STUDIES WITH RADIOACTIVE TRACERS

VIII. The Incorporation of S^{35}-Labeled Sulfate by Maturing Thatcher Wheat[1]

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ABSTRACT

Sulfate-S^{35} was injected into the stems of maturing Thatcher wheat plants, and the distribution of S^{35} in the mature plants was examined. The kernels appeared to be the most active site of S^{35} assimilation, but only about one-half of the administered sulfate was utilized. Gluten was the most radioactive component of flour milled from the labeled kernels. The activity per mg. of flour or per mg. of gliadin or glutenin was greater when the tracer was present in the plant for longer periods of time. The results indicated that at any stage during the growth period studied, the gliadin fraction was increasing in specific activity about 20% faster than the glutenin fraction. It is suggested that sulfate is utilized slowly and that an excess is constantly available to the plants.

The sulfur contents of 21 varieties of wheat grown on a soil rich in sulfur have been reported to range between 0.15 and 0.22% (3). Analysis of Marquis wheat and its milling products also showed sulfur contents of this order of magnitude (8). With such small amounts of total sulfur, the application of the tracer technique may present a useful tool for a more extensive investigation of the sulfur-containing components of wheat and flour. The assimilation of injected phosphate-P^{32} by maturing Thatcher wheat was recently reported from this laboratory (6). The present paper deals with a similar study with S^{35}-labeled sulfate. In view of the continued interest of the cereal chemist on sulfur-containing proteins of flour, particular attention was paid to the incorporation of S^{35} into the gliadin and glutenin fractions. Moreover, the S^{35}-labeled flour obtained in this work was used for a study on the reaction between flour sulfhydryls and oxidative improvers (4).

Materials and Methods

Administration of S^{35}. Wheat plants of the Thatcher variety were grown in the greenhouse. At various stages of growth, an aliquot (0.05 or 0.1 ml.) of a solution initially containing 0.2 to 0.4 mc. of carrier-free sulfuric acid-S^{35} [3] in approximately 0.01*N* hydrochloric

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[3]Supplied by Atomic Energy of Canada Limited. The actual weight of S^{35} injected per plant would be of the order of 10^{-9} g.

acid was injected into the stem of each plant according to the method of McConnell and Ramachandran (7). At maturity, the kernels appeared to be of comparable quality to those obtained from greenhouse-grown plants that had not been injected with radioactivity. After harvesting, the kernels were milled with a mill designed by Geddes and Frisell (2).

Two crops of wheat were grown; the first crop received the S^{35} injections 20 days before harvest. For the second crop, the activity was injected to five groups of plants, respectively, at 28, 23, 18, 13, and 8 days before harvest. Three individual plants chosen at random from the first crop were each separated into leaf, stem, chaff, and grain and assayed to ascertain the distribution of radioactivity in the various parts.

Fractionation of Active Flour. Two procedures were employed. The first was the method of McConnell and Ramachandran (7), with the fractions designated as lipid, albumin, water-soluble nonprotein, gluten, NaOH-soluble nonprotein, and starch as described previously (6). The second method was that of Bilinski and McConnell (1) and was used for the preparation of the gliadin and glutenin fractions.

Radioactivity Determination. Appropriate amounts of material (40–100 mg.) were placed between two layers of benzoic acid (about 200 mg.) and made into a pellet with a press. The benzoic acid was used primarily to enhance the ease of combustion; but it also served to prevent mechanical loss of active material during pellet preparation. The pellet was burnt in a Parr bomb under 25 atmospheres of oxygen and in the presence of 10 ml. of distilled water. After combustion, the contents of the bomb were washed out and made up to a known volume. An aliquot was withdrawn, acidified with 2.0 ml. of 3*N* hydrochloric acid containing 50 mg. of sulfuric acid as carrier, heated on a steam bath, and the sulfate precipitated by the addition of 5.0 ml. of solution containing 150 mg. of barium chloride. The mixture was digested on the steam bath and then allowed to stand overnight. The precipitate was filtered through a Tracerlab stainless-steel filtration apparatus, giving an "infinitely thick" sample of barium sulfate of constant geometry. Three such samples prepared from each combustion were dried under an infrared lamp and counted with a thin-window Geiger counter. The mean activity of the three samples was used in all calculations. Corrections for decay of the S^{35} were made the usual way by counting a standard amount of S^{35} along with the samples (5,6).

Results and Discussion

Distributions of S^{35} in the Plant, the Milling Products, and Flour Fractions. Results from the analyses of the leaf, stem, chaff, and grain are shown in Table I. It was found that a considerable amount of the

TABLE I

SULFUR-35 IN VARIOUS PARTS OF WHEAT PLANT (% OF S^{35} RECOVERED)

PLANT	LEAF	STEM	CHAFF	GRAIN
	%	%	%	%
1	0.4	72.2	2.5	24.9
2	0.4	51.3	8.9	39.4
3	0.5	54.7	5.9	38.9

activity was incorporated into the grain; but the largest portion still remained in the stem. These findings indicated that during the last 20 days of growth, the kernels were the most active sites for the assimilation of the injected sulfate. The administered sulfate, however, was not completely utilized in 20 days. More than one-half of the activity injected into the stem remained there at maturity, although a part of this activity might have become unavailably bound in the scar tissue or in the collodion used to seal off the injection wound.

The S^{35} contents in the milling products from wheat harvested 20 days after the administration of sulfate-S^{35} are given in Table II.

TABLE II

DISTRIBUTION OF S^{35} IN MILLING PRODUCTS

	WEIGHT	SPECIFIC ACTIVITY	TOTAL ACTIVITY	
	g.	*reg.*[a]*/min./g.*	*reg.*[a]*/min.*	%
Bran	10.3	5,332	54,920	25.1
Shorts	6.0	5,009	30,050	13.7
Flour	37.6	3,572	134,300	61.3

[a] In this and in all subsequent tables, one register equals 64 counts.

It may be noted that the flour contained the highest total activity but showed the lowest specific activity.

Distributions of S^{35}, after fractionation of the active flour by the method of McConnell and Ramachandran (7), are given in Table III. The largest amount of activity was found in the gluten, which, together with the albumin, accounted for about 60% of the flour activity. This observation is in accord with the finding of Sullivan and Howe (8) that wheat and flour samples with higher protein contents also showed higher contents of sulfur.

TABLE III

DISTRIBUTION OF S^{35} IN FRACTIONS OBTAINED FROM 1.0 G. FLOUR

FRACTION	ACTIVITY [a]	RECOVERY	
		Based on Total S^{35} [b]	Based on Recovered S^{35} [c]
	reg./min.	%	%
Lipid	31	0.9	0.9
Albumin	338	9.5	10.0
H_2O-soluble nonprotein	926	26.0	27.2
Gluten	1825	51.1	53.7
NaOH-soluble nonprotein	131	3.7	3.8
Starch	151	4.2	4.4
Total	3402	95.4	100.0

[a] Average of duplicate experiments.
[b] Total activity of 1.0 g. of flour was 3572 reg./min.
[c] Sum of recovered activities in all fractions taken as 100%.

Effects of S^{35} Duration in the Plant on Its Incorporation into Flour and into the Gliadin and Glutenin Fractions. The S^{35} activities of the flours derived from the five groups of wheat to which the labeled sulfate was administered at different stages of maturity are shown in Table IV. The data indicated that for the period studied in these

TABLE IV

EFFECT OF S^{35} DURATION IN PLANT ON S^{35} INCORPORATION IN FLOUR

GROUP [a]	S^{35} IN PLANT	FLOUR	
		Weight	Specific Activity
	days	*g.*	*reg./min./mg.*
1	28	22.0	8.86
2	23	23.3	8.08
3	18	23.1	6.14
4	13	22.0	2.50
5	8	23.1	0.60

[a] Each group consisted of 75 plants.

experiments (8 to 28 days before harvest), a longer duration of the S^{35} in the plant resulted in a greater incorporation of the isotope into the flour. A similar trend was found in the incorporation of injected phosphate-P^{32} by maturing Thatcher wheat (6). The present results are also illustrated graphically in Fig. 1. The rise in flour specific activities between 13 and 23 days before harvest likely corresponded to a vigorous growing period of the kernels. When the activity was injected shortly before maturity, kernel growth was practically complete, thus resulting in a low degree of incorporation of S^{35} into the flour.

The activities in the gliadin and glutenin fractions (1) showed a

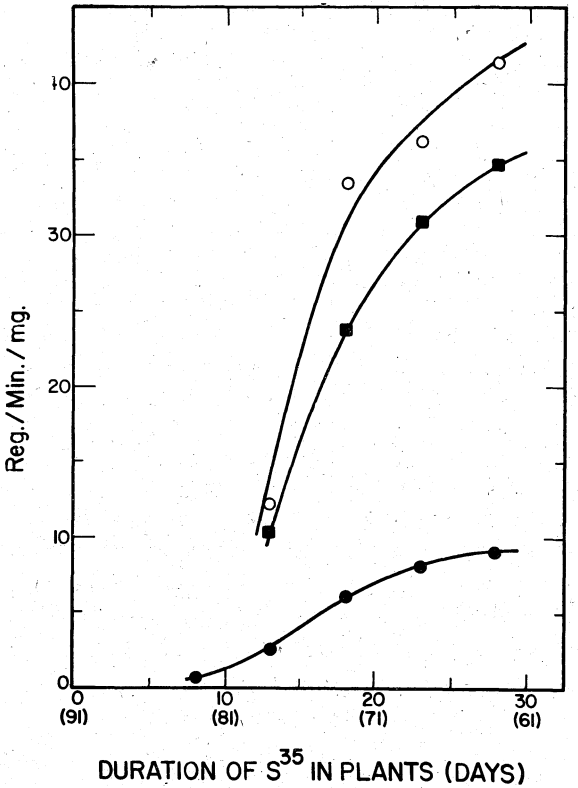

Fig. 1. Specific activities of gliadin, glutenin, and flour following injection of sulfate-S^{35} into wheat plants at different stages of maturity. Open circles, gliadin; closed squares, glutenin; closed circles, flour. Numbers in parenthesis are injection time, in days, after seeding.

trend similar to that of the flour, in that earlier administration or longer duration of activity in the plant resulted in greater incorporation (Table V). The activities per mg. for these fractions are also shown in Fig. 1, illustrating that flour with higher specific activity gave more highly active gliadin and glutenin.

It is of interest to note that when the activity per mg. of the gliadin or glutenin fraction is compared with that of flour, or with each other, nearly constant ratios are obtained regardless of the time of administration of S^{35} (Table V). These findings suggest that, at any stage during the growth period studied, the gliadin fraction was increasing in specific activity about 20% faster than the glutenin fraction. Moreover, if the utilization of the injected sulfate-S^{35} were slow, as indicated by the fact that much of the injected activity remained in the stem of the plant even after it reached maturity (Table I), it may be

TABLE V

EFFECT OF DURATION OF S^{35} IN PLANT ON THE INCORPORATION OF S^{35} INTO GLIADIN AND GLUTENIN FRACTIONS OF FLOUR

S^{35} IN PLANT	GLIADIN [a]		GLUTENIN [a]		ACTIVITY RATIOS		
	Weight [b]	Activity [b]	Weight [b]	Activity [b]	Gliadin/ Flour [c]	Glutenin/ Flour [d]	Gliadin/ Glutenin [e]
days	*mg.*	*reg./min.*	*mg.*	*reg./min.*			
28	82.2	3,377	45.8	1,574	4.6	3.9	1.2
23	74.2	2,672	47.8	1,473	4.5	3.8	1.2
18	61.3	2,050	52.4	1,239	5.4	3.9	1.4
13	71.9	870	45.6	465	4.8	4.1	1.2

[a] From 1.0 g. of flour.
[b] Average of duplicate experiments.
[c] (Reg./min./mg. gliadin)/(reg./min./mg. flour), reg./min./mg. flour from Table IV.
[d] (Reg./min./mg. glutenin)/(reg./min./mg. flour).
[e] (Reg./min./mg. gliadin)/(reg./min./mg. glutenin).

visualized that injected inorganic ions such as sulfate, or even phosphate (6), may remain constantly available to the plant in the period between administration and maturity. Incorporation into kernel constituents may, therefore, proceed while an excess supply of the injected ions is present for utilization. Thus earlier administration and longer duration of the sulfate or phosphate activity in the plant would result in a greater extent of incorporation as observed.

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