THE OXIDATION-REDUCTION ENZYMES OF WHEAT

I. A Qualitative Investigation of the Dehydrogenases[1]

G. R. HONOLD, G. L. FARKAS,[2] AND M. A. STAHMANN

ABSTRACT

A qualitative investigation of dehydrogenases in whole wheat and five milling fractions of two hard red winter (Triumph and Bison) and spring (Lee and Selkirk) wheats was made. Homogenates were prepared in aqueous sucrose and assayed by polyacrylamide gel electrophoresis, followed by incubation of the gels in substrate solutions and coupling of the enzymatic reaction with reduction of nitro-blue tetrazolium. Isoenzyme bands were detected for the dehydrogenases of glucose-6-phosphate, 6-phosphogluconate, malate, isocitrate, alpha-ketoglutarate, succinate, glutamate, alcohol, and lactate. Significant activities were seen in flour, particularly of the spring wheats. The number of observed isoenzyme bands varied from one for lactate, alpha-ketoglutarate, and isocitrate dehydrogenases to eleven for malate dehydrogenase. The number of isoenzyme bands and level of dehydrogenase activity were generally higher in the two spring wheats than in the two winter wheats.

Although considerable research has been conducted on respiratory enzymes in root and leaf tissue, few investigations have been made on the dehydrogenases in wheat seed. Past investigations have usually involved only the germ fraction. Barnett *et al.* (1) and Anderson *et al.* (2) reported detection of 6-phosphogluconate, and of isocitric and glucose-6-phosphate dehydrogenases in wheat germ. Loewus *et al* (3) studied incorporation of deuterium into malate upon reduction of oxalacetate with labeled DPNH (DPND) and malate dehydrogenase-containing extracts from wheat germ.

Using water-soaked wheat seeds that were germinated in the dark, Sisakyan and Vasil'eva (4) detected isocitric, glutamic, malic, and succinic dehydrogenases in the endosperm and the germ of hard and soft wheats. They reported that all investigated dehydrogenases had about the same activities. Conflicting results (5) suggested no glutamic dehydrogenase activity in water-soluble extracts of wheat germ; however, the variety and method of assay were not specified. Oparin and Gel'man (6) reported the presence of glutamic and succinic dehydrogenases in wheat sprouts.

In a survey of pyridine nucleotide dehydrogenases in higher plants,

[1]Manuscript received November 22, 1965. Contribution from Department of Biochemistry, University of Wisconsin, Madison, 53706. This study was supported in part by the Agricultural Research Service. U.S. Department of Agriculture Grant No. 12-14-100-7654(74) administered by the Western Utilization Research and Development Division, Albany, California. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2]Present address: Institute of Plant Physiology, Eötvös University, Budapest, Hungary.

Stafford and Vennesland (7) observed considerable alcohol dehydrogenase activity in wheat germ. Wheat germ also contains a dehydrogenase capable of reversible conversion of hydroxymalonate to ketomalonate (8). The distribution of this activity was found to be similar to that of malate dehydrogenase.

Although various enzymes in wheat germ have been studied, little attention has been paid to the enzymes of the wheat endosperm other than amylases and proteases. A little information exists on lipases and lipoxidases, the latter mostly in connection with the stability of xanthophyll or other pigments in the endosperm of durum wheat.

Oxidizing agents in relatively small amounts are used to bleach flour and to improve the dough properties. The latter changes result from oxidation by added oxidizing chemicals, such as chlorine dioxide, potassium bromate, or iodate (9). More attention should be directed toward the enzymes in wheat flour that catalyze oxidation or reduction reactions, for these may function in the maturing of flour to influence its baking properties. It was the purpose of this investigation to extract the soluble enzyme from wheat and various milling fractions, separate these enzymes by zone electrophoresis, and qualitatively assay dehydrogenases by the nitro-blue tetrazolium reduction method to determine the number of isoenzymes and their relative intensities.

Materials and Methods

Two hard red winter wheats, Triumph and Bison, and two hard red spring wheats, Lee and Selkirk, were used. All four wheats were milled with a MIAG Multomat experimental milling unit that had three breaks and five reductions, one sifter section for each break and reduction, plus two sections for middlings grading and dusting. The grinding part of the Multomat consisted of two groups of four pairs of rolls. The whole wheat and five milling fractions (flour, break shorts, reduction shorts, bran, and red dog) were stored at −20°C. until extractions were made. In preparation for extraction, the whole wheat (about 500 ml. volume) was ground in a Wiley No. 1 mill with a fine screen for 20 min. The sieved whole-wheat flour and the five milling fractions were used for subsequent preparation of crude enzyme extracts.

All water was redistilled in glass for solutions required in this work.

Twenty-five different extraction media and five mechanical methods of extraction were compared in a search for optimal extraction conditions. The extraction media included combinations or individual solutions selected from the following: water, sodium phosphate buffer

at pH 7.5 and ionic strength of 0.1, 12.5% aqueous sucrose, 1% ascorbic acid, 0.1% cysteine, 0.5% mercaptomethanol, *tris*-hydrochloride (*tris*[hydroxymethyl]aminomethane-hydrochloride) buffer at pH 7.5 and ionic strength of 0.1, 0.5*M* sodium chloride, 0.05*M* sodium chloride, and 0.03*M* acetic acid. Five mechanical methods of extraction were investigated: (a) the dry material was mixed with the extraction medium and hand-ground with mortar and pestle for 5 min. at 5°C.; (b) the suspension of material was stirred with a magnetic stirrer for 12 hr. at 5°C.; (c) the suspension along with a marble was placed in a 25 × 100-mm. test tube that was mechanically inverted on a rotating plate at 5°C. for 12 hr.; (d) the suspension was homogenized in a Potter-Elvehjem homogenizer for 5 min. at 5°C.; and (e) the suspension was homogenized with a VirTis homogenizer for 5 min. at 5°C.

The extraction method selected was the one which extracted the most soluble protein as determined by (a) the biuret method (10), and (b) amido black dye stain of soluble proteins of the crude extract separated on polyacrylamide disk gels, and oxidase activity as determined by the *nadi* reaction (11,12). The *nadi* reaction involves oxidation and condensation of alpha-naphthol and N,N-dimethyl-*p*-phenylenediamine to form indophenol. The reaction is not specific and was used in these investigations because of the large number of oxidases that react to it.

Optimal extraction was obtained with an aqueous 12.5% sucrose medium and the VirTis homogenizer. All extraction steps were carried out at 5°C. Fifteen grams of dry material was homogenized in 75 ml. of an aqueous 12.5% sucrose solution (13) for 5 min. with a VirTis homogenizer; centrifuged for 30 min. at 3,600 × *g*; filtered through Whatman No. 1 filter paper; and stored in 2-ml. vials at −20°C. until needed. Samples were rapidly thawed immediately before use. In the case of succinate dehydrogenase, the thawed samples were treated with 1% digitonin for 10 min. before electrophoresis to rupture the mitochondrial membranes.

The polyacrylamide disk electrophoresis technique of Ornstein (14) and Davis (15) was used to analyze the crude extracts because of its high resolving power, transparency, and chemical inertness of the gel. Also, this procedure allows the use of crude tissue extracts. In all experiments, 0.35 ml. of crude extract was applied to the gel. Electrophoresis was performed at 5°C. for 2 hr. at 3 ma. of constant current per gel tube. The gel was at pH 8.9 at the beginning of electrophoresis and the reservoir buffer was at pH 8.3.

The assay used for dehydrogenases was reduction of nitro-blue tetrazolium (NBT) to a fine diformazan precipitate (12,16,17,18). For

a specific dehydrogenase, electrophoresis was performed and the gel was then incubated in a solution containing the appropriate substrate, metal ion, nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) as required, electron-transfer agent, sodium cyanide, buffer, and tetrazolium. Twenty-five milliliters of substrate incubation solution for the individual enzymes contained the reagents indicated in Table I.

TABLE I

VARIOUS COMPONENTS PRESENT IN 25 ML. OF SUBSTRATE INCUBATION SOLUTION FOR EACH ENZYME[a]

ENZYME (DEHYDROGENASE)	SUBSTRATE	SUBSTRATE AT PH 7.5	$MgCl_2$	NAD OR NADP	NaCN	TRIS-HCl BUFFER AT PH 7.5	NBT	PM
		μmoles	*μmoles*	*μmoles*	*μmoles*	*μmoles*	*μmoles*	*μmoles*
Glucose-6-PO_4[b]	Glucose-6-PO_4	100	100	NADP, 5	30	80	6	2
Malate[c]	Sodium malate	200	100	NAD, 5	30	80	6	2
Isocitrate[d]	Trisodium isocitrate	120	100	NADP, 5	30	80	6	2
Glutamate[c]	Sodium glutamate	200	100	NAD, 5	30	80	6	2
6-Phospho-gluconate[d]	Sodium 6-phospho-gluconate	100	100	NADP, 5	30	80	6	2
Lactate[c]	Lactic acid	330	100	NAD, 5	30	80	6	2
Alcohol[e]	Ethyl alcohol	15,000	100	NAD, 5	30	80	6	2
Succinate[e]	Succinic acid	400	100	None	None	400	12	2
Alpha-keto-glutarate[f]	Sodium alpha-keto-glutarate	160	100	NAD, 5	30	80	6	2

[a] NBT = Nitro-blue tetrazolium; NAD = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; PM = phenazine methosulfate.
[b] See references 18, 19.
[c] See references 18–21.
[d] See references 18–20.
[e] See references 11, 18, 20, 21.
[f] Two μmoles of reduced coenzyme A and 1 μmole of thiamine pyrophosphate chloride were present in the reaction mixture for this enzyme in addition to those components listed.

The gels were incubated with the substrate mixture until clearly observable bands developed. The reactions were then stopped with 7% acetic acid. The incubation time was different for the various substrates and various milling fractions. The control in each case consisted of the experimental mixture with water instead of substrates to ascertain that the bands were formed by reaction with the given substrate.

Two methods of evaluating and recording gel electrophoresis data were used: (a) The distance that each band migrated relative to that of the marker dye or the R_F value of the band migration was recorded. The R_F was calculated as follows:

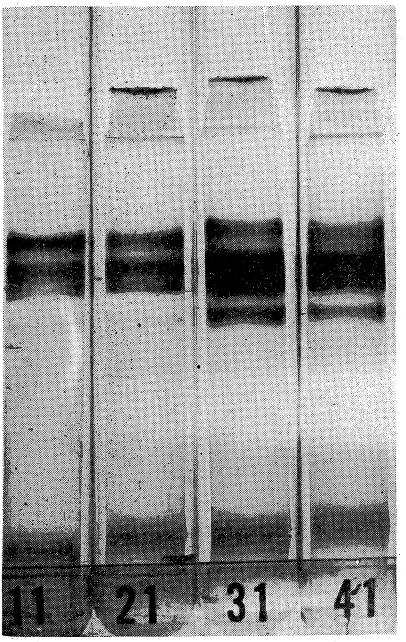
$$R_F = \frac{\text{distance that enzyme band migrates}}{\text{distance that marker dye migrates}}$$

(b) Photographs of the polyacrylamide gels were taken with high-contrast microfile film. In addition, the gels were observed visually and the relative intensities of bands were graded.

Results and Discussion

Dehydrogenases are important in carbohydrate metabolism. Sensitive and specific methods are available for detection of most dehydrogenases which involve coupling to the reduction of nitro-blue tetrazolium. These dehydrogenases are involved in the metabolism of glucose-6-phosphate through the citric acid cycle and include the associated reactions of alcohol, lactate, and glutamate dehydrogenases.

Dehydrogenases of the Pentose Shunt. 1. *Glucose-6-phosphate dehydrogenase.* Isoenzyme (22) bands of glucose-6-phosphate dehydrogenase



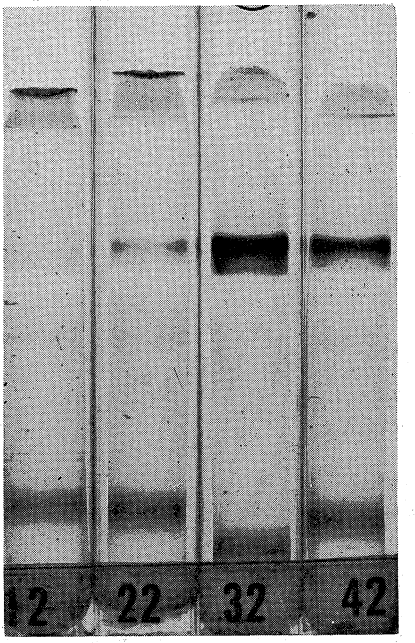


Fig. 1 (left). Isoenzyme bands of glucose-6-phosphate dehydrogenase in ground whole-wheat extracts of two hard red winter wheats (Triumph, 11, and Bison, 21) and two hard red spring wheats (Lee, 31, and Selkirk, 41).

Fig. 2 (right). Isoenzyme bands of glucose-6-phosphate dehydrogenase in extracts of flour of two hard red winter wheats (Triumph, 12, and Bison, 22) and two hard red spring wheats (Lee, 32, and Selkirk, 42).

activity detected in extracts of whole wheat on polyacrylamide gels are shown in Fig. 1 and the relative intensities of isoenzyme bands observed in extracts from flours are given in Fig. 2. A sharp contrast was noted in both the level of activity and the number of isoenzymes detected in whole wheat and in flour. A maximum of six bands was detected in the whole-wheat fraction of Lee wheat as compared to only two bands in the flour.

The photographic process has some limitations in reproducing the bands and often does not show all that can be observed visually on the original gel. This is evident when Fig. 1 is compared with the data presented in Table II. Six isoenzyme bands were observed in both Lee

TABLE II

RELATIVE INTENSITIES OF ISOENZYME BANDS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN MILLING FRACTIONS OF FOUR VARIETIES OF WHEAT

FRACTION	TRIUMPH		BISON		LEE		SELKIRK	
	R_F	Intensity[a]	R_F	Intensity	R_F	Intensity	R_F	Intensity
Whole wheat					0.45	xx	0.45	x
	0.37	x	0.37	x	0.37	xxxx	0.37	xxx
	0.31	xx	0.31	xx	0.32	xxxxx	0.32	xxxx
	0.25	xx	0.25	x	0.25[b]	xx	0.24[b]	xx
Flour		0			0.37	x		
			0.32	x	0.33	xxxx	0.32	xx
Break shorts		0	0.33	x	0.45	xx	0.44	x
					0.38	xxxx	0.36	xx
					0.32	xxxxx	0.32	xxx
					0.19	x		
Reduction shorts					0.41	xx		
					0.37	xxxx	0.35[c]	xxxxx
	0.32	x	0.31	x	0.31	xxxxx		
	0.24	x	0.24	x	0.24	x	0.26	x
Bran					0.45	x		
					0.41	x		
					0.37	xxx		
	0.32	x	0.32	x	0.31	xxxxx	0.32	xxxx
	0.24	x			0.24	x	0.24	xx
Red dog					0.45	x		
					0.40	xx	0.41	x
	0.37	x	0.37	x	0.36	xxx	0.37	xxx
	0.33	xxx	0.33	xx	0.30	xxxxx	0.32	xxxxx
	0.26	x	0.26	x			0.24	xx

[a] Increasing intensity of bands is represented from x to xxxxx.
[b] A group of three bands is clustered close together.
[c] A broad band extends from R_F 0.32 to 0.37.

and Selkirk whole wheats, whereas only three were detected in Triumph and Bison. No activity was detected in Triumph flour, and very low activity was detected in Bison flour, compared to more intense

bands observed for the spring wheats. In all fractions, the spring wheats contain more isoenzymes than the winter wheats.

2. *6-Phosphogluconate dehydrogenase.* The isoenzyme bands of 6-phosphogluconate dehydrogenase are indicated in Table III. Two

TABLE III

RELATIVE INTENSITIES OF ISOENZYME BANDS OF 6-PHOSPHOGLUCONATE DEHYDROGENASE IN MILLING FRACTIONS OF FOUR WHEAT VARIETIES

Fraction	Triumph		Bison		Lee		Selkirk	
	R_F	Intensity[a]	R_F	Intensity	R_F	Intensity	R_F	Intensity
Whole wheat	0.40	x	0.40	x	0.40	xxxx	0.41	xxx
	0.44	x	0.44	x	0.44	xx	0.45	xx
Flour		0		0	0.28	x	0.28	x
					0.33	x	0.34	x
					0.40	xxxx	0.40	xx
					0.44	x	0.44	x
Break shorts	0.28	x	0.28	x	0.30	x		
					0.36	x	0.36	xx
	0.40	xx	0.40	xx	0.41	xxxxx	0.43	xxxxx
	0.44	x	0.44	x	0.46	x		
Reduction shorts	0.28	x	0.30	x	0.29	x	0.29	x
	0.40	xx	0.42	xx	0.35	x	0.35	x
	0.45	x	0.45	x	0.40	xxxx	0.40	xxx
					0.46	x	0.44	x
Bran	0.43	xx	0.44	xx	0.30	x	0.30	x
					0.37	x	0.37	x
					0.42	xxxxx	0.42	xxxx
					0.46	x	0.46	x
Red dog	0.30	x	0.31	x	0.32	xx	0.32	x
	0.37	x	0.38	x	0.38	x	0.38	x
	0.42	xxxx	0.43	xxxx	0.43	xxxxx	0.44	xxxxx
	0.46	x	0.46	x	0.48	xx	0.49	xx

[a] Increasing intensity of bands is represented from x to xxxxx.

bands were detected in each of the whole-wheat extracts, with the apparent intensity much higher in the spring wheats than in the winter wheats. No activity was detected in the flours of the winter wheats, whereas four bands of activity were detected in each of the spring wheat flours. A large difference was also noted in the bran fractions. Only one band was detected in each of the winter wheat brans, whereas four bands occur in the spring wheat bran. In the latter it appears that most of the activity is in one band with an R_F of 0.42. Four bands were detected in many fractions.

Dehydrogenases of the Citric Acid Cycle. 1. *Malate dehydrogenase.* Numerous bands of malate dehydrogenase activity were detected on the gels as indicated by Fig. 3 and Fig. 4. In Fig. 4, heavy bands occur in

TABLE IV

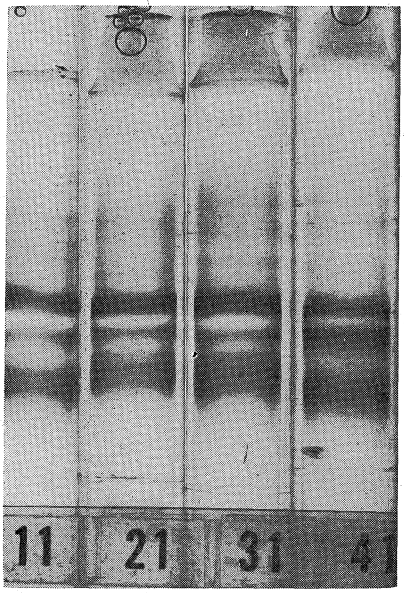
RELATIVE INTENSITIES OF ISOENZYME BANDS OF MALATE DEHYDROGENASE IN MILLING FRACTIONS OF FOUR VARIETIES OF WHEAT

Fraction	Triumph		Bison		Lee		Selkirk	
	R_F	Intensity[a]	R_F	Intensity	R_F	Intensity	R_F	Intensity
Whole wheat	0.59	xxxx	0.59	xxx	0.59	xxx	0.59	xxxx
	0.57	xxx	0.57	xxxx	0.56	xxxx	0.56	xxx
			0.54	xx	0.54	xxx		
	0.50	xxx	0.50	xxx	0.50	xx	0.50	xx
	0.42	xxxxx	0.42	xxxxx	0.42	xxxxx	0.42	xxxxx
	0.37	x	0.33	x	0.37	x	0.37	x
	0.27	x	0.25	x	0.30	x	0.26	x
	0.17	x			0.22	x	0.21	x
					0.15	x		
Flour			0.52	xxxx	0.52	xxx	0.52	xxx
	0.50	x	0.50	xxxx	0.50	xxx	0.50	xxx
	0.37	xxxx	0.38	xxxxx	0.38	xxxxx	0.38	xxxxx
	0.35	xxxx	0.36	xxxx	0.35	xxxx	0.35	xxxx
			0.28	xx	0.30	x	0.30	x
	0.26	xx	0.26	xx	0.26	x	0.26	x
			0.24	xx				
			0.21	x	0.22	x	0.22	x
	0.16	x	0.16	x	0.15	x	0.15	x
			0.10	x				

[a] Increasing intensity of bands is represented from x to xxxxx.

pairs, and in this photograph two bands appear as a single band. The R_F's and relative intensities of the bands of malate dehydrogenase in the various fractions are presented in Table IV. Seven isoenzyme bands were observed on gels containing winter wheat extracts and extract of the Selkirk variety, whereas ten bands were detected in the Lee variety. A striking feature of these data is the large number of bands detected in Bison flour as compared to Triumph flour. The isoenzyme patterns of Lee and Selkirk flours were identical. Because of the large number of malate dehydrogenase bands, only the data for the whole wheat and flour fractions are included in Table IV. From eight to eleven bands were detected in most of the other fractions. All fractions except whole wheat and flour showed a considerable amount of smearing of activity on the gel. This was especially pronounced in the bran and red dog fractions. Malate dehydrogenase, which has almost universal distribution, apparently occurs in considerably more molecular forms in wheat than in animal tissue extracts, where from two to six isoenzyme bands have been reported.

2. *Isocitrate dehydrogenase.* Isocitrate dehydrogenase activity was detected as a single band of R_F 0.39. The level of activity is generally low as compared to malate dehydrogenase and glucose-6-phosphate dehydrogenase. Bands observed for the spring wheats were more intense than those for the winter wheats.



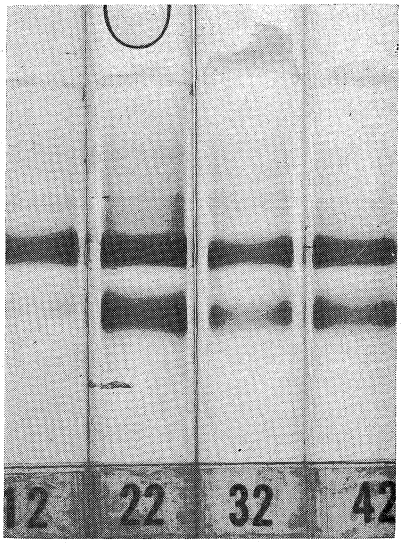


Fig. 3 (left). Isoenzyme bands of malate dehydrogenase in ground whole-wheat extracts of two hard red winter wheats (Triumph, 11, and Bison, 21) and two hard red spring wheats (Lee, 31, and Selkirk, 41).

Fig. 4 (right). The isoenzyme bands of malate dehydrogenase in extracts of flour of two hard red winter wheats (Triumph, 12, and Bison, 22) and two hard red spring wheats (Lee, 32, and Selkirk, 42).

3. *Succinate dehydrogenase.* Succinate dehydrogenase activity was observed on gels of all milling fractions except the flours of Triumph, Bison, and Selkirk, as indicated in Table V. The intensity and number of bands varied considerably among the various milling fractions. The bands on the gels were sharply separated from one another, even though they were close together in some instances. A substantially longer period of incubation was required for the gels containing extracts of whole wheat and flour than for the other fractions, which contained higher levels of activity as well as a greater number of bands.

4. *Alpha-Ketoglutarate dehydrogenase.* Alpha-ketoglutarate dehydrogenase activity was detected in most fractions. No more than one band was detected in any case. The R_F varied from 0.42 to 0.44. A very slight amount of activity was observed in whole wheat and red dog of Triumph and Bison and none in the other fractions of these two varieties. Wide variations in the detectable levels were observed in samples from the various fractions of Lee and Selkirk varieties. The break shorts of Selkirk is the only mill fraction of this variety in which no activity was detected. To the knowledge of the authors, alpha-

TABLE V

RELATIVE INTENSITIES OF ISOENZYME BANDS OF SUCCINATE DEHYDROGENASE IN MILLING FRACTIONS OF FOUR WHEAT VARIETIES

FRACTION	TRIUMPH		BISON		LEE		SELKIRK	
	R_F	Intensity [a]	R_F	Intensity	R_F	Intensity	R_F	Intensity
Whole wheat	0.45	x			0.46	xxxx	0.47	x
	0.39	x	0.39	x	0.40	x	0.42	x
Flour		0		0	0.40	x		0
Break shorts	0.55	x			0.56	x	0.55	x
	0.51	x			0.54	x	0.53	x
	0.47	x	0.48	x	0.50	xxxx	0.51	xx
							0.49	xx
Reduction shorts	0.56	xx	0.57	xxx	0.56	xxx	0.56	xxx
	0.53	xx	0.54	xxx	0.52	xxx	0.54	xxxxx
	0.50	x	0.51	x	0.49	xxxxx	0.52	xxxx
					0.45	x	0.49	xxx
					0.43	x		
Bran	0.54	xx	0.54	x	0.54	x	0.52	x
	0.51	x			0.51	xx	0.50	xx
					0.47	xxxx	0.48	xx
					0.43	x	0.46	xx
					0.41	x		
Red dog	0.53	x	0.47	x	0.54	x	0.52	xx
	0.50	x			0.50	x	0.50	xx
	0.47	x			0.47	xxxxx	0.48	xx
					0.44	x	0.46	xx

[a] Increasing intensity of bands is represented from x to xxxxx.

ketoglutarate dehydrogenase activity had not been previously demonstrated by using tetrazolium salts.

The R_F values of the bands of alpha-ketoglutarate dehydrogenase on acrylamide gels and the distribution of the intensities of bands among the various fractions bear a close resemblance to that of alcohol dehydrogenase. We suspect that alcohol dehydrogenase may have catalyzed the alpha-ketoglutarate dehydrogenase reaction. Alcohol dehydrogenase is known to have multiple substrate specificities, and several examples can be cited. The enzyme in yeast and liver extracts is known to react not only with ethanol but also with allyl, n-propyl, n-butyl, n-amyl, isopropyl, and other higher aliphatic alcohols. In addition, it can act as an isomerase converting lactaldehyde to acetol and glyceraldehyde-3-phosphate to dihydroxyacetonephosphate.

Other Related Dehydrogenases. 1. *Alcohol dehydrogenase.* Alcohol dehydrogenase activity was detected in a surprisingly high level in the Lee variety. Except for the Lee break shorts and red dog fractions, only one band of alcohol dehydrogenase activity was detected which had an R_F from 0.42 to 0.44. The winter wheats contain very little activity

as compared to the spring wheats. Activity was detected in extracts of whole wheat and red dog from the winter wheats, but no activity was detected in other winter wheat fractions. No activity was detected in break shorts of Selkirk. A notable observation is the extremely low activity in the bran fraction as compared to the other fractions.

2. *Lactate dehydrogenase.* Lactate dehydrogenase activity was detected in all fractions except flour. The R_F ranged from 0.67 to 0.69. The level of lactate dehydrogenase activity is very low as compared to many other dehydrogenases in wheat and could not be detected in flour even after long incubation with substrate. It appears that the fast-moving single band is the only molecular form of the enzyme that occurs in wheat seed. This is interesting when one compares these data with the normal occurrence of five isoenzyme bands of lactic dehydrogenase that have been demonstrated with starch gel electrophoresis of extracts from animal tissue (24).

3. *Glutamate dehydrogenase.* Glutamate dehydrogenase activity was detected in all milling fractions. In general, the level of activity of this enzyme was relatively low in all varieties. A few interesting points were revealed by the assay of this enzyme in the milling fractions. There is a similar level of activity in the four varieties. This is contrary to what was observed for many other enzymes. It is unusual that the level of glutamate dehydrogenase was high in the flour fraction of all four varieties relative to the other fractions. There also is a relatively high activity of this enzyme in the winter wheats as compared to the spring wheats. This is the opposite of what was found for many other enzymes. In most cases, only one band of activity was observed which had an R_F from 0.17 to 0.20. The flour and reduction shorts of Triumph and the break shorts and reduction shorts of Bison contained two bands of activity; the second had an R_F of 0.24 to 0.27. If the samples were allowed to incubate with substrate for a long period (24 hr.), a very light band with an R_F of about 0.38 developed in most all samples. The level of activity in this light band was almost negligible as compared to the heavy band.

One of the significant points demonstrated in this study is that flour is not an enzymatically inert mixture of starch and protein. Many enzymes are present and active in all milling fractions of wheat. The dehydrogenases in flour may alter the fermentation process, as these enzymes react with the same substrates as many yeast enzymes. These enzymes use either di- or triphosphopyridine nucleotides in their reactions and, therefore, would favor an accumulation of reduced nucleotides in flour doughs. The accumulation of reduced nicotinamide adenine dinucleotide or reduced nicotinamide adenine dinucleo-

tide phosphate may alter the fermentation process and affect baking quality. Also, these cofactors are oxidation-reduction reagents which reduce nitro-blue tetrazolium for dehydrogenase detection. These same cofactors may possibly affect the enzymatic and nonenzymatic reactions that are involved in polymerization of proteins in the dough. Future work is planned to elucidate the role of these enzymes in flour dough improvement.

Acknowledgment

The authors thank J. W. Pence and H. P. Binger for their advice and help in initiating this study. Thanks are also expressed to J. A. Shellenberger for providing the wheats and the milling fractions.

Literature Cited

1. BARNETT, R. C., STAFFORD, HELEN A., CONN, E. E., and VENNESLAND, BIRGIT. Phosphogluconic dehydrogenase in higher plants. Plant Physiol. **28:** 115–122 (1953).
2. ANDERSON, D. G., STAFFORD, HELEN A., CONN, E. E., and VENNESLAND, BIRGIT. The distribution in higher plants of triphosphopyridine nucleotide-linked enzyme systems capable of reducing glutathione. Plant Physiol. **27:** 675–684 (1952).
3. LOEWUS, P. A., TCHEN, T. T., and VENNESLAND, BIRGIT. The enzymatic transfer of hydrogen. III. The reaction catalyzed by malic dehydrogenase. J. Biol. Chem. **212:** 787–800 (1955).
4. SISAKYAN, N. M., and VASIL'EVA, N. A. Oxidation-reduction processes in hard and soft wheat. Biokhimiya **19:** 730–737 (1954).
5. PROSKURYAKOV, N. I., and RODIONOVA, I. V. Enzymatic activity of the water-soluble proteins of wheat germ. Biokhim. Zerna, Sbornik **5:** 108–120 (1960). (Chem. Abstra. **54:** 228661.)
6. OPARIN, A. I., and GEL'MAN, N. S. Comparative characteristics of respiratory enzymes of sprouts of wheat and rice. Biokhim. Zerna, Akad. Nauk S.S.S.R., Sbornik **1:** 7–16. (Chem. Abstr. **46:** 11336i.)
7. STAFFORD, HELEN A., and VENNESLAND, BIRGIT. Alcohol dehydrogenase of wheat germ. Arch. Biochem. Biophys. **44:** 404–414 (1953).
8. STAFFORD, HELEN A. Dehydrogenase activity of hydroxymalonate and related acids in higher plants. Plant Physiol. **31:** 135–141 (1956).
9. BLOKSMA, A. H., and HLYNKA, I. Basic considerations of dough properties. *In* Wheat: Chemistry and technology, I. Hlynka, ed.; pp. 465–526. American Association of Cereal Chemists: St. Paul, Minn. (1964).
10. GORNALL, A. G., BARDAWILL, C. S., and DAVID, M. M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177:** 751–766 (1949).
11. GOMORI, G. Microscopic histochemistry, pp. 137–167. University of Chicago Press: Chicago, Ill. (1952).
12. BURSTONE, M. S. Enzyme histochemistry and its application in the study of neoplasms, pp. 429–521. Academic Press: New York (1962).
13. HEITEFUSS, R., BUCHANAN-DAVIDSON, DOROTHY J., and STAHMANN, M. A. The stabilization of extracts of cabbage leaf proteins by polyhydroxycompounds for electrophoretic and immunological studies. Arch. Biochem. Biophys. **85:** 200–208 (1959).
14. ORNSTEIN, L. Disc electrophoresis. I. Background and theory. Ann. N.Y. Acad. Sci. **121:** 321–349 (1964).
15. DAVIS, B. J. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121:** 404–427 (1964).
16. QUASTEL, J. H. Use of artificial electron acceptors in the study of dehydrogenases. *In* Methods in enzymology, S. P. Colowick and N. O. Kaplan, eds.; vol. IV, pp. 334–336. Academic Press: New York (1957).
17. SHAW, C. R. Electrophoretic variation in enzymes. Science **149:** 936–943 (1965).
18. BARKA, T., and ANDERSON, P. J. Histochemistry, pp. 296–318. Harper & Row: New York (1963).

19. GOMORI, G. Histochemical methods for enzymes. *In* Methods in enzymology, S. P. Colowick and N. O. Kaplan, eds.; vol. IV, pp. 381–382. Academic Press: New York (1957).
20. FINE, I. H., and COSTELLO, L. A. The use of starch electrophoresis in dehydrogenase studies. *In* Methods in enzymology, S. P. Colowick and N. O. Kaplan, eds.; vol. VI, pp. 968–969 Academic Press: New York (1963).
21. SELIGMAN, A. M. Histochemical methods for dehydrogenases. *In* Methods in enzymology, S. P. Colowick and N. O. Kaplan, eds.; vol. VI, pp. 889–894. Academic Press: New York (1963).
22. WEBB, S. C. Nomenclature of multiple enzyme forms. Nature **203:** 821 (1964).
23. VAN EYS, J. Aldehyde-ketone isomerization activity of liver alcohol dehydrogenase. J. Biol. Chem. **236:** 1531–1533 (1961).
24. WIELAND, T., and PFLEIDERER, G. Chemical differences between multiple forms of lactic acid dehydrogenases. Ann. N.Y. Acad. Sci. **94:** 691–700 (1961).