

Relationships of Free Lipids with Quality Factors in Hard Winter Wheat Flours¹

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ABSTRACT

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Hard winter wheat (*Triticum aestivum* L.) flours ($n = 72$) were analyzed for free lipids (FL) and their relationships with quality parameters. The two main glycolipid (GL) classes showed contrary simple linear correlations (r) with quality parameters. Specifically, kernel hardness parameters, flour yields, and water absorptions had significant negative correlations with monogalactosyldiglycerides (MGDG) but positive correlations with digalactosyldiglycerides (DGDG). MGDG showed negative correlations with gluten content but positive correlations with gluten index. The percentages of DGDG in FL had significant positive correlations among

cultivars ($n = 12$) with mixograph and bake mix times ($r = 0.71$, $P < 0.01$ and $r = 0.67$, $P < 0.05$, respectively), mixing tolerance ($r = 0.67$, $P < 0.05$), and bread crumb grain score ($r = 0.71$, $P < 0.01$). These results suggest that increasing DGDG in FL could contribute to enhancing wheat quality attributes including milling, dough mixing, and breadmaking quality characteristics. FL content and composition (ratio of MGDG or DGDG to GL) supplement flour protein content to develop prediction equations of mixograph mix time ($R^2 = 0.89$), bake mix time ($R^2 = 0.76$), and loaf volume ($R^2 = 0.72$).

Although native wheat (*Triticum aestivum* L.) lipids are a minor component in flour, these lipids have significant effects on baking, interacting with other components even at the high level of fat that is included in the baking formula (Chung and Pomeranz 1981). Free lipids (FL) are defined as those that extracted with nonpolar solvents such as petroleum ether, hexane or diethyl ether. FL in wheat flour have been recognized as important factors affecting quality in breadmaking (Chung et al 1978; Chung and Ohm 1997). Daftary et al (1968) reported that free polar lipids (PoL) improved loaf volume while free nonpolar lipids (NL) decreased loaf volume and impaired crumb grain. They also indicated that galactosylglycerides of free PoL increased loaf volume substantially among PoL.

The amount of glycolipids (GL) differs among wheat cultivars, which influences breadmaking. Chung et al (1980a) selected petroleum ether and Skellysolve B as the best solvents to extract lipids from flour for differentiation of wheat flours according to loaf volume potentials. Chung et al (1982) reported that loaf volume was significantly correlated with PoL, the ratio of NL to PoL, and GL extracted by petroleum ether in hard red winter wheats. They also found that the mixing time requirement was curvilinearly related with PoL, the ratio of NL to PoL, and GL in hard red winter wheats. The significant relationships of flour lipids and loaf volume have been reported by other researchers (Lin et al 1974; Tweeten et al 1981; Zawistowska et al 1984; Bekes et al 1986; Larsen et al 1989; McCormack et al 1991; Graybosch et al 1993).

Flour FL bind to proteins, especially gluten, as flour mixing proceeds, which develops dough (Zawistowska et al 1984; Chung 1986). Chung et al (1980b) suggested that free PoL would interact with proteins and with added shortening to enhance adequate protein aggregation, which produce a bread with good loaf volume and crumb grain. Interactions of free PoL and proteins increase and stabilize the dough gas cells by decreasing surface tension (Gan et al 1990, 1995;

Carr et al 1992; Larsen 1993). The free PoL also enhance gas retention and stabilize gas cells by forming PoL film at the gas-liquid interface of gas cells (Gan et al 1990; Larsen 1993). MacRitchie and Gras (1973) suggested that flour lipids could stabilize foam structure of dough.

PoL in flour have genetic relationships with wheat kernel hardness. Morrison et al (1984, 1989) found that free PoL content was under the control of genes located on the short arm of chromosome 5D. They also reported that the genes controlling PoL were allelic or linked to the genes controlling wheat kernel hardness.

The significant relationships between PoL and quality factors are due mainly to the variation in GL. The main GL classes include monogalactosyldiglycerides (MGDG) and digalactosyldiglycerides (DGDG). MGDG and DGDG have different effects on breadmaking. Among native flour GL classes, DGDG is the best lipid class for improving breadmaking attributes (Chung and Pomeranz 1981). Eliasson and Larsson (1993) suggested that the high ratio of MGDG to DGDG in flour could be detrimental to bread quality.

This study was performed to investigate whether variations in GL classes, specifically DGDG and MGDG, could be correlated with quality factors in hard winter wheats.

MATERIALS AND METHODS

Materials

Straight-grade flours were obtained by milling 72 wheat samples. Winter wheat cultivars were: 'Arlin', 'Cimmaron', 'Discovery', 'Karl', 'Karl 92', 'Newton', 'Scout 66', 'TAM 107', 'TAM 200', 'Tomahawk', 'Voyager' and '2163'. Each of the 12 cultivars were harvested in: Ellis, Franklin, Greeley, Labette, Stafford, and Thomas counties in Kansas in 1993, for a total of 72 samples used in this study. The information on quality characteristics and FL were described previously (Ohm et al 1998; Ohm and Chung 1999a,b, 2000).

Extraction and Fractionation of FL

FL were extracted from flour with petroleum ether using a Soxhlet system (Chung et al 1980a). Flour samples (12.5 g, db) were extracted for 16 hr at a solvent condensation rate of 2–3 drops/sec. After petroleum ether was reduced to ≈ 3 mL by a rotary evaporator, the remaining material was dried under a nitrogen gas stream. A solid phase extraction (SPE) system with a prepacked column (Mega Bond Elut Bonded Phase SI 1GRM, Varian, Harbor City, CA) with the help of a vacuum manifold (Visiprep Solid Phase Extraction Vacuum Manifold, Supelco Inc., Bellefonte, PA) was used to fractionate FL into NL, GL, and phospholipid (PL) fractions (Ohm and Chung 1999b). The MGDG and DGDG were analyzed as described by Ohm and Chung (1999b).

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Statistical Analysis

Statistical analyses including stepwise multiple regression were performed (v. 6.11, SAS Institute, Cary, NC). Correlations among cultivars were calculated using cultivar means over growing regions (Souza et al 1993; Ohm et al 1998).

RESULTS AND DISCUSSION

Table I shows mean, standard deviation, and range for the flour FL contents, kernel hardness, gluten properties, and mixograph and baking parameters of samples analyzed. Mean values of FL content and composition of each cultivar grown in six locations are given in Table II. The related lines Karl and Karl 92 had similar GL, MGDG, and DGDG contents (mg/10 g flour): 14.6, 2.6, and 6.5 (mg/10 g flour) for Karl and 14.8, 2.6, and 6.3 for Karl 92, respectively. However TAM 107 and TAM 200, both carrying the 1A1.1RS wheat-rye chromosomal translocation, significantly differed in GL, MGDG, and DGDG contents (mg/10 g flour, db): 11.3, 1.9, and 5.3 for TAM 107 and 14.5, 2.9, and 6.1 for TAM 200, respectively. Chung and Tsen (1974) studied GL contents of rye, triticale, and wheat and reported that lower GL contents in triticale might be due to the lower GL content in rye. Further research is needed to investigate whether the low GL contents of TAM 107 could be related to low GL contents of rye.

Simple linear correlation coefficients ($n = 72$) were calculated between FL fractions and quality parameters. Significant simple linear correlations occurred between flour FL and near-infrared reflectance hardness score (NIR-HS) and single kernel hardness index (SK-HI) (Table III). NIR-HS and SK-HI both showed a significant negative correlation with MGDG. The negative correlations of NIR-HS and SK-HI with MGDG partly support the hypothesis that the genes controlling free PoL in flour could be linked closely to genes that control wheat hardness on the group 5 chromosome (Morrison et al 1984). The relationships also were represented by significant correlations among cultivars between NIR-HS and GL composition (mg/100 mg of FL) (Fig. 1A) and MGDG content (mg/10 g of flour) (Fig. 1B). Karl and Karl 92, which showed significant differences in GL and MGDG, did not significantly differ in NIR-HS (60 and 56, respectively). TAM 200 showed more GL in FL (mg/100 mg of FL) than TAM 107; TAM 107 also showed a higher NIR-HS (70) compared with TAM 200 (52). The DGDG contents in flour, FL, and GL had a positive correlation with NIR-HS and SK-HI (Table III), suggesting that it could contribute to an increase in wheat kernel hardness. A positive correlation of DGDG (mg/100 mg GL) but a negative correlation of MGDG (mg/100 mg GL) with NIR-HS and SK-HI also suggest that GL composition could affect variation in wheat kernel hardness. The contrary correlation of MGDG and DGDG to wheat hardness might have resulted from higher polarity in DGDG than MGDG or complex genetic linkages as stated by Morrison

TABLE I
Wheat Hardness and Flour Characteristics

Characteristic	Mean	Standard Deviation	Range
Kernel hardness			
NIR hardness score	61.6	11.7	38.0-85.7
SKCS hardness index	62.0	11.6	43.9-92.0
Flour yield (%)	70.8	2.0	65.9-76.0
Flour protein content (mg/100 mg of flour) ^a	10.0	1.6	7.8-14.8
Gluten properties			
Wet gluten (mg/100 mg flour) ^a	27.4	5.8	15.8-40.8
Dry gluten (mg/100 mg flour) ^a	9.5	2.0	5.8-14.3
Gluten index (%)	93.7	4.9	74.0-99.5
Mixograph			
Water absorption (mL/100 mg of flour) ^a	60.6	2.7	56.4-67.2
Mix time (min)	5.2	1.2	2.9-9.2
Mix tolerance	3.8	0.6	2.0-5.0
Baking characteristics			
Water absorption (mL/100 mg of flour) ^a	63.3	3.2	56.1-71.7
Bake mix time (min)	7.2	2.5	3.1-19.1
Loaf volume (cm ³)	811	83	656-1070

^a 14% flour moisture basis.

TABLE II
Flour Free Lipid Contents and Compositions of Hard Winter Wheat Cultivars^a

Cultivars	Amounts in Flour (mg/10 g of flour, db)						% FL (mg/100 mg of FL)					% GL (mg/100 mg of GL)	
	FL	NL	GL	MGDG	DGDG	PL	NL	GL	MGDG	DGDG	PL	MGDG	DGDG
Karl	99.2	74.3	14.6	2.6	6.5	7.6	77.0	15.1	2.6	6.8	7.9	17.5	44.7
Karl 92	99.0	75.4	14.8	2.6	6.3	6.3	78.2	15.3	2.6	6.5	6.5	17.5	42.1
TAM 107	90.2	70.8	11.3	1.9	5.3	5.8	80.6	12.8	2.1	6.0	6.6	16.6	46.4
TAM 200	98.2	74.7	14.5	2.9	6.1	6.5	78.0	15.2	3.0	6.4	6.8	20.6	42.0
Cimmaron	103.4	77.8	15.1	2.6	6.4	7.3	77.7	15.0	2.6	6.4	7.3	17.6	42.8
Newton	106.9	79.9	16.3	2.9	6.5	6.8	77.6	15.8	2.8	6.3	6.6	18.1	40.9
Scout 66	93.6	72.2	11.8	2.0	5.4	6.1	80.2	13.0	2.3	6.0	6.8	17.7	45.9
2163	108.9	84.8	15.3	2.8	6.2	6.2	79.8	14.4	2.6	5.8	5.8	18.6	40.1
Arlin	100.3	78.0	14.5	2.5	5.9	6.0	79.2	14.7	2.5	6.0	6.1	17.3	40.2
Tomahawk	101.2	78.7	12.8	1.7	5.4	6.3	80.4	13.1	1.7	5.6	6.5	13.6	42.1
Voyager	96.2	73.7	12.7	2.1	5.7	6.5	79.3	13.7	2.2	6.1	7.0	16.0	44.2
Discovery	100.6	73.6	16.1	3.1	6.2	7.3	75.9	16.6	3.1	6.4	7.6	18.9	38.5
Mean	99.8	76.2	14.1	2.5	6.0	6.6	78.7	14.6	2.5	6.2	6.8	17.5	42.5
LSD	1.7	1.6	0.7	0.3	0.4	0.6	0.9	0.6	0.3	0.4	0.7	2.2	3.1

^a Mean value of each cultivar grown at six locations in Kansas. FL = total free lipids, NL = nonpolar lipids, GL = glycolipids, MGDG = monogalactosyl-diglycerides, DGDG = digalactosyldiglycerides, PL = phospholipids, and LSD = least significant difference at $P < 0.05$.

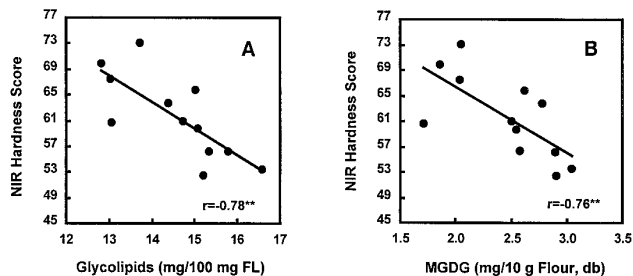


Fig. 1. Correlations among cultivars (r) between wheat kernel hardness and percentage of glycolipids in total free lipids (FL) (mg/100 mg of FL) (A) and flour monogalactosyldiglycerides (MGDG) content (mg/10 g of flour, db) (B). Significant at $P < 0.01$, $n = 12$.

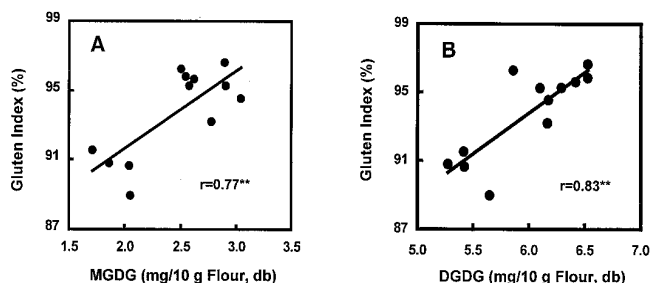


Fig. 2. Correlations among cultivars (r) between gluten index and amounts of monogalactosyldiglycerides (MGDG) (A) and digalactosyldiglycerides (DGDG) (B). Significant at $P < 0.01$, $n = 12$.

TABLE III
Simple Linear Correlation Coefficients (r) Between Free Glycolipids in Flour, or Extracted Lipids and Kernel Hardness and Flour Yield^{a,b}

Free Lipids	Kernel Hardness		Flour Yield (%)
	NIR Score	SKCS Index	
Monogalactosyldiglycerides			
Amount (mg/10 g of flour)	-0.57***	-0.36**	-0.25*
% FL (mg/100 mg of FL)	-0.60***	-0.43***	-0.25*
% GL (mg/100 mg of GL)	-0.65***	-0.54***	-0.49***
Digalactosyldiglycerides			
Amount (mg/10 g of flour)	0.25*	0.33**	0.41***
% FL (mg/100 mg of FL)	0.31**	0.30**	0.47***
% GL (mg/100 mg of GL)	0.49***	0.34**	0.30**

^a *, **, *** = $P < 0.05$, 0.01 , and 0.001 , respectively ($n = 72$).

^b SKCS = Single Kernel Characterization System; FL = total free lipids; GL = glycolipids.

(1988). It is also uncertain whether the influence of free GL on kernel hardness is related to the association between GL of starch surface lipids and kernel hardness in hard and soft wheats, as reported by Greenblatt et al (1995).

The flour DGDG and MGDG contents (mg/10 g of flour) or GL composition (%GL wt) showed contrary correlations with flour yield similar to kernel hardness (Table III). Straight-grade flour yields were negatively correlated with MGDG but positively correlated with DGDG. Break flour yields were also negatively correlated with DGDG content in GL (mg/100 mg of GL) but positively correlated with MGDG (mg/100 mg of GL): $r = -0.35$ ($P < 0.01$) and $r = 0.31$ ($P < 0.01$), respectively. The contrary association of MGDG and DGDG with either straight-grade or break flour yields could be influenced by their relationships with kernel hardness. Kernel hardness had a positive correlation with flour yield when milling condition was optimized for hard winter wheats (Ohm et al 1998). The positive effect of kernel hardness among hard winter wheats on flour yield could be due to easier separation of harder endosperm from bran during the milling process (Eliasson and Larsson 1993).

Gluten contents showed negative correlations with MGDG, suggesting that they could have an adverse effect on gluten formation

TABLE IV
Simple Linear Correlation Coefficients (r) Between Monogalactosyldiglycerides in Flour or Extracted Lipids and Gluten Parameters^a

	Wet Gluten (%) ^b	Dry Gluten (%) ^b	Gluten Index (%)
Amount (mg/10 g of flour)	-0.57***	-0.53***	0.40***
%FL (mg/100 mg of FL)	-0.55***	-0.49***	0.38***
%GL (mg/100 mg of GL)	-0.56***	-0.51***	0.26*

^a *, **, *** = $P < 0.05$ and 0.001 , respectively ($n = 72$). FL = total free lipids, GL = glycolipids.

^b 14% flour moisture basis.

TABLE V
Simple Linear Correlation Coefficients (r) Between Glycolipid Contents or Flour Composition or Extracted Lipids and Mixograph and Bake Water Absorption^{a,b}

Free Lipids	Water Absorption (% flour wt)	
	Mixograph	Bake
Monogalactosyldiglycerides		
Amount (mg/10 g of flour)	-0.52***	-0.52***
%FL (mg/100 mg of FL)	-0.50***	-0.50***
%GL (mg/100 mg of GL)	-0.65***	-0.58***
Digalactosyldiglycerides		
Amount (mg/10 g flour)	0.25*	ns
% FL (mg/100 mg of FL)	0.37**	0.24*
% GL (mg/100 mg of GL)	0.39***	0.31**

^a *, **, *** = $P < 0.05$, 0.01 , and 0.001 , respectively. NS = not significant, FL = total free lipids, GL = glycolipids, $n = 72$.

^b 14% flour moisture basis.

during mixing (Table IV). However, DGDG contents of flour (mg/10 g of flour), FL (mg/100 mg of FL), or GL (mg/100 mg of GL) showed no significant correlation with gluten contents. The GL contents appear to increase gluten index, as indicated by positive correlations with MGDG and DGDG (Fig. 2). Scout 66, TAM 107, and Tomahawk, showed low DGDG contents also had low gluten index values. Karl and Karl 92 showed similar gluten index values of 96%. But the rye translocation lines, TAM 107 and TAM 200, differed in gluten index, showing mean values of 90 and 95%, respectively. The positive effects of GL contents on gluten index could be due to the amphiphilic nature of GL that could contribute to an interaction of gluten proteins. Free PoL could enhance protein aggregation by interacting with proteins and with added shortening (Chung et al 1980b).

The flour GL were correlated significantly with mixograph and bake water absorptions (Table V). Mixograph and bake water absorption had negative correlations with MGDG. Specifically, %MGDG (mg/100 mg of GL) showed a significant negative correlation with mixograph or bake water absorptions. However, water absorptions were positively correlated with DGDG in FL (mg/100 mg of FL) or in GL (mg/100 mg of GL). The correlation coefficients of GL with water absorptions could be influenced by contrary correlations with kernel hardness. Kernel hardness showed a positive correlation with water absorption among hard winter wheat cultivars (Ohm et al 1998). Hard wheats require more water for mixing than soft wheats because of higher damaged starch contents in the flour (Pomeranz et al 1984). Phase behavior in water differs between MGDG and DGDG. Eliasson and Larsson (1993) suggested that a higher percentage of NL and of MGDG in FL could also have an adverse effect on breadmaking in relation to lipid phase behavior in water.

Loaf volume was negatively correlated with NL ($r = -0.40$ ***, $n = 72$). Chung et al (1982) reported that loaf volumes had a negative correlation with NL but a positive correlation with PoL among hard winter wheat cultivars. Flour protein content had $R^2 = 0.64$ with loaf volume in this sample set. The strong influence of the protein contents of the current sample set on breadmaking might have masked the beneficial effects of free PoL contents, especially DGDG

TABLE VI
Parameters and Coefficient of Determination (R^2) of Estimation Equation for Water Absorption and Loaf Volume

	Parameters ^a	R^2	RMSE ^b	$P < F^c$
Water absorption (% flour weight) ^d	PC,	0.79	1.4	***
	PC, MGDG/GL	0.87	1.1	***
	PC, MGDG/GL, DGDG/GL	0.89	1.0	*
Bake	PC	0.71	1.9	***
	PC, MGDG/GL	0.76	1.8	**
Loaf volume (cm ³)	PC,	0.64	56	***
	PC, FL,	0.68	54	**
	PC, FL, NL/MGDG	0.72	50	**

^a PC = flour protein content (mg/100 mg of flour, 14% moisture); MGDG = monogalactosyldiglycerides DGDG = digalactosyldiglycerides GL = glycolipids FL = total free lipid, and NL = nonpolar lipids (all mg/10 g flour, db, $n = 54$).

^b Root mean square of error.

^c *, **, *** = F value of parameter included in prediction equation is significant at $P < 0.05$, 0.01, and 0.001, respectively.

^d 14% flour moisture basis.

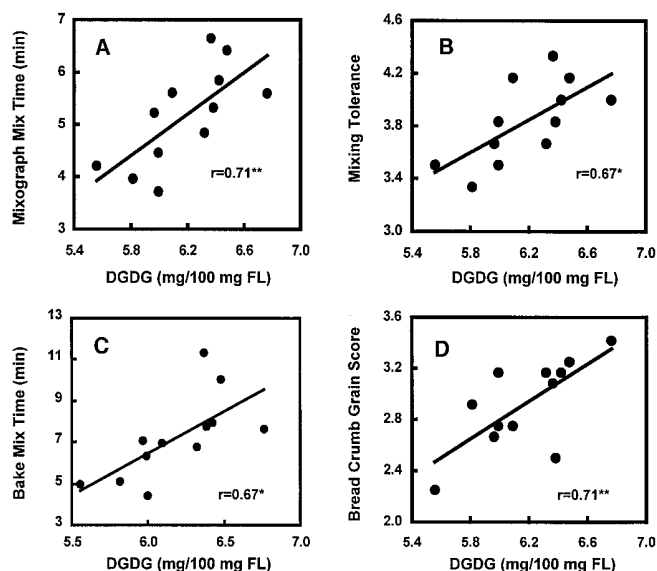


Fig. 3. Correlations among cultivars (r) between percentages of digalactosyldiglycerides (DGDG) in free lipids (FL) (mg/100 mg of FL) and quality parameters, including mixograph mix time (A), mixing tolerance (B), bake mix time (C), bread crumb grain score (D). *, ** = $P < 0.05$ and 0.01, respectively, $n = 12$.

contents, on loaf volume, as reported by Chung and Pomeranz (1981) and Chung et al (1982).

The lipid composition in flour showed significant correlations with quality parameters among cultivars ($n = 12$) (Fig. 3). Mixograph mix time and mixing tolerance and bake mix times had significant positive correlations with percentage of DGDG in FL (mg/100 mg of FL) among cultivars ($n = 12$). This result suggests that a higher percentage of DGDG in total FL could increase these parameters, which are very important breadmaking quality attributes. A high percentage of DGDG in FL might contribute to increased mix time and tolerance by interacting with protein. The free PoL have been reported to have beneficial effects on baking by enhancing the adequate aggregation of proteins (Chung et al 1980b).

The percentage of DGDG in FL also was correlated significantly with bread crumb grain scores among cultivars (Fig. 3D), suggesting that a higher percentage of DGDG in FL could enhance bread crumb. Flour PoL decrease the surface tension of gas cells and increase the number of gas cells in dough so that the coalescence of cells can be reduced during baking (Carr et al 1992; Larsen 1993). DGDG could decrease the surface tension of gas cells and thus contribute to the formation and stabilization of more cells during mixing and baking, respectively. The DGDG could increase mixing time and tolerance that could also function to formation of more gas cells in the

dough. This could account for the significant correlation of DGDG and crumb grain scores.

Estimation equations of mixograph and bake water absorption and loaf volume were derived using flour protein and FL contents and compositions by stepwise multiple regression (Table VI). The estimation of mixograph water absorption derived using flour protein content and the ratios of MGDG to GL and DGDG to GL had a coefficient of determination (R^2) = 0.89 and root mean square error (RMSE) = 1.0. The estimation of bake water absorption derived using flour protein content and the ratio of MGDG to GL had an R^2 = 0.76 and RMSE = 1.8 (Table V). The estimation of loaf volume had R^2 = 0.72 and RMSE = 50. These results suggest that FL fractions could supplement flour protein content for better predicting bake parameters.

Because 12 hard winter wheat cultivars were studied in this experiment, genetic variation in quality parameters could be narrow. A further experiment using wheat lines that encompass greater variations would be helpful to elucidate the relationships between FL and quality characteristics.

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