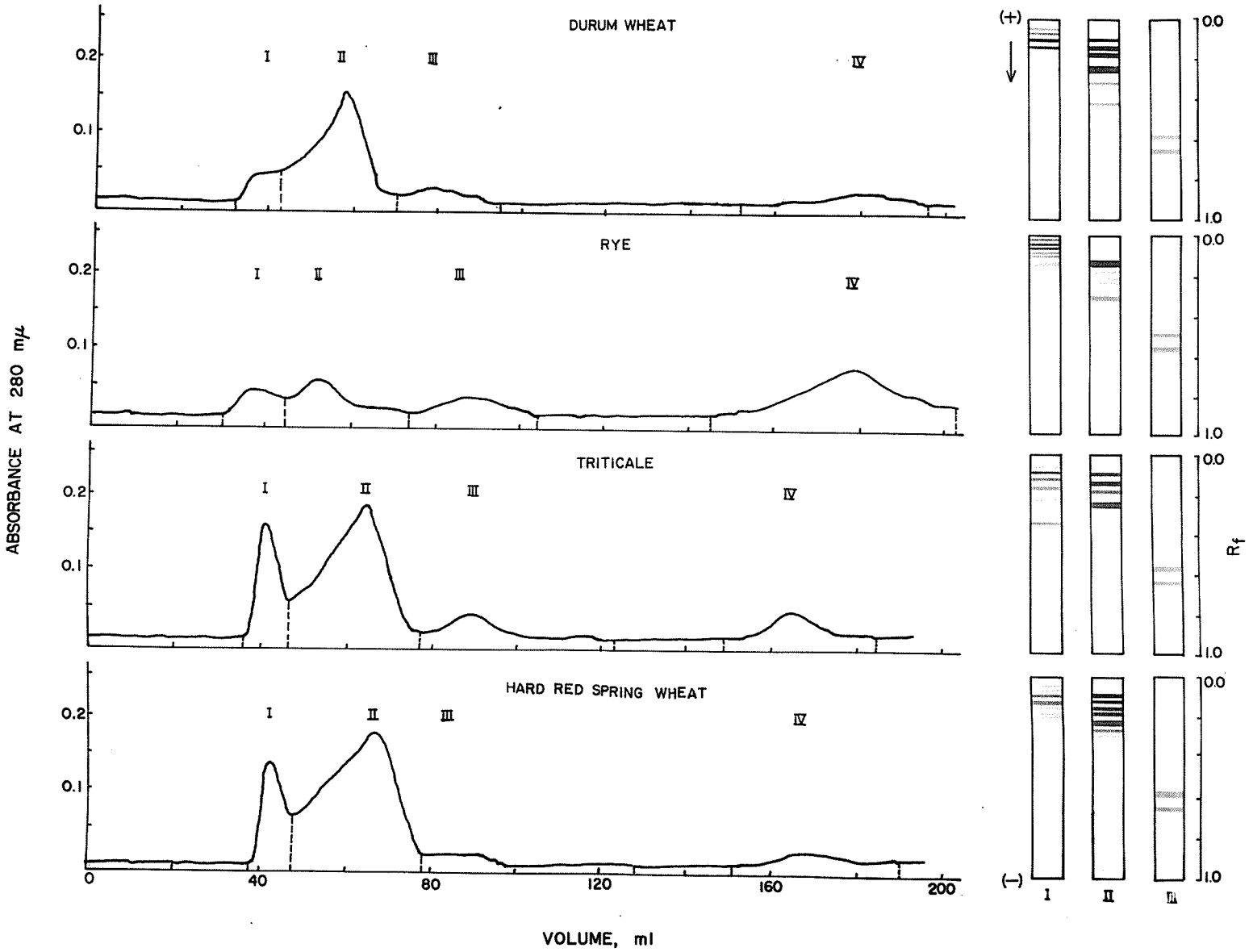
For gel filtration, the alcohol-soluble proteins were dissolved in 2M DMF-0.05M acetic acid solution, and eluted with 1M DMF-0.05M acetic acid solution at a flow rate of 9-10 ml. per hr. Sodium chloride could not be used with this extract since gliadins are highly insoluble in salt solutions (18).

The results of gel filtration and disc electrophoresis of the alcohol-soluble proteins for the four species are shown in Fig. 4. As in the case of the water-solubles, the alcohol-soluble proteins were divided into four fractions. Relatively small peaks were obtained in the gel filtration spectrum for the rye extract. Presumably this resulted from the low solubility of the protein of this extract in the solvent used.

Fraction I contained proteins of molecular weight above the exclusion limit of the gel. Electrophoresis pattern for this fraction showed a number of faint, slow moving bands. The main protein components of the alcohol-soluble fraction were separated as fraction II. The molecular weight of fraction II proteins is in the range of 80,000-90,000 for the four species. This estimated molecular weight is somewhat higher than the 50,000 reported to be the average molecular weight of wheat gliadins (9).

Disc electrophoresis results indicated that fraction II consists of several slow moving protein bands. On the basis of molecular weights and electrophoretic mobility, fraction II proteins appear to be gliadins. The

Fig. 4. Gel filtration and disc electrophoretic patterns for the alcohol-soluble proteins from durum wheat, rye, Triticale, and HRS wheat.


DURUM WHEAT
RYE
TRITICALE
HARD RED SPRING WHEAT
I
II
III
IV
0.2
0.1
ABSORBANCE AT 280 mμ
0
40
80
120
160
200
VOLUME, ml
(+)
(−)
0.0
1.0
Rf

durum wheat and the Triticale showed similar patterns comprising at least five slow moving bands. Fraction II of the rye contained one major and several minor bands. The HRS wheat fraction II contained at least seven protein bands. The patterns for the rye, the durum wheat, and the HRS wheat were all distinctly different. On the other hand, the pattern for the Triticale was similar to that of its tetraploid parent. A comparison of the electrophoresic patterns of fractions I and II showed some overlap in these fractions.

Fraction III appeared as a small peak with approximate molecular weight of 40,000 to 50,000. In disc electrophoresis, this fraction showed two minor protein bands with relatively high mobilities for the four species studied. Disc electrophoresis of fraction IV showed that it did not contain any proteins.

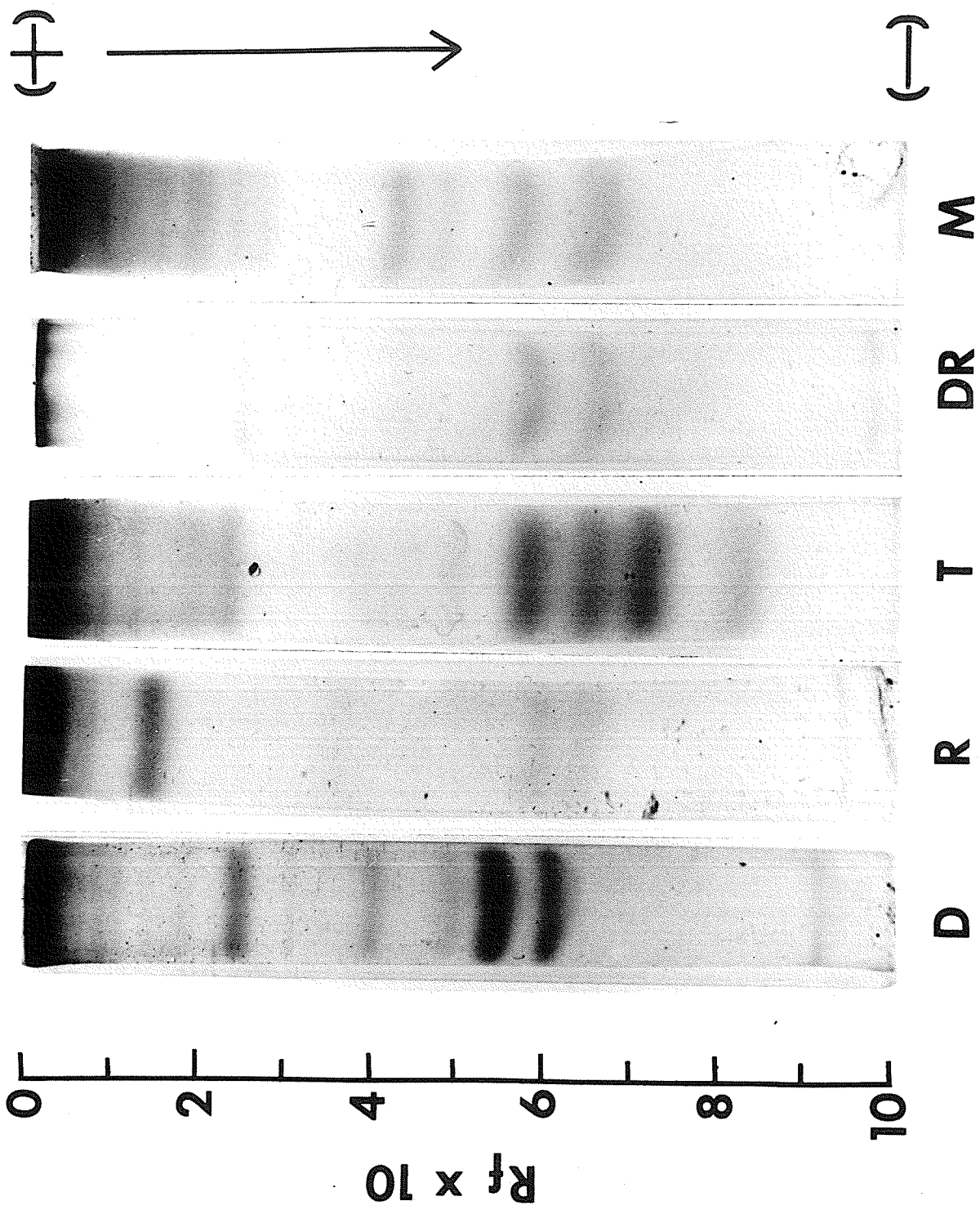
Acetic Acid-Soluble Proteins

The proteins of the acetic acid-soluble fraction were dispersed in 4M DMF-0.1M acetic acid solution. In gel filtration, most of the protein material could not be fractionated on Bio-Gel P-150. Presumably the molecular weights of this group of proteins were above 150,000, the exclusion limit of the gel used. When this dispersion was examined by disc electrophoresis, a number of faint bands were obtained for all four species (Fig. 5), but most of the protein remained at the point of application. The pattern for Triticale showed two fast moving bands which were not present in the analogous dispersions for the parents. The significance of these apparently "new" bands in relation to inheritance of proteins will be discussed in the accompanying paper (5).

GENERAL DISCUSSION

By means of gel-filtration chromatography, three of the four solubility extracts discussed in the first paper (4) were separated further into

Fig. 5. Disc electrophoretic patterns of the acetic acid-soluble proteins from durum wheat, rye, Triticale, and HRS wheat.


M
DR
T
R
D
0
2
4
6
8
10
Rf x 10

fractions which were identified as albumins, globulins, gliadins, and glutenins. This identity was based on approximate molecular weights, on solubility, and on electrophoretic mobility. Fractions of similar molecular weight obtained by gel filtration were found to be relatively heterogeneous by disc electrophoresis. As expected, there was some overlap among fractions of each species. Further studies are necessary to identify particular bands among the various species. This information is essential for the determination of the inheritance of the proteins in the interspecific hybrid, Triticale, from its parents.

The quantitative distributions of the protein components in the various solubility groups (Table 2) show a wide variation among the species studied. As was expected, the water soluble protein fraction contains considerable amounts of gliadin-like proteins in addition to the albumins. The composition of the fractions vary among the four species examined. In general, the protein composition of Triticale is intermediate between those for its parents. A similar relationship was found for the total extracts (4).

The number of protein bands in the various solubility groups for the three related species is different. However, in no case does the extract from the Triticale contain more components than the sum of components (bands) in analogous extracts from the parents. Experiments designed to establish qualitative identity between protein components of Triticale with those of its two parents will be discussed in the next paper.

It is interesting to note that the gel-filtration spectrum for the line of Triticale examined was similar to that for the HRS variety. However, both qualitative and quantitative differences in solubility, gel filtration and disc electrophoresis patterns between the Triticale and the HRS wheat proteins were observed. Accordingly, both factors might be responsible for the lack of breadmaking quality of Triticale.

Table 2. Fractionation of the proteins by gel filtrations (in total weight recovered)

Fractions	Rye	Triticale	Durum wheat	HRS	Major proteins
	%	%	%	%	
A. Water-soluble proteins					
I	36	31	28	45	gliadins and glutenins
II	29	27	26	29	albumins
III	24	30	31	16	albumins
IV	11	12	15	10	albumins
B. Salt-soluble proteins					
I	31	27	22	26	globulins
II	23	32	33	39	globulins
III	46	41	45	35	globulins
C. Alcohol-soluble proteins					
I	27	14	8	15	glutenins
II	33	55	62	68	gliadins
III	22	15	19	12	gliadins
IV	18	16	11	5	nonproteins
D. Acetic-acid-soluble proteins (not separated)					glutenins

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NATURE OF PROTEINS IN TRITICALE AND ITS PARENTAL SPECIES

III. A COMPARISON OF THEIR ELECTROPHORETIC PATTERNS

C. H. Chen and W. Bushuk

ABSTRACT

Comparison of the disc electrophoretic patterns for the albumins, globulins, gliadins, and glutenins of one line Triticale and its durum wheat and rye parents showed that all the protein components of the interspecific hybrid are present in the parents. It is concluded that the proteins of Triticale are simply inherited from its parents. The patterns for the albumin and globulin groups for the variety of durum wheat (Stewart 63) and the variety of hard red spring wheat (Manitou) investigated were identical. Differences were observed in the patterns for the gliadins and glutenins of these two wheats. These qualitative differences might account for the difference in their breadmaking qualities, and provides additional evidence in support of the hypothesis that the genes for breadmaking quality factors are in the D genome.

INTRODUCTION

Previous papers(1, 2) presented results on some chemical and physical properties of the proteins of one line of Triticale, its durum wheat and rye parents, and one variety of Canadian hard red spring wheat. In all properties examined, the proteins of Triticale appeared intermediate between those of its two parents.

To establish homology of proteins in related species, it is desirable first of all to establish the identity of specific protein components of the progeny and the parents. In studies of grain proteins by various workers (1), mobility in starch-gel electrophoresis has been used as the main criterion of homology. Disc electrophoresis, which gives better resolution and is more convenient for examining a large number of samples, was used to obtain the results discussed in this paper.

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[1]Contribution No. 185 of the Department of Plant Science, University of Manitoba, Winnipeg 19, Manitoba, Canada, with financial assistance from the Canada Department of Agriculture, Research Branch.

MATERIALS AND METHODS

Protein extracts from one line of Triticale (6A190), and one variety of durum wheat (T. turgidum cv. Stewart 63), rye (S. cereale cv. Prolific), and hard red spring (HRS) wheat (T. aestivum L. em Thell cv. Manitou), were prepared as described previously (1). All chemicals used were of reagent grade unless otherwise stated.

Disc Electrophoresis

The procedure used was described in the second paper of this series (2), and salt-soluble proteins were dissolved in 2M dimethylformamide (DMF) - 0.5M sodium chloride-0.05M acetic acid solution and the alcohol- and acetic acid-soluble proteins were dissolved in 4M DMF-0.1M acetic acid solution. Higher concentration of DMF was used with the latter fractions to increase the solubilization; the additional DMF had no effect on the number of protein bands or their mobility.

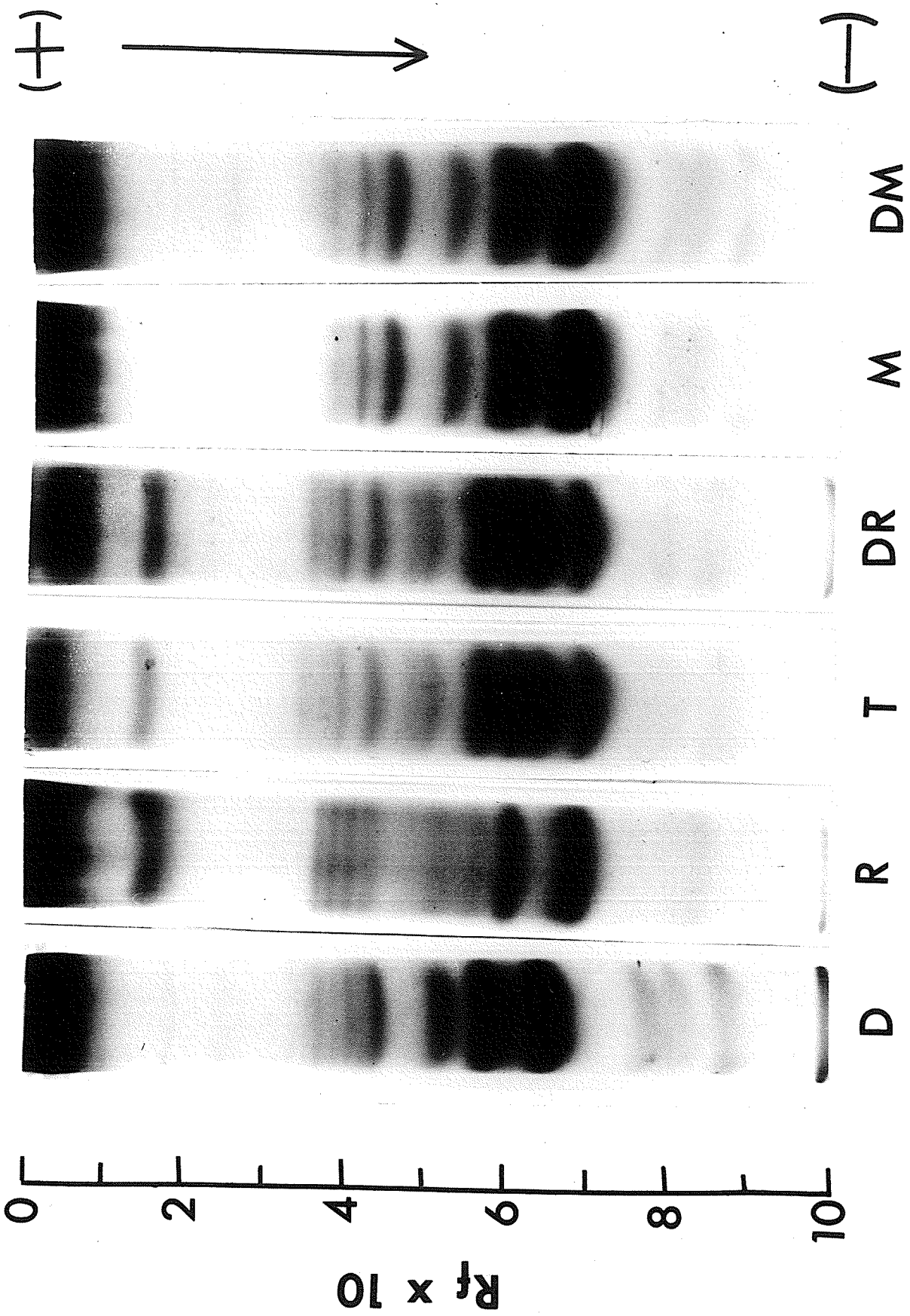
RESULTS AND DISCUSSION

Albumins

Electrophoretic patterns for the water-soluble proteins of the four species and mixtures of equal parts of the extracts from durum wheat and rye, and from durum and hard red spring wheats show some distinct differences and similarities among the four species (Fig. 1). The components with relative mobilities (R_f) from 0.35 to 0.9 are considered to be albumins (2).

The patterns for durum wheat and rye were similar in the number of bands but there were differences in R_f values, especially for the two major bands. Triticale pattern appeared to be almost an exact composite of the patterns for its parents. It showed four major albumin bands with R_f values from 0.6 to 0.7. The pattern for the durum wheat and rye mixture (DR) appeared essentially the same as the Triticale pattern. On the basis

Fig. 1. Disc electrophoretic patterns for albumins from durum wheat (D), rye (R), Triticale (T), and HRS wheat (M), and mixtures of albumins from durum wheat and rye (DR), and from durum and HRS wheats (DM).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(−)
DM
M
DR
T
R
D
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2
4
6
8
10
Rf x 10

of these results, it is concluded that the line of Triticale studied does not have any "new" proteins in the albumin group not present in the same solubility group of proteins from its parents. This finding is contrary to that of Yong and Unrau (7) who detected several "new" proteins by starch-gel electrophoresis with mobilities in the range of values for the albumin group.

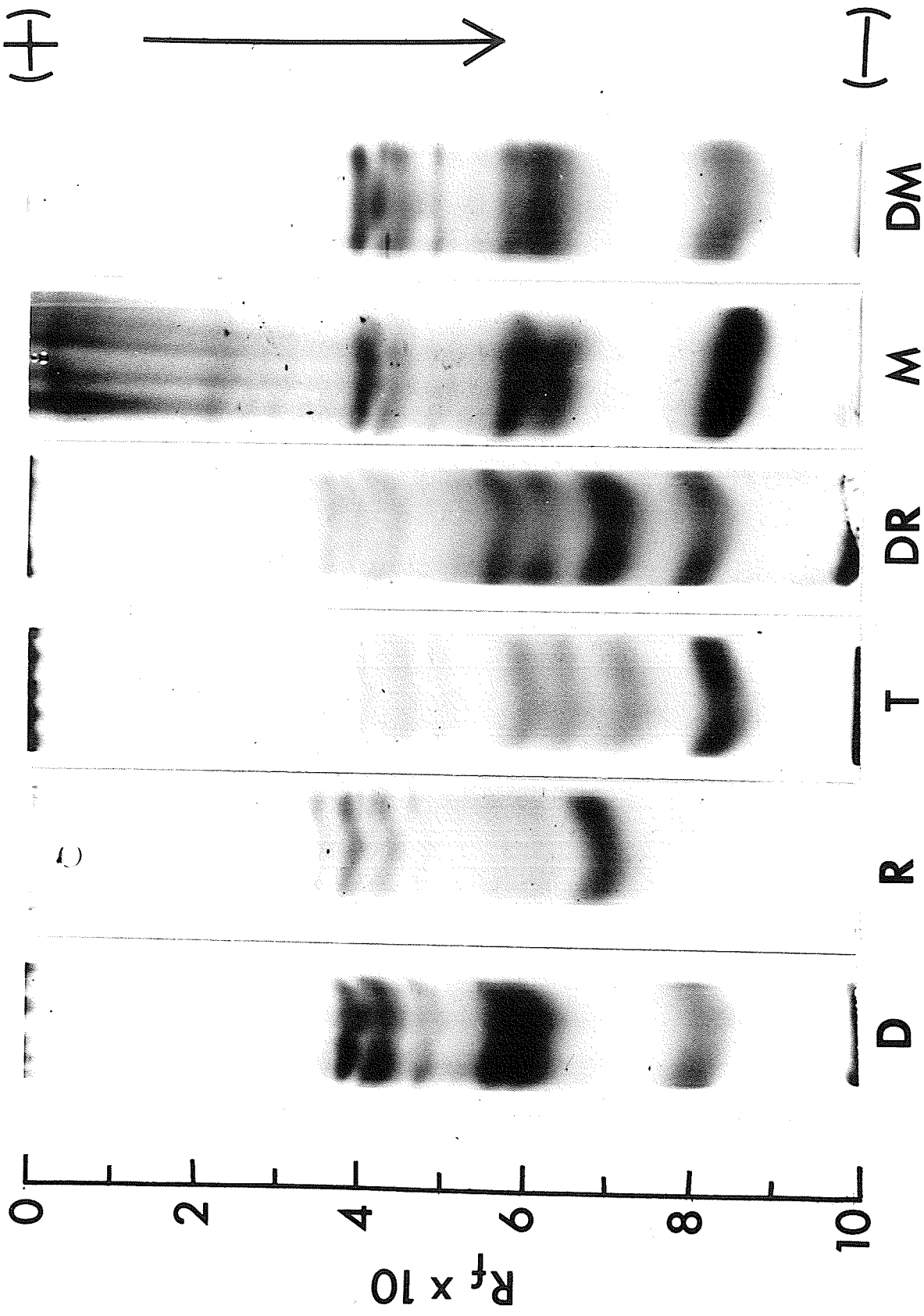
Comparison of albumin patterns for the durum and HRS wheats showed that all the albumins of the durum wheat were present in the HRS wheat. The HRS wheat had two additional minor fast moving bands (R_f 0.62 and 0.70) not present in durum wheat. The mixture (DM) produced the same pattern as that for the HRS wheat. Accordingly, it appears that the D genome contributed two minor protein components that could be differentiated by the technique used.

Globulins

Disc electrophoresis patterns for the salt-soluble proteins (globulins) (Fig. 2) are arranged in the same order as for the albumins shown in Fig. 1. A comparison of Figs. 1 and 2 shows that the patterns for the two fractions are significantly different although both groups of proteins migrate in the same range of mobility. There is a possibility that some of the bands of the globulin group represent proteins of the albumin group.

Results of Fig. 2 show that the patterns for durum wheat and rye are quite different, especially in the high mobility region. Several of the slow moving bands appear common to both species. As was found for the albumins, the globulins pattern for the Triticale appears to be a composite of the patterns for its parents. All the major bands of the durum wheat and the rye were found in the Triticale pattern. One minor, slow moving band of the rye extract was absent from the Triticale pattern. The composite nature of the Triticale pattern and the lack of one of the rye bands was confirmed by comparing the patterns for Triticale (T) with that for the

Fig. 2. Disc electrophoretic patterns for globulins from durum wheat (D), rye (R), Triticale (T), and HRS wheat (M), and mixtures of globulins from durum wheat and rye (DR), and from durum and HRS wheats (DM).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DM
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R_f x 10

mixture of durum wheat and rye (DR) extracts. There were no bands in the Triticale pattern that were not represented in the patterns for its parents.

The globulin pattern for the HRS wheat (M) was essentially identical to that for the durum wheat. Identity of the protein bands was established by running a mixture of the two extracts in a single tube (DM). The genetic control of the biosynthesis of the globulins in durum and HRS wheats appears to be entirely in the A and B genomes.

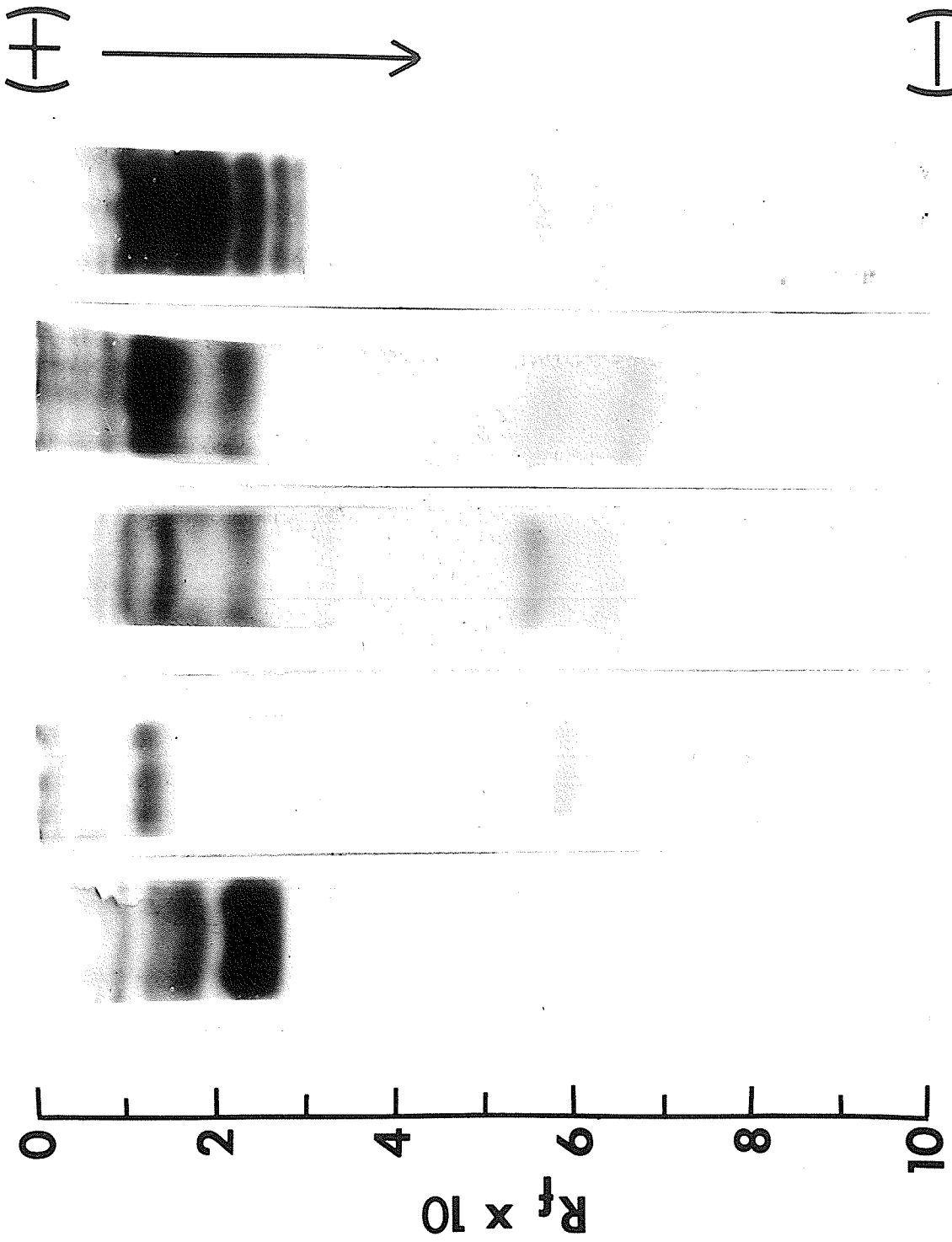
In contrast to the results for the albumins, the globulin patterns for Triticale and the HRS wheat are different. One major fast moving band in Triticale, which appears to have originated from the R genome of the rye, is absent from the pattern for the HRS wheat.

Gliadins

Disc electrophoresis patterns for the alcohol-soluble proteins (gliadins) of the four species, and a mixture of durum wheat and rye extracts (DR) are shown in Fig. 3. Major protein components of this group migrate very slowly under the conditions used. Accordingly the resolution is not very high. In addition to the major, slow moving bands, all species showed a number of minor, fast moving components which are probably albumins or globulins. Distinct differences in this group of proteins were observed among the species examined. Some of the bands could be detected only with fluorescent underlighting and are not visible in the photographs.

Durum wheat extracts showed four major gliadin bands in the R_f region from 0.1 to 0.3 and six minor, faster-moving bands with mobility values in the range 0.3 to 0.65. The gliadin group of the rye comprises one major component with R_f of 0.12 and six (only the two fastest are visible in the photographic reproduction) minor components of higher mobility. Triticale extract showed four major and the two minor fast-moving components similar to those of the durum wheat extract. The major band of the rye extract

Fig. 3. Disc electrophoretic patterns for gliadins from durum wheat (D), rye (R), Triticale (T), and HRS wheat (M), and a mixture of gliadins from durum wheat and rye (DR).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R_f x 10

was also present in the Triticale pattern however the two fast-moving minor components of rye were not detected. The pattern for the mixture of durum wheat and rye extracts was essentially to be the same as the Triticale pattern. Although the two fast bands of durum wheat and rye appear slightly different in the patterns for these species, the pattern for the mixture showed only two, somewhat diffuse bands. Apparently these two pairs of proteins are quite similar.

On the basis of the electrophoretic patterns obtained in the present study, no "new" proteins could be detected in the gliadins of Triticale not present in the analogous protein groups of its parents. However, it is obvious from Fig. 3 that the resolution of bands under the experimental conditions used was quite low. Accordingly the conclusion that Triticale does not have any "new" gliadins should be considered as tentative.

The gliadin pattern for the HRS wheat (M) appears to be significantly different from that for durum wheat (D). Durum wheat extract had a very broad band with R_f of 0.2 to 0.3 whereas the HRS wheat extract showed three distinct narrow bands. On the basis of these results, the gliadins of the durum and HRS wheats studied appear to be distinctly different. This finding should be confirmed by more detailed studies before it is implicated in genetic and breadmaking quality relationships between the two wheat species.

Glutenins

It was reported in the second paper of this series (2) that most of the protein of the glutenin group could not be resolved both by gel filtration and disc electrophoresis because of their high molecular weight. Accordingly detailed comparative studies of glutenins of related species must be preceded by chemical reduction or degradation to molecules of smaller size. This technique has been used in studies of the structure of gluten (3, 4) however it requires further refinements before it can be used in studies

of the homology of proteins in genetically related species. The possibility of producing artifacts by the non-specific modification is extremely high.

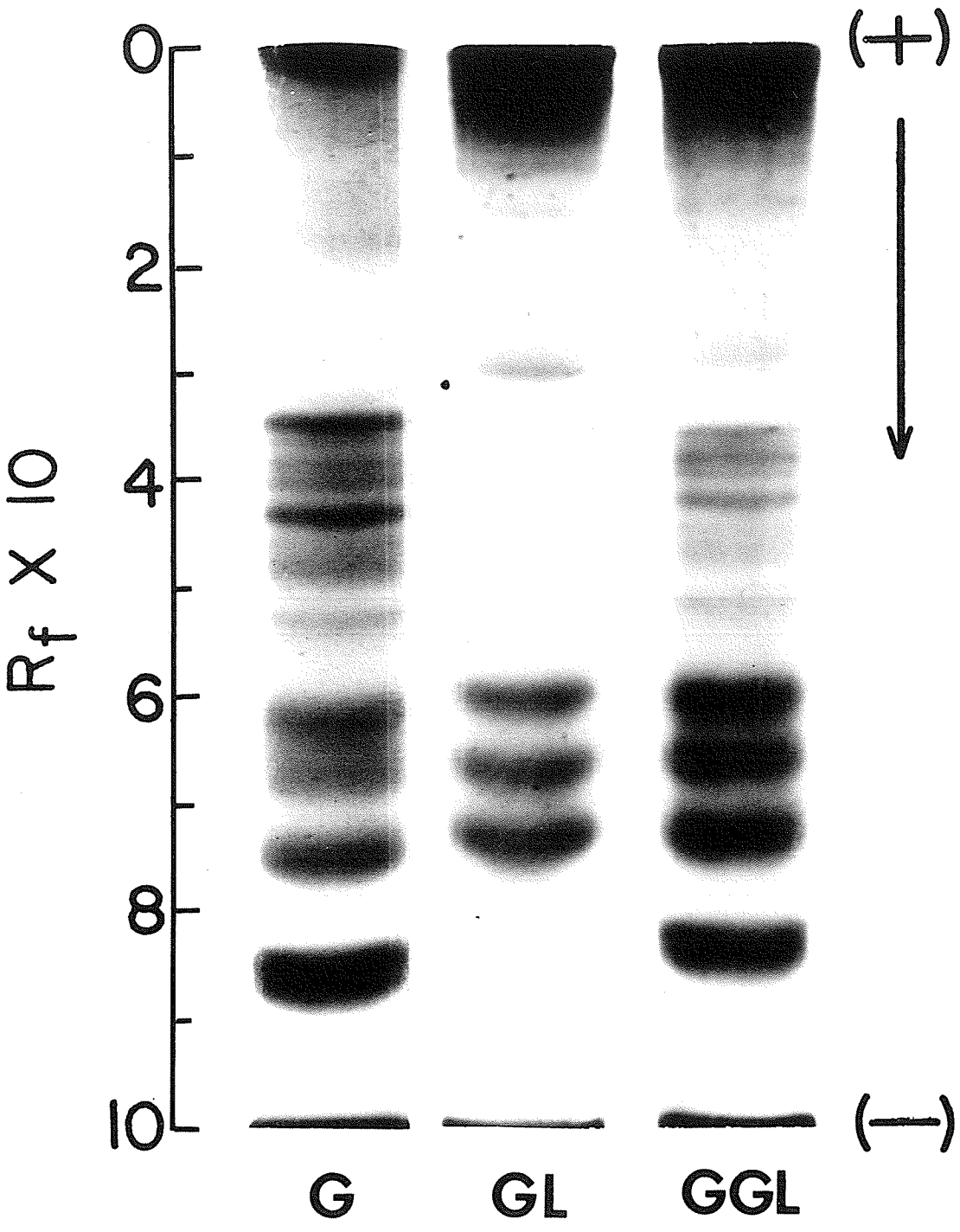
Figure 6 of the previous paper (2) showed that a dispersion of the glutenin fraction in 4M DMF-0.1M acetic acid solution contained a number of components that migrated into the gel in disc electrophoresis. Furthermore it was found that the Triticale extract showed two fast moving components that were completely absent in analogous extracts from its parent species. On the basis of mobility, the two "new" bands in Triticale appeared to be similar to two of its globulin bands. Although these two bands represented only a small fraction of the glutenin group, it seemed essential to establish their identity.

Figure 4 shows disc electrophoresis patterns for the globulins (G), glutenins (GL), and a mixture of globulins and glutenins (GGL) of the Triticale. Examination of the patterns showed that the two apparently "new" bands of the glutenin group are electrophoretically identical with two of the globulin components. Presumably the extraction of the salt-soluble proteins from Triticale flour was not as complete as for the other species. On the basis of these results it is concluded that the glutenins of Triticale that migrate into the gel in disc electrophoresis do not contain any "new" proteins not present in its parents.

GENERAL DISCUSSION

Comparison of the disc electrophoretic patterns of various soluble protein fractions from one line of Triticale and patterns for the analogous fractions from its durum wheat and rye parents showed that there are no "new" proteins in the interspecific hybrid not present in its parents. This conclusion is based on results with one technique used to examine about 80% of the total proteins. It is possible that "new" proteins might be detected by other techniques in the soluble fractions examined or in the residual proteins not examined in the present study. All the albumins of

Fig. 4. Disc electrophoretic patterns for the globulins (G), the acetic acid-soluble proteins (GL), and the mixture (GGL) of the globulins and the acetic acid-soluble proteins from Triticale.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G
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the parent species were detected in Triticale. One rye globulin component and a number of minor gliadin and glutenin components of rye were not detected in Triticale. Two, apparently "new" bands were detected in the soluble portion of Triticale glutenin group; however, it was shown that these were identical with two of its globulin components. Apparently the removal of globulins from Triticale flour by salt solution was not as efficient as in the other species. Accordingly, it appears that the proteins of the one line of Triticale studied, were simply inherited from its parents.

The observations reported in this study are at variance with those of Yong and Unrau (7) for the same line of Triticale. These workers found four "new" proteins in the hybrid by starch-gel electrophoresis and suggested that there must be interactions between alien genomes. Their extraction procedure was slightly different from that used in the present study, and it is quite possible that the "new" proteins resulted from incomplete extraction of various solubility groups of proteins as was obtained for the glutenin group in the present study.

In relation to inheritance of proteins in bread wheat, it might be noted that the albumins and globulins of durum and HRS wheats are essentially identical. The components of these groups could well be homologous. Their biosynthesis would be controlled by genes in the A and B genomes. Some differences between the gliadins and between the glutenins of the durum and HRS wheats were detected. These might reflect the importance of the D genome in the breadmaking quality of bread wheats (5, 6). However this hypothesis remains to be confirmed by more detailed studies.

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PART THREE

ADDENDUM

ADDITIONAL RESULTS AND DISCUSSION

1. Reduced and Alkylated Gliadins and Glutenins

Introduction

It has been reported (1) that high molecular weight gliadins of wheat are similar to low molecular weight glutenins. Furthermore, cleavage of the disulfide linkages of glutenin proteins produces a number of smaller protein units similar to gliadin components (2, 3). In the present studies, it was found that most of the glutenin group of proteins could not be fractionated or separated by gel filtration and disc electrophoresis. Presumably the molecular weights of these proteins are too high to permit their penetration into the supporting media used in the two techniques. An attempt was therefore made to examine these proteins further by decreasing their molecular size by reducing the disulfide crosslinkages. Results of these preliminary experiments are discussed in this section.

Material and Methods

Gliadins and glutenins from the four species of grain studied were obtained in crude form as the alcohol- and acetic acid-soluble proteins respectively as described in the second paper of Part II. Reduction and alkylation was carried out according to the method of Nielsen *et al* (4). The protein (50-100 mg.) was dissolved in 5 ml. of 8M urea-0.05M Tris buffer solution adjusted to pH 8.2. 2-Mercaptoethanol (0.5 ml.) was added to make a 1M solution. The mixture was stirred for 2 hr. at room temperature. Acrylonitrile (0.56 ml.) was then added to 2M concentration and stirred for additional hour. The resulting solution was adjusted to pH 4.0 with acetic acid, dialysed against 0.05M acetic acid for 24 hr., and freeze-dried.

Sedimentation patterns were obtained with the Spinco Model E analytical ultracentrifuge using a solution containing 0.25% (w/v) of protein in 6M guanidine hydrochloride-0.1M acetic acid solution. The ultracentrifuge was operated at 60,000 r.p.m. and 20°C.

Disc electrophoretic procedures were the same as described in the previous paper (second paper of Part II).

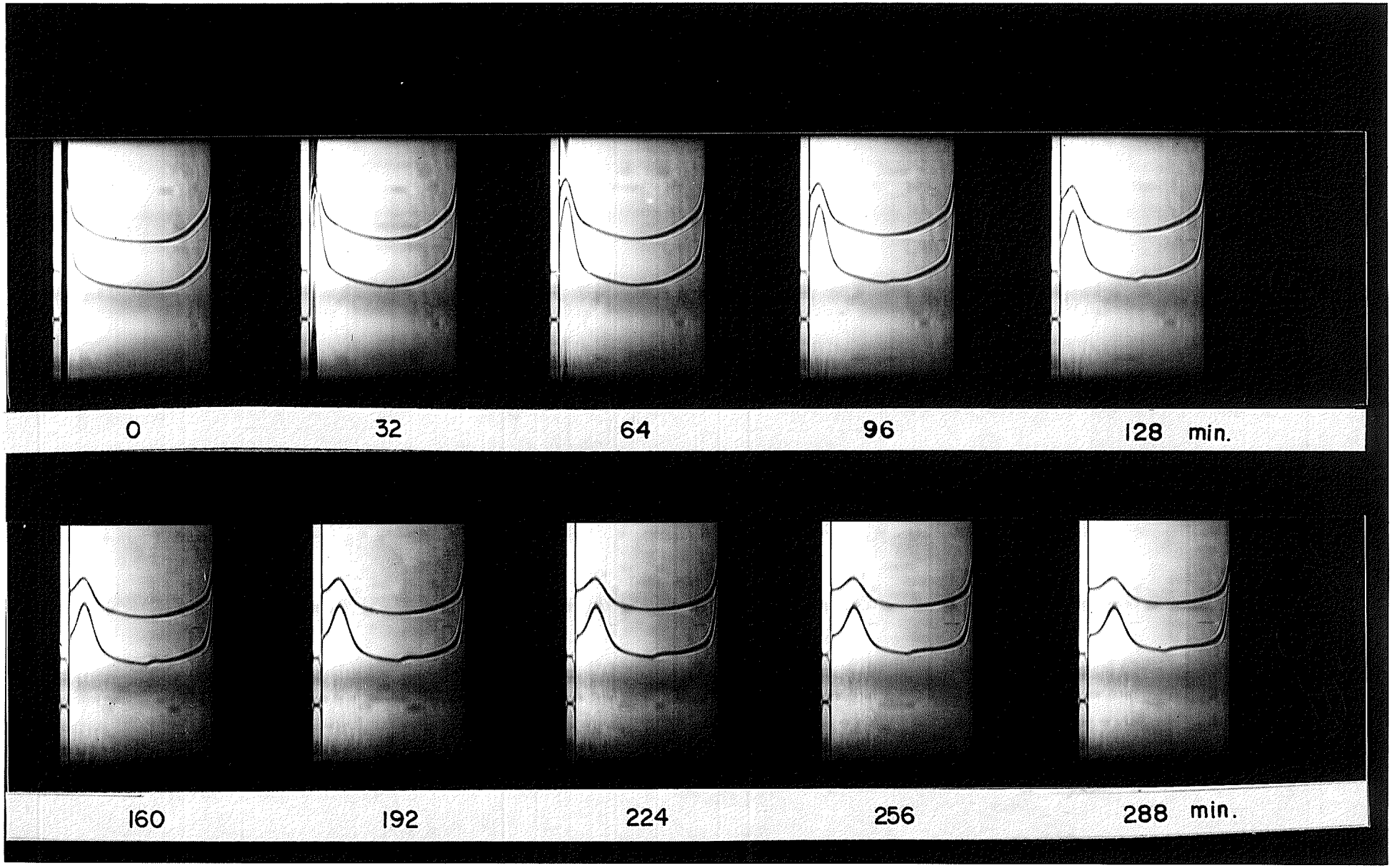
Results and Discussion

Sedimentation patterns

The rate of sedimentation in an ultracentrifuge gives a measure of the size and shape of macromolecules in the solution under investigation. In the present study, the reduced-alkylated gliadins and glutenins of durum wheat were examined by the analytical ultracentrifuge to determine possible changes in molecular size compared with the native gliadins and glutenins. Figure 1 shows that both native and reduced-alkylated gliadins of durum wheat appeared as one peak with the similar apparent sedimentation coefficient, S=0.50. These results suggest that reduction of disulfide bonds in gliadins did not produce any significant change in size of molecules. Accordingly, the disulfide bonds in gliadin appear to be mainly intramolecular.

Glutenins from durum wheat showed one slow sedimenting peak with apparent sedimentation coefficient, S=0.6, (Fig. 2). The proteins of this peak presumably represent small molecules in the acetic acid extract. The large molecular weight glutenins settled at the bottom of the ultracentrifuge cell during the acceleration to equilibrium speed. Reduced-alkylated glutenins appeared with two peaks of apparent sedimentation coefficient of 0.7 and 1.5. The proteins of the sedimentation

Fig. 1. Sedimentation patterns of native gliadin (upper) and reduced-alkylated gliadins (lower) from durum wheat.


0
32
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96
128 min.
160
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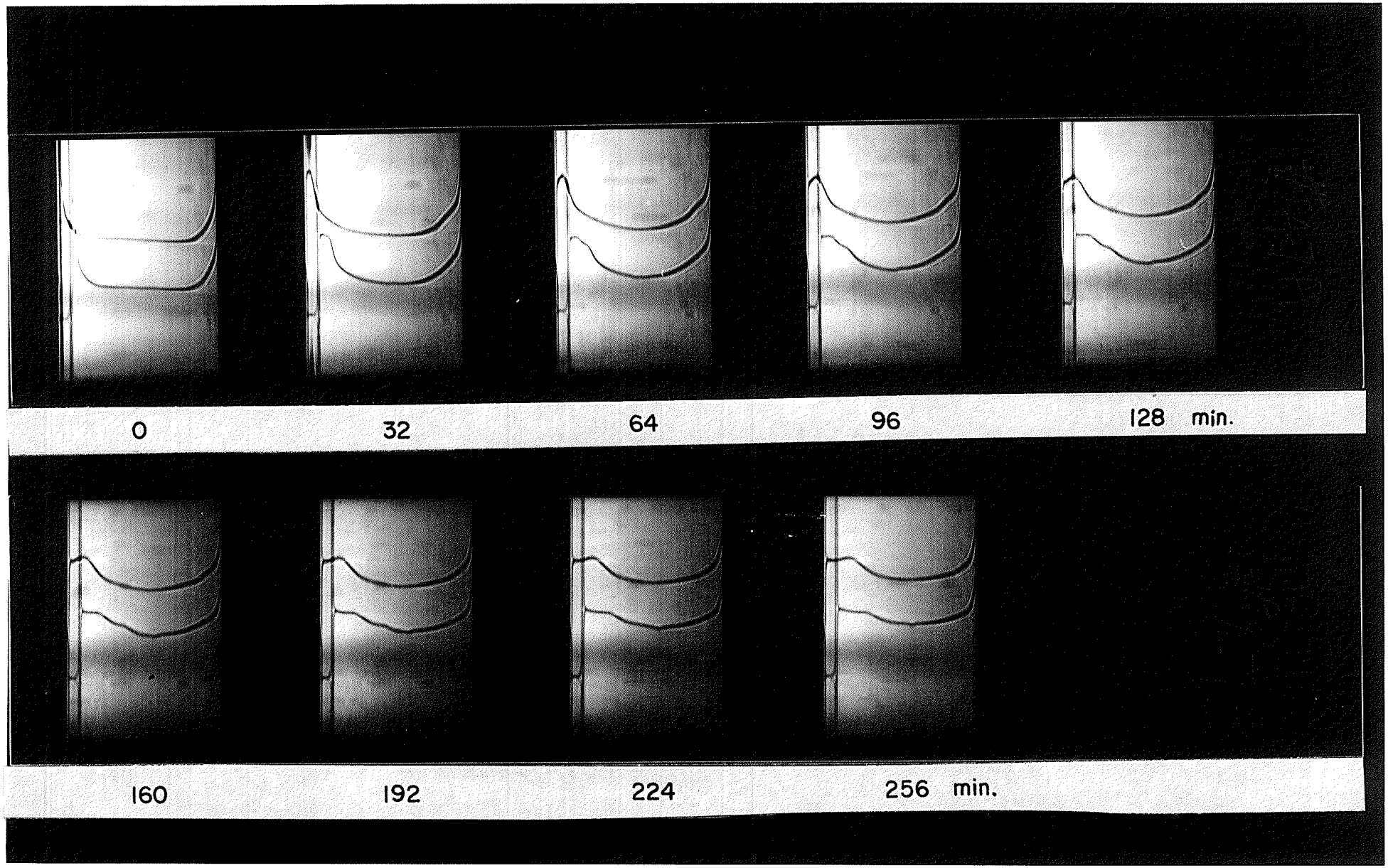
224
256
288 min.

Fig. 2. Sedimentation patterns of native glutenin (upper) and reduced-alkylated glutenin (lower) from durum wheat.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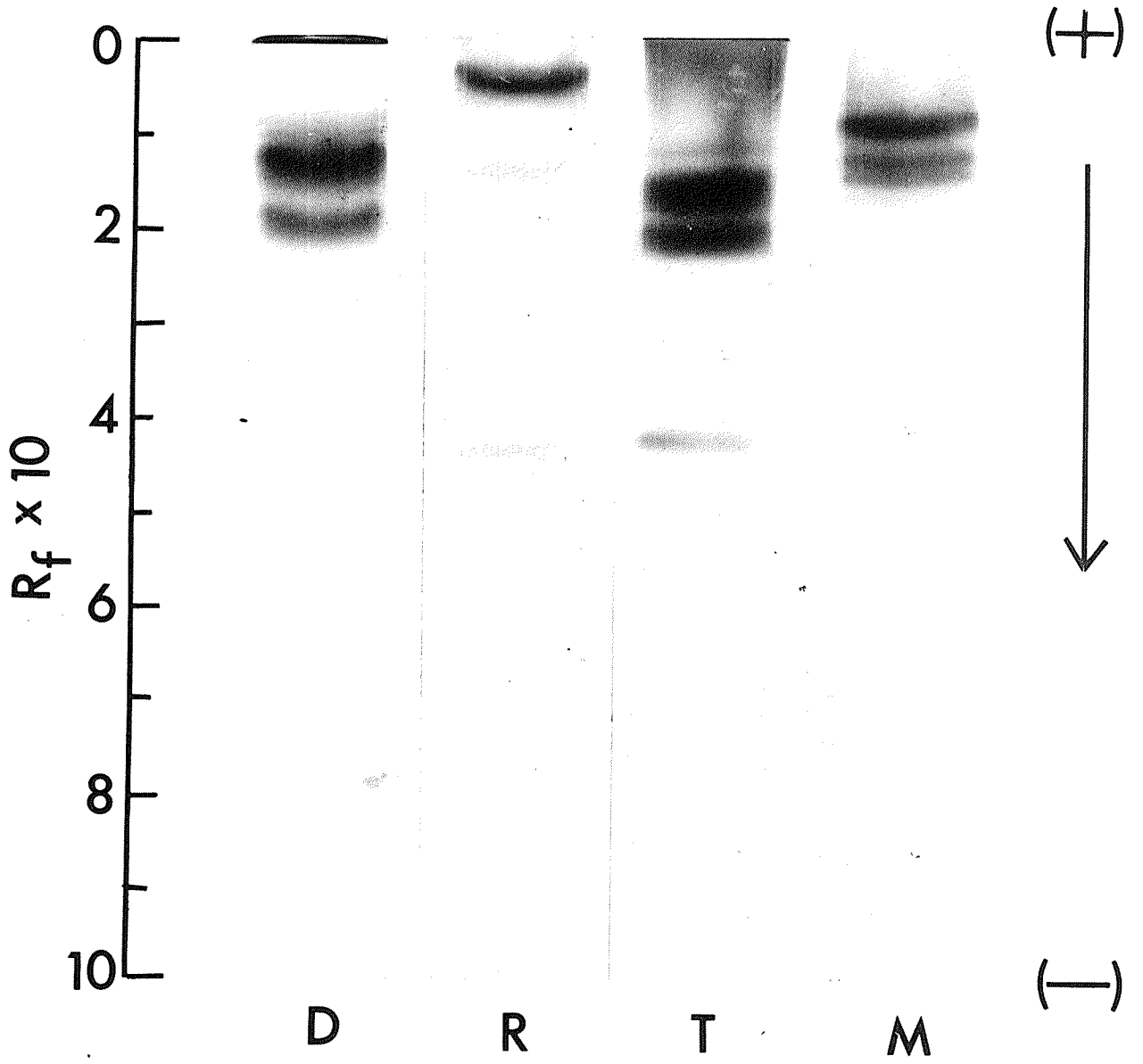
coefficient of 1.5 appear to be the proteins obtained from the large molecular-weight glutenins by the cleavage of disulfide bonds.

Disc electrophoretic patterns

Electrophoretic patterns of reduced-alkylated gliadins of durum wheat, rye, Triticale and the HRS wheat are shown in Fig. 3. It was shown in the third paper of Part II that the gliadins of durum wheat and Triticale have the similar electrophoretic patterns. The patterns for the reduced-alkylated gliadin of these two species also appeared to be similar. It is interesting to note that the patterns for the reduced-alkylated gliadins of the four species appeared with the same number of protein bands as those for the corresponding native gliadins; however, the mobilities of the reduced-alkylated gliadins were lower than those of the native gliadins. This could result from cleavage of intramolecular disulfide linkages in the native gliadin to produce a more open conformation of the polypeptide chain.

The electrophoretic patterns of reduced-alkylated glutenins of the four different cereal species are shown in Fig. 4. The major reduced-alkylated glutenins were represented by several protein bands in the slow moving region of the electrophoresis. In all cases, there was a heavy band on the top of the gel which was too large to migrate into the gel. Protein bands in the center region of gel were probably proteins of water- or salt-soluble groups which were not removed during the preparation of glutenin, as shown in paper III of Part II. Patterns of reduced-alkylated glutenins were quite different from those of corresponding reduced-alkylated gliadins for all four species.

Fig. 3. Disc electrophoretic patterns of reduced-alkylated gliadins from durum wheat (D), rye (R), Triticale (T), and HRS wheat (M).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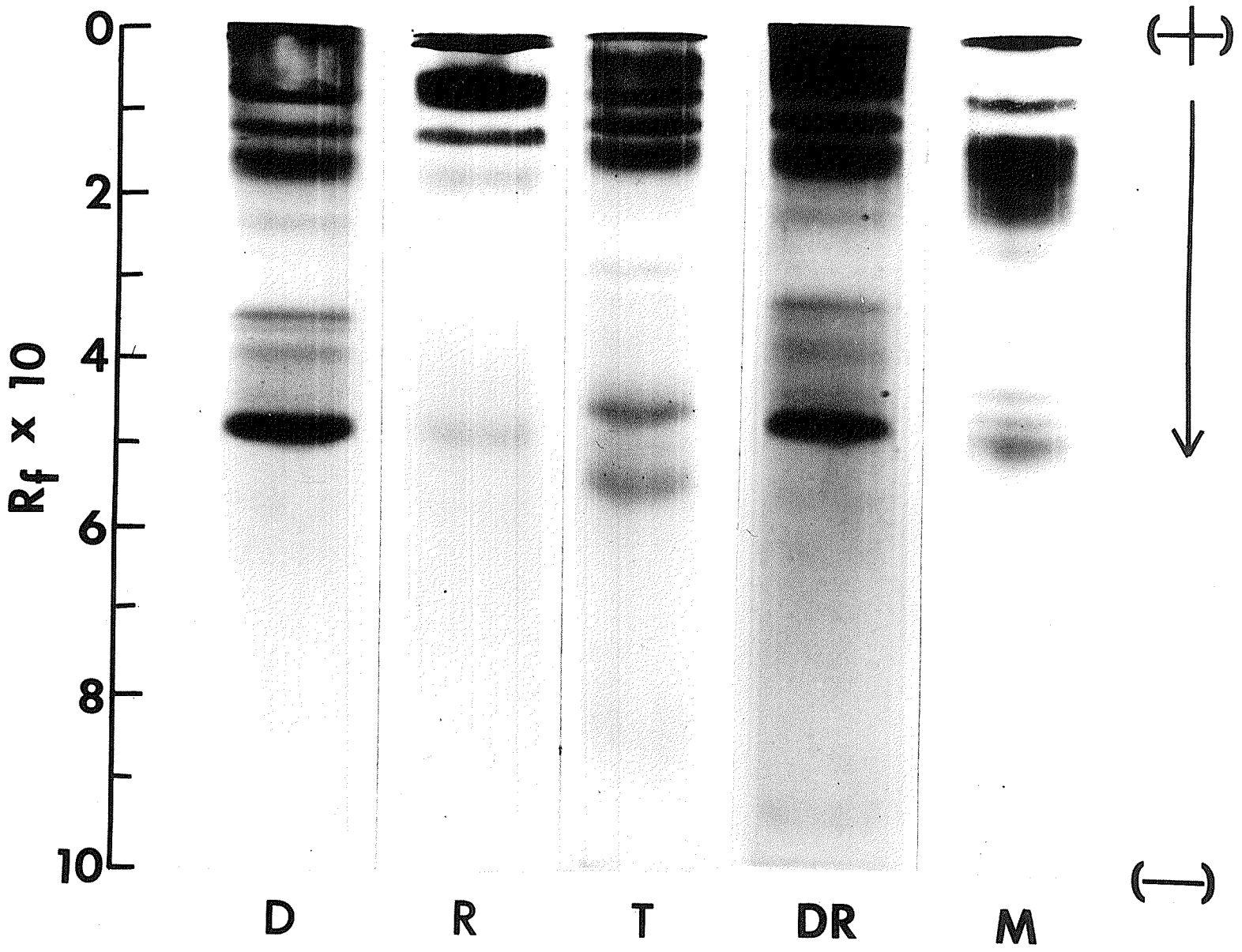
R_f x 10
(+)
(—)
D
R
T
M

Fig. 4. Disc electrophoretic patterns of reduced alkylated glutenins from durum wheat (D), rye (R), Triticale (T), HRS wheat (M), and mixture of glutenins from durum wheat and rye (DR).


0
2
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Rf x 10
D
R
T
DR
M
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(−)

Durum wheat and Triticale had similar patterns of the reduced-alkylated glutenins in the slow moving region, but Triticale appeared with one extra band in the center region of the gel. Rye showed a completely different pattern. Accordingly, it seems that the rye genome does not contribute extensively to the glutenins of Triticale. The pattern of reduced-alkylated glutenins from the HRS wheat was distinctly different from those of the other species.

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II. SUMMARY AND CONTRIBUTIONS TO KNOWLEDGE

1. The proteins of one line of Triticale (6A190), its parental species (T. turgidum L., ssp durum, cv. Stewart 63 and Secale cereale, cv. Prolific), and one variety of T. aestivum L. em Thell, (ssp. aestivum, cv. Manitou) were studied.

2. The content of most of the amino acids in Triticale was intermediate between those of its parents durum wheat and rye; the amounts of glycine, cystine, tyrosine and arginine were slightly higher in Triticale than in the parent species.

3. Protein solubility distribution and protein content in each solubility fraction varied significantly among the four cereal species studied. The solubility characteristics of proteins of Triticale are intermediate between those of its two parents. Rye contained the highest amount of water-soluble proteins; durum wheat contained the highest amount of alcohol-soluble proteins (gliadin); and the HRS wheat contained the highest amount of insoluble-residual proteins.

4. For fractionation of the proteins studied by gel filtration, it was found that 2M N,N-dimethylformamide solution gave the better resolution than 3M urea solution.

5. Relatively pure albumins and gliadins can be prepared by gel filtration.

6. Molecular weights, estimated by the gel filtration method, for albumins and globulins were 1×10^4 to 3×10^4; for gliadins and glutenins were 5×10^4 to 9×10^4 and above 1.5×10^5 respectively for all the four species studied.

7. The gel filtration patterns of various protein solubility groups for Triticale were similar to those for the corresponding groups from the HRS wheat.

8. Disc electrophoresis at pH 3.8 on 7.5% polyacrylamide gel separated albumins and globulins in the R_f region 0.35 to 0.90, and gliadins in the R_f region 0.10 to 9.30. Glutenins did not migrate into the gel under these conditions.

9. Albumin and glubulin patterns of Triticale were found to be exactly the composite of the patterns of its two parents; gliadin pattern of Triticale was similar to that of durum wheat.

10. No "new" proteins which were not detected in the parent species were found in Triticale.

11. Similar albumin and globulin patterns were found between durum and the HRS wheats: this suggests the dominant contribution of A and B genome in the biosynthesis of these proteins in the HRS wheat.

12. Gliadin pattern of the HRS wheat was quite different from that of all other species; these differences might be contributed by the D genome.

13. Disc electrophoretic patterns of the reduced and alkylated gliadins and glutenins of Triticale and its two parents indicated that gliadins and glutenins of Triticale are similar to those of durum wheat parent.

14. It is concluded that proteins of Triticale have the intermediate characteristics between those of the two parent species and they are directly inherited from its two parents.

III. SUGGESTION FOR FURTHER WORK

The author feels that the present study should be extended to give more detailed information on the proteins of *Triticale*. Some suggestions for future work are the following:

1. Soluble protein components compared among the various species in the present work were of molecular weight smaller than 150,000. Proteins with molecular size larger than this value were not examined in detail in this study.

2. Residual proteins (insoluble in dilute acetic acid) were not investigated in the present work. These proteins are known to be glutenin-like proteins. Since these form a significant fraction of the total protein, their comparative structure must be resolved before homology of the total proteins in the related species can be established.

3. More detailed physicochemical investigation of the proteins of *Triticale* and its parental species are required to assess the relationship between protein composition and breadmaking quality.

4. The present study should be repeated with other lines of hexaploid *Triticale*.

5. Similar study of octaploid *Triticale* and its parental species would be useful.