COMPOSITIONAL CHANGES IN THE DEVELOPING GRAIN OF HIGH- AND LOW-PROTEIN WHEATS. I. CHEMICAL COMPOSITION[1]

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ABSTRACT

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The kernel development of high- and low-protein wheat cultivars (cv. Timgalen and cv. Heron, respectively) was examined in an attempt to account for their genotypically determined differences in protein content. The high-protein cultivar yielded higher amounts of protein (N × 5.7) per kernel and lower kernel weights than did the low-protein cultivar. Up to day 30, nonprotein-nitrogen levels were higher in developing grain of the high-protein cultivars. The quantity of starch per kernel was only marginally different in corresponding crops of the two cultivars, and differences in kernel weight appeared to be largely due to increased levels of carbohydrates other than starch in the low-protein cultivar. All crops showed a maximum dry weight and maximum nitrogen per kernel approximately two weeks before the grain was mature. It is suggested that the loss of dry weight and total nitrogen during the later stages of maturation is due to movement of soluble material out of the grain to other parts of the head.

The protein content of wheat, one of its principal quality parameters, is influenced primarily by the environmental conditions during growth but also by the genotype and the interaction of genotype with growth environment. There are "high-protein" wheat cultivars which consistently yield grain of higher protein content than "low-protein" wheats grown under the same conditions. The basic physiological and biochemical reasons for these genotypically determined differences in protein content are not clearly understood. The aim of the work described in this and the succeeding paper (1) is to locate points or areas of difference in the composition and metabolic activity of the developing grain of a high- and a low-protein wheat. Little has been published on the overall compositional changes in developing wheat grain since the extensive work of Morton and coworkers (2–5).

MATERIALS AND METHODS

Plant Material

The two Australian white spring wheats, *Triticum aestivum* cv. Timgalen and cv. Heron, used in this study were grown in plots at North Ryde, N.S.W., Australia, in 1974. When grown under similar conditions, Timgalen consistently produces grain 2 to 3% higher in protein content than does Heron. Planting times (late May and late July) were arranged such that grain matured in either early November or mid-December. Plots were heavily fertilized with a mixed NPK fertilizer at planting and plants were never allowed to suffer from water stress. Heads were labeled at anthesis and harvests were made at either 2-day or twice-weekly intervals.

Growth Curves

At each harvest, 20 heads were cut for each variety. Five grains were removed from the central portion of each side of every head so that 200 grains were

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sampled. These were weighed, freeze-dried, and weighed again. Growth curves were constructed from these data. Freeze-dried samples were ground in a Wiley mill with a 0.05-mm screen and used for chemical analysis. All analyses were conducted in duplicate.

Total Nitrogen

The micro-Kjeldahl method (6) was used for the determination of total nitrogen.

Nonprotein Nitrogen

Freeze-dried samples of wheat (25 mg) were extracted with 10 ml 80% v/v aqueous ethanol using a Branson Model S110 Ultrasonifier to disperse solid material. The supernatant obtained from centrifuging ($8000 \times g$ for 10 min) this extract was dialyzed against aqueous ethanol in Visking 18/32 cellulose casing and the concentration of nonprotein nitrogen (NPN) which diffused was determined by the ninhydrin procedure (7).

Cytoplasmic and Storage Protein

Cytoplasmic proteins (albumins plus globulins) were removed from freeze-dried wheatmeal (50 mg) by extracting three times with 10 ml 10 m*M* sodium pyrophosphate HCl, pH 7.5, containing 0.5*M* sodium chloride, centrifuging ($8000 \times g$ for 10 min), and pooling supernatants. Following removal of cytoplasmic protein, storage (gluten) protein was extracted with three successive 10-ml portions of 50 m*M* sodium hydroxide. In all cases, samples were dispersed in the extraction solvent using a Branson Model S110 Ultrasonifier on setting 3 for 20 sec. Protein determinations were made on pooled extracts using the Lowry (8) method with appropriate cytoplasmic or storage protein preparations as standards.

Glucose and Sucrose

Sugars were removed from freeze-dried wheatmeal (100 mg) by extracting twice with 5-ml portions of boiling 70% v/v aqueous ethanol. The combined extracts were evaporated to dryness and the residue taken up in 10 ml 0.1*M* acetate buffer pH 4.6. After clarification of the extract by centrifugation, glucose was determined by the glucose oxidase procedure using "Glucostat" reagent (Worthington Biochemical Corp.). Sucrose was hydrolyzed by incubating 0.5 ml of the acetate buffer-sugar solution with 50 μl invertase concentrate (1000 I.U./ml) (British Drug Houses, Poole, England) in a total volume of 2 ml for 2 hr at 37° C. The concentration of sucrose was then determined from the increase in glucose level brought about by hydrolysis with invertase.

Starch

Wheatmeal (25 mg) was first treated with 2 ml of 0.2*M* potassium hydroxide at 100° C for 10 min to solubilize starch. The dispersion was neutralized with 2*M* acetic acid and 0.5 ml of 0.5*M* acetate buffer at pH 5.5 was added. Starch was hydrolyzed by incubating for 24 hr at 37° C with 0.2 mg Rhizopus amyloglucosidase (Sigma). The glucose formed was estimated using "Glucostat" reagent with the inclusion of appropriate blanks in the assay protocol.

RESULTS AND DISCUSSION

The changes in dry weight and fresh weight observed for the two crops of both cv. Timgalen and cv. Heron are shown in Fig. 1.

It is of interest to note that in all cases both the fresh and dry weight per kernel reached maxima 1 to 2 weeks before maturity (12% grain moisture). The extent of the drop in dry weight following the maximum differed between the two varieties and the two crops, but qualitatively it was always observable.

The accumulation of nitrogen in the two cultivars is shown in Fig. 2.

If comparison is made first within each cultivar, it can be seen that the maximum weight of nitrogen per kernel was identical for each crop, whereas maximum kernel dry weights were different. This means that the differences in weight per kernel are entirely responsible for differences in percentage nitrogen. When comparison is made between cultivars, it can be seen that both the weight of nitrogen per kernel and the kernel dry weight influenced the nitrogen content (per cent dry weight). It should be emphasized that all plants were grown in similar soil, as the only difference between the first and second crops was the time of year during which grain development occurred. The results in Fig. 2 show that the weight of nitrogen per grain reached a maximum at a time coincident with that for maximum dry weight. It is not possible to account for the decrease in dry

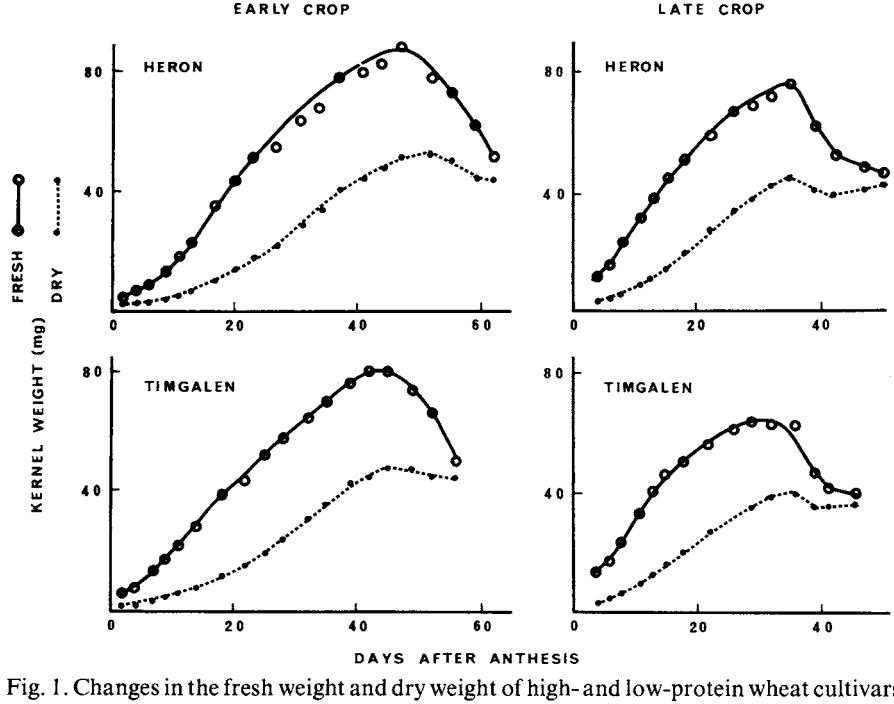

Fig. 1. Changes in the fresh weight and dry weight of high- and low-protein wheat cultivars (cv. Timgalen and cv. Heron, respectively) during kernel development. Early crop matured early November 1974, and late crop matured in mid-December.

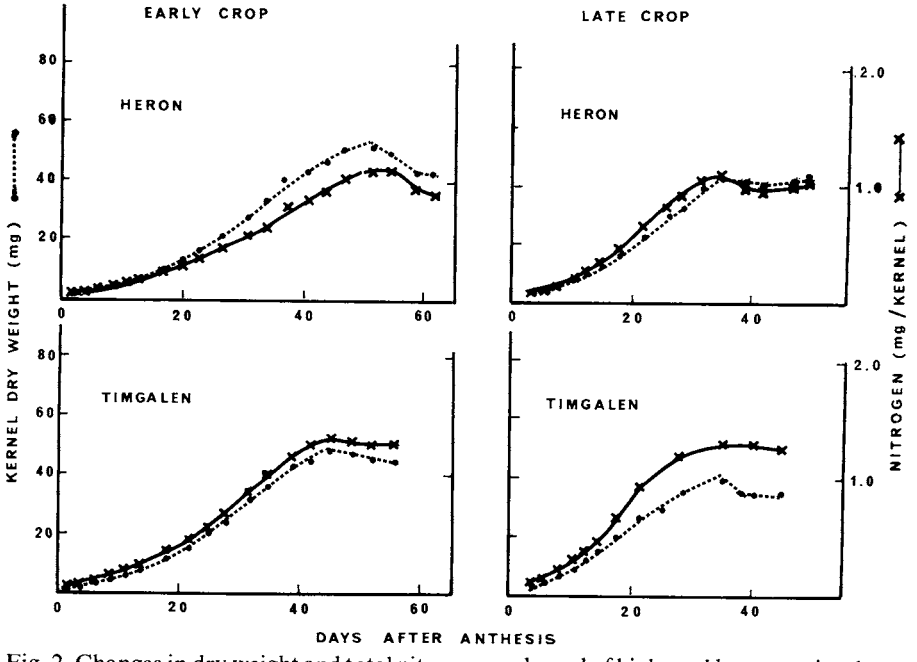

Fig. 2. Changes in dry weight and total nitrogen per kernel of high- and low-protein wheat cultivars during kernel development.

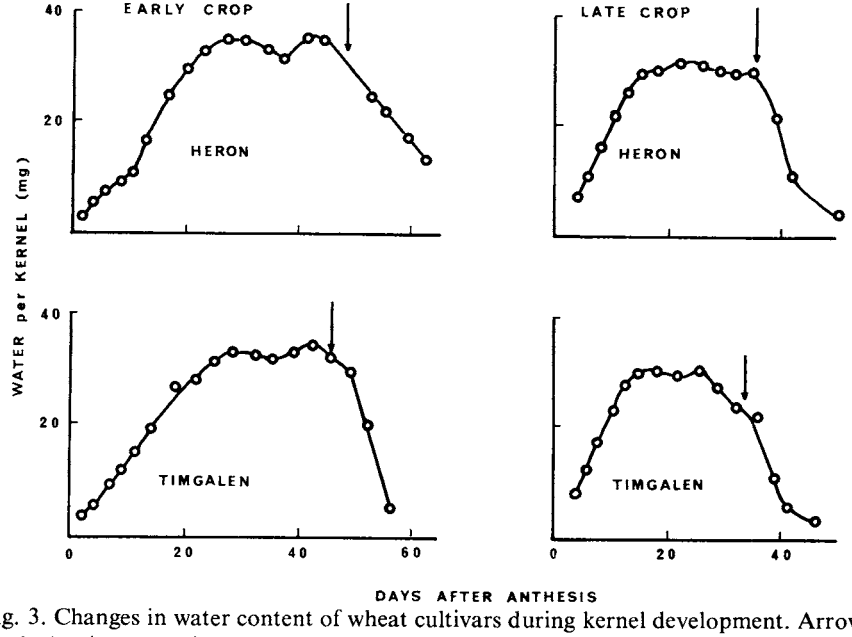

Fig. 3. Changes in water content of wheat cultivars during kernel development. Arrows mark the time at which maximum dry weight was reached.

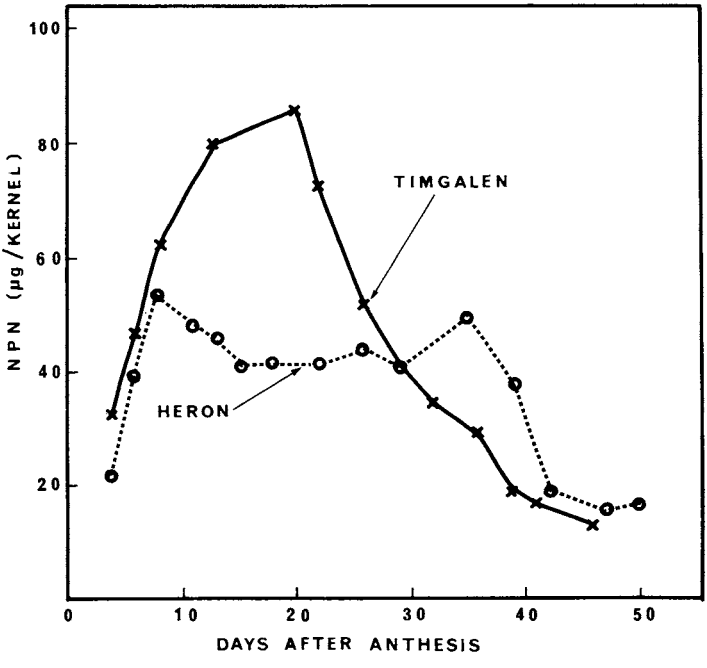

Fig. 4. Changes in nonprotein nitrogen (NPN) during kernel development. Data are for the late crop only.

TABLE I
Changes in Storage and Cytoplasmic Protein during Kernel Development of High- and Low-Protein Wheat Cultivars[a]

Days Post Anthesis	Timgalen (High-Protein)		Heron (Low-Protein)	
	Protein %[b]	Storage/Cytoplasmic Protein Ratio	Protein %[b]	Storage/Cytoplasmic Protein Ratio
8	14.1	0.31	12.1	0.26
11	13.7	0.31	10.4	0.20
15	14.1	0.37	10.3	0.30
18	15.0	0.48	10.1	0.47
22	15.7	0.62	9.7	0.49
26	14.8	0.60	10.1	0.50
29	15.2	0.63	9.9	0.55
32	15.8	0.65	10.4	0.57
36	15.3	0.65	11.9	0.57
39	16.9	0.66	11.8	0.59
41	17.0	0.65	12.2	0.63
46	16.2	0.68	12.0	0.64
50	...	...	12.3	0.63

[a]Data for late crop only.
[b]Expressed as total N per cent × 5.7 on a moisture-free basis.

weight and nitrogen during the last stages of grain maturation other than by assuming movement of material from the grain to other parts of the head. The loss of dry weight and nitrogen occurs at a time of very rapid desiccation (Fig. 3) and it may be that soluble materials are moved out of the grain through the rachilla.

The total nitrogen of wheat grain may be conveniently divided into protein and nonprotein portions. Figure 4 shows the changes in NPN of the two cultivars during development. Only data from the late crop are included; however, the early crop showed similar trends.

Nonprotein nitrogen constituted a continuously decreasing proportion of the total nitrogen as the grain developed. The NPN level in the high-protein variety Timgalen rose to a maximum of 88 μg/kernel at about 18 days and then fell continuously until the grain matured. The data for Heron show that NPN did not reach the levels seen in Timgalen but the decline as maturity approached was slower. If the level of NPN is taken as a measure of the size of the amino acid pool used for protein synthesis, then the higher NPN figures for Timgalen may indicate one reason for its higher protein content. The other prerequisite for this higher NPN level to be a determinant of higher protein content is, of course, the capacity of the protein synthetic system to make use of this substrate, and this is discussed in the succeeding paper (1).

The two principal classes of protein in wheat endosperm are those which are soluble in dilute salt solutions (cytoplasmic proteins) and those which are only soluble in dilute acid, alkali, or disaggregating solvents (storage or gluten protein). The relative proportions of these two protein classes in developing grain of the two wheat varieties are shown in Table I.

The proportion of storage protein relative to cytoplasmic protein in each

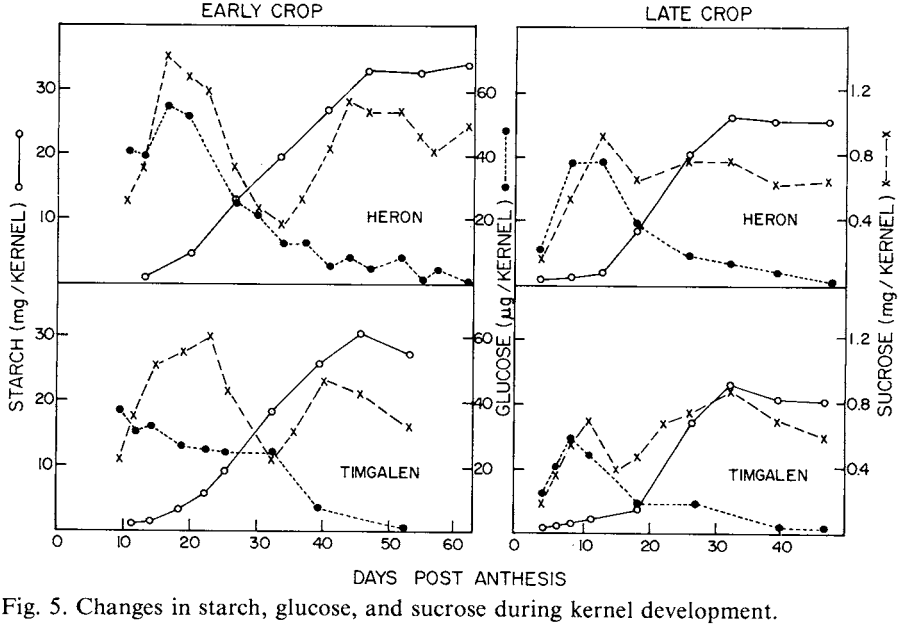

Fig. 5. Changes in starch, glucose, and sucrose during kernel development.

variety increases as grain development proceeds. The differences in the proportion of storage protein in Timgalen and Heron, at corresponding stages, are very minor. The proportions of storage protein in mature grain are lower than those commonly reported for flour (9). There are two reasons for this. Firstly, the values in Table I are for whole wheat, not flour, and secondly, the use of an ultrasonifier results in increased efficiency of extraction of salt-soluble protein.

The final protein content (%) of mature wheat grain is essentially determined by the relative rates and durations of both protein and carbohydrate synthesis. The principal carbohydrate in wheat endosperm is, of course, starch. Accordingly, starch was determined in developing grain of the two crops of both Heron and Timgalen to see if differences in the final protein contents, as a percentage of dry weight, could in part be accounted for by differences in starch content. The changes in starch level together with those for sucrose and glucose are given in Fig. 5.

In the early crop, net starch weight per kernel increased linearly from approximately 20 to 45 days and the maximum levels of starch per kernel were essentially identical for each variety. In the late crop, both the duration of starch accumulation and the maximum starch levels were less than in the early crop, but the rate of synthesis was faster. In the second crop, as in the first, the maximum levels of starch in each variety were very similar. Sucrose, which is the primary source of carbon reaching the endosperm (10), became high within 10 to 20 days but dropped at the onset of rapid starch synthesis. Sucrose levels increased again and then underwent a gradual decline toward maturity of the crop. Maximum levels of sucrose in the kernel before initiation of starch synthesis may bear some relation to final starch levels in the grain, as is most evident when comparing the early and late crop. Jenner (10) has shown a relation between the concentration of sucrose in the endosperm and the rate of starch synthesis in detached ears of wheat cultured on solutions of sucrose. Glucose showed an early peak at approximately 10–15 days, followed by a steady decrease.

Duration rather than rate of starch deposition appeared to be the critical factor affecting final starch content of the grain. This was particularly evident when comparing crops, whereas differences between the two varieties were small. Sofield *et al.* (11) have found that higher temperatures induce faster rates of growth in wheat but shorten the duration of growth. The shortened duration of starch deposition in the late crop may have been the result of increased temperature, as the crop did not mature until mid-December.

From the data presented in Fig. 2, it was concluded that the differences in nitrogen content (per cent of dry weight) between corresponding crops of Timgalen and Heron, were due partly to the greater amount of protein per kernel in Timgalen and partly to the greater kernel weight of Heron. Despite these differences in kernel weight between the two varieties, the differences observed in the maximum weight of starch per kernel were minor. It would seem, then, that the kernel weight differences must be largely attributed to constituents other than starch.

CONCLUSION

The differences observed in the grain nitrogen contents (per cent dry weight) of a high- and a low-protein wheat can be attributed partly to higher amounts of

nitrogen per kernel in the high-protein cultivar and partly to its lower kernel weight. Associated with these higher amounts of grain nitrogen were higher levels of NPN, particularly in the early stages of grain development. It is possible that these higher levels of NPN reaching the grain by translocation from other parts of the plant may lead to a higher rate of grain protein accumulation and, thus, a higher final grain protein content, provided that the duration of synthesis is maintained. The differences in the maximum amounts of starch per kernel in corresponding crops of the high- and low-protein cultivars were minor. However, the amounts of nonstarch, nonprotein material were greater in the low-protein cultivar and accounted in part for its lower percentage of grain nitrogen.

The crude protein content (per cent N × 5.7) of wheat is essentially a ratio between the amount of protein and the amount of carbohydrate per kernel. In breeding new wheat cultivars, it is undesirable to increase grain protein percentage by decreasing carbohydrate synthesis, since this leads to lower yields. Perhaps the best overall aim would be to increase the yield of grain protein per unit growing area first, and be concerned with the protein concentration in the grain later.

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