3. Amylases in $\underline{Sh_1}$ and $\underline{sh_1}$.

The hypothesis that the phenotypic hollowness observed in $\underline{sh_1}\underline{sh_1}\underline{sh_1}$ kernels is due to the action of a starch digesting amylase that is not present in the full-kernel $\underline{Sh_1}\underline{Sh_1}\underline{Sh_1}$ types was investigated. In developing endosperm of maize kernels that had 0, 1, 2, and 3 doses of the $\underline{Sh_1}$ allele, no differences were observed in amylolytic activity. Thus, the $\underline{Sh_1}$ protein band observed in disc-gel electrophoresis of endosperm proteins and absent in $\underline{sh_1}$ tissue is not associated with amylase activity.

Amylase is active in developing normal and $\underline{sh_1}$ endosperm from twelve to thirty-six days after pollination. The level of the amylolytic activity decreases slightly as the kernels mature as seen in the table. Amylase activities were measured by the decrease in iodine blue color of starch.

Amylase Activities of Maize Endosperm

Dose of $\underline{Sh_1}$ Gene	0	1	2	3
Genotype	$\underline{sh_1}\underline{sh_1}\underline{sh_1}$	$\underline{Sh_1}\underline{sh_1}\underline{sh_1}$	$\underline{Sh_1}\underline{Sh_1}\underline{sh_1}$	$\underline{Sh_1}\underline{Sh_1}\underline{Sh_1}$
Phenotype	shrunken	non-shrunken		
Specific Activity (relative units/mg protein)				
13-day	8.5	8.5	8.5	9.3
18-day	8.3	8.1	7.6	6.8
24-day	6.2	6.6	5.5	6.7
30-day	6.3	6.3	5.0	6.0
36-day	5.0	4.0	6.5	5.3

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1. Genetic and biochemical studies of chlorophyll deficient mutants.

For the past few years we have been accumulating mutants that are defective in the chlorophyll but which might have near normal carotenoid synthesis. Mutants of this type would be expected to have a luteus phenotype, but we have also included pale yellow, yellow-green as well as some near albino types in this study. If the mutant had not been previously located to chromosome we attempted to do this and for most of

Table 1
Phenotype, chromosome location and chlorophyll, carotene, and xanthophyll concentrations for 23 chlorophyll deficient mutants grown at 80°F. and under 2,000 foot candles of light

Mutant	Phenotype	Chromo-some	Chlorophyll mg/gm	Carotene mg/gm	Xanthophyll mg/gm
normal	green	-	1.674	.0980	.1140
$\underline{l}_3$	almost albino	-	0	.0015	.0055
$\underline{l}_4$	very slight yellow	10	trace	trace	.0066
$\underline{l}_6$	yellow-green	9?	-	-	-
$\underline{l}_7$	pale yellow-green	9	.3220	.0077	.0395
$\underline{l}_{10}$	good yellow	6	trace	.0029	.0140
$\underline{l}_{1106}$	yellow, leaf tips slightly green	4	.0992	.0019	.0187
$\underline{l}_{4106}$	light yellow-green	-	.0781	.0028	.0103
$\underline{l}_{4117}$	yellow-green	-	.1824	.0051	.0335
$\underline{l}_{4120}$	yellow, leaf tips green	6	.0935	.0021	.0175
$\underline{l}_{4920}$	pale yellow, leaf tips green	-	.0486	.0021	.0117
$\underline{l}_{4923}$	dark yellow, some green	-	.2667	.0205	.0350
py PI 177593	pale yellow, leaf tips green	4	.1914	.0059	.0165
yg PI 183367	yellow with some green	-	.2380	.0070	.0375
yel nec PI 217486	yellow, necrotic leaf tips	8	.1639	.0128	.0369
$\underline{l}_{Blandy\ \#1}$	yellow, leaf tips green	-	-	-	-
$\underline{l}_{Blandy\ \#2}$	yellow	-	-	-	-
$\underline{l}_{Blandy\ \#3}$	yellow with some green	6	.3115	.0176	.0432
$\underline{l}_{Blandy\ \#4}$	good yellow	-	.0055	.0240	.0195
$\underline{l}_{Brawn\ \#1}$	yellow-green	6	.5939	.0222	.0800
yellow dwarf	yellow-dwarf	3	.0140	.0015	.0070
$\underline{w}_1$	very pale yellow	6	.0180	.0008	.0091
$\underline{w}_{IT.\ \#1}$	very pale yellow	-	.0140	.0000	.0030
$\underline{w}_{8896}$	pale yellow	6	trace	.0006	.0090

them we determined chlorophyll, carotene, and xanthophyll concentrations. For the pigment analysis, the mutants were grown at 80° F. and 2,000 foot candles of light. Table 1 summarizes the results of these studies.

It is obvious from the results reported in Table 1 that most of the mutants make some chlorophyll although they all fall far short of that made by the normals. Five of them, $\underline{1}_3$, $\underline{1}_4$, $\underline{1}_{10}$, $\underline{1}_{Blandy\ \#4}$, and $\underline{w}_{8896}$, are very deficient in chlorophyll formation and come the closest to being true chlorophyll mutants. It is equally obvious that although some of the mutants looked quite yellow none of them approximate the level of carotenoid observed in the normal. The low levels of carotenoids could be due to pigment bleaching in the bright light.

Six of the mutants in Table 1 were grown in the dark and checked for their ability to make protochlorophyllide and to convert it to chlorophyllide and chlorophyll. These tests were run by using whole leaves in a reflectance attachment on a Bausch and Lomb 505 recording spectrophotometer. Protochlorophyllide was indicated by the presence of a peak at 630 mu in a dark grown leaf. A peak at about 684 mu after the leaves were exposed to one minute of light indicated that chlorophyllide was formed. After one hour in the dark the peak shifts to approximately 673 mu, which is thought by some to be the result of phytylation of the chlorophyllide, producing chlorophyll. Table 2 summarizes the results of these studies. Grown under dark conditions $\underline{1}_{Blandy\ \#4}$ evidently does not make chlorophyll or its precursors and $\underline{1}_3$ and $\underline{1}_{Blandy\ \#2}$ make only a trace. The other three mutants make significant amounts of these pigments, although quantitative values cannot be determined from these tests. Mutants which showed an ability to synthesize some chlorophyll in the light (Table 1) also seem to be able to synthesize it in the dark. Mutants with marked chlorophyll deficiency when grown in the light produce little or no chlorophyll precursors in the dark. In this regard they differ from normal plants, the white-albino mutants, and others of the luteus type mutants.

Table 2
Protochlorophyllide, chlorophyllide, and chlorophyll production as measured in vitro in six "chlorophyll" mutants

Mutant	Protochlorophyllide peak (484 mu)	Chlorophyllide peak (684 mu)*	Chlorophyll**
$\frac{1}{-}3$	trace	trace	trace
$\frac{1}{-}4920$	trace	+	+
$\frac{1}{-}$Blandy #2	+	+	+
$\frac{1}{-}$Blandy #3	+	+	+
$\frac{1}{-}$Blandy #4	0	0	0
$\frac{w}{-}8896$	trace	trace	trace

*684 mu is an average value. In different samples the peak might vary two or three mu from this value.

**As measured by a shift in the spectrum peak from the 684 mu value to a peak of shorter wave length.

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2. Metabolic block in porphyrin synthesis.

The seedling mutant $\frac{w}{-}8896$ forms at most only a trace of protochlorophyll when grown in the dark. It also has a reduced amount of carotenoids in the present genetic background (see accompanying report). This pigment relationship is the opposite of that found in the group of albino mutants which we have been investigating. In these latter mutants the formation of protochlorophyll, chlorophyllide, etc., is normal but there are metabolic blocks in carotenoid biosynthesis.

The enzyme catalase is a porphyrin enzyme so it was of interest to measure the amount of this enzyme and compare the results with the findings obtained with the porphyrin chlorophyll. When dark or dim light grown leaves of $\frac{w}{-}8896$ were ground and assayed they were found to contain about one-third the catalase of normal leaves. When the extract was centrifuged there was obtained a chloroplast pellet and a chloroplast free supernatant. No catalase was found in the chloroplast pellet of $\frac{w}{-}8896$. All of the catalase of the mutant was in the supernatant. In the normal about two-thirds of the catalase activity was in the chloroplast and one-third in the supernatant fraction.