COMPARATIVE PHYSIOLOGY OF GREEN AND ALBINO CORN SEEDLINGS[1,2]

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Previous comparative studies of green, albino, and variegated plants have shown higher levels of soluble nitrogenous compounds and lower levels of protein nitrogen in albino or in variegated than in green tissues (6, 14). The soluble nitrogen level was reduced and the protein level was increased when carbohydrates were supplied to albino tissues and plants. Groner (6), on the basis of such findings, postulated that when the supply of carbohydrate from the endosperm of an albino corn seedling became limiting, the seedling would hydrolyze its proteins, utilizing some of the products of hydrolysis in metabolism. The soluble nitrogenous substances, notably some amino acids, that could not be metabolized were thought to accumulate.

Respiratory studies with green and albino plants have yielded conflicting results. Lebedeff (8) reported that rates of respiration of albino corn seedlings were 17 % less than that of green seedlings. Schumacker (14) used various variegated dicotyledonous leaves and found lower respiratory rates in albino tissues. He attributed this difference to the lower reducing-sugar content of albino tissues. He found that the rate increased in both kinds of tissues when supplied with glucose. Greater increases occurred in the albino tissues. Groner (5) reported little difference in respiratory rates of green and albino corn seedlings.

It seemed desirable to investigate further the respiration and the changes of various nitrogen fractions with the possible occurrence of proteolysis in the albino corn seedlings. The findings reported here are results of two studies: (a) a time-course study of the distribution of certain nitrogenous substances in light- and dark-grown green and albino corn seedlings and (b) some aspects of the intermediary metabolism of these tissues. This study has suggested new aspects of metabolism of albino tissues, none of which were investigated completely. Consequently, this paper should be considered as a preliminary study.

MATERIALS AND METHODS

The plant material used here consisted of green individuals and " white " albino individuals of a strain of corn (*Zea mays* L.). One fourth of the seeds from a selfed, heterozygous parent had a pale-yellow marker indicating an albino germling. The pale-yellow marker and the albinism in the individuals germinating from such seeds were controlled by the homozygous recessive condition of the factor designated lw_1 (lemon-white$_1$) (13). Seeds of green and albino plants were placed in water at room temperature. This was designated as zero time of growth. After soaking 24 hours, groups of 10 seeds of each type were planted in garden loam in 10-inch pots. All seedlings were grown in a constant-temperature chamber at 25 to 26° C. Seedlings grown in light were subjected to a 12-hr photoperiod using one " white " and one " daylight " 100-watt fluorescent tube as the light source yielding 450 fc at pot level.

For nitrogen analyses, 40 plants each of the green and albino populations were harvested after 4, 6, 8, 10, 12, 15, and 20 days of growth. The seedlings were cut off as close to their seeds as possible and dried in vacuo at 65° C over P_2O_5. Each population was ground to 40 mesh in a Wiley mill. Soluble N was extracted with water from the dry powder according to Vickery et al (19). Total and soluble N were determined by a semi-micro Kjeldahl procedure using selenium oxychloride as the catalyst. (Nitrate nitrogen was not determined.) Protein N was calculated as the difference between total and soluble N. The soluble N fraction was analyzed for asparagine, glutamine, and ammonium N according to Vickery et al (18) and Pucher et al (12), and for α-amino N according to Frame et al (3). All determinations were in duplicate.

For manometric studies the expanded portions of the second and third leaves of 9-day-old green and albino seedlings were used. The leaves were split down the midvein and cut transversely with a razor blade into pieces 1 to 2 mm wide. Leaf segments from a number of plants were mixed thoroughly in distilled water, drained and placed into Warburg vessels containing 0.01 M phthalate-phosphate buffer plus 0.002 M $MgSO_4$ and 0.002 M $CaCl_2$. All solutions were adjusted to the desired pH with NaOH. Standard Warburg procedure was followed. Experiments were performed in duplicate in the dark at 26° C with the appropriate gas phase. The respiratory quotient (RQ) was obtained by a one-vessel method (10).

Etiolated plants were grown in the dark from " green " and " albino " seeds. Etiolated tissues for chemical and manometric determinations were prepared in the semi-darkened laboratory to minimize any changes in the tissues due to light. For N analyses 40 plants each of dark-grown green and albino populations were harvested after 6, 9, and 12 days of growth. For manometric studies 9-day-old, dark-grown green and albino plants were used.

RESULTS

GENERAL GROWTH HABIT: The albino seedlings used in these investigations were the same in form, but smaller than the green seedlings. Both types formed 4 leaves during the first 20 days of growth. After 12 days of growth the tips of the older leaves

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of the albino seedlings showed necrotic areas. Rates of growth of the albino plants decreased from 12 to 15 days and death occurred approximately 20 days after zero time (table I).

When etiolated, green and albino corn seedlings showed similar structural changes (fig 1). The plants became prostrate because of a relative lack of sclerenchymatous cells in the greatly elongated first internodes. Leaves of etiolated plants were longer and narrower and somewhat longitudinally curled. Etiolated "potentially green" seedlings were devoid of chlorophyll and their carotenoid pigments were visible. Etiolated albino seedlings had a slight blue-green hue which disappeared in light. Leaf material was prepared for microscopic examination from 9-day-old, light-grown plants by a freeze-dry method (7). No plastids or plastid-like bodies could be detected in the albino. Green tissues, however, had well-formed, undistorted chloroplasts.

NITROGEN METABOLISM: Total N per gram dry weight (per gm DW) (fig 2) was essentially the same in green and albino corn seedlings up to 12 days of growth. Soluble N per gm DW was the same in both tissues at 4 days of growth, increased slightly in green tissues by 6 days and then decreased. In the albino, however, soluble N content increased above that in the green seedlings at 8 days of growth, fell slightly until 12 days and then increased, paralleling the increased necrobiosis. Protein N per gm DW decreased rapidly from 4 to 6 days of growth. During the period of study the wet- and dry-weight values of the tissues increased. The rapid changes of N values from 4 to 6 days was due to a dilution through the rapid increase in non-nitrogenous dry-weight per seedling (table I).

Asparagine, glutamine, α-amino, and ammonium N per gm DW were essentially the same in both green and albino epicotyls after 4 days of growth (fig 3). Asparagine showed parallel increases in both tissues from 4 to 6 days of growth. The large

TABLE I

DRY WEIGHT IN MILLIGRAMS PER KERNEL OF LIGHT-GROWN GREEN AND ALBINO SEEDLINGS AND PER EPICOTYL OF LIGHT-GROWN AND DARK-GROWN GREEN AND ALBINO SEEDLINGS FROM ZERO TO TWENTY DAYS OF GROWTH *

	DAYS GROWTH									
	0	2	4	6	8	9	10	12	15	20
Kernels of light-grown plants										
Green	260	250	230	190	130	...	80	60	...	...
Albino	260	250	230	190	140	...	110	70	...	...
Light-grown epicotyls										
Green	...	...	8	25	46	...	66	80	82	85
Albino	...	...	8	20	33	...	46	56	61	58
Dark-grown epicotyls										
Green	...	...	...	25	...	54	...	113	...	...
Albino	...	...	...	22	...	43	...	61	...	...

* 40 seedlings per sample.

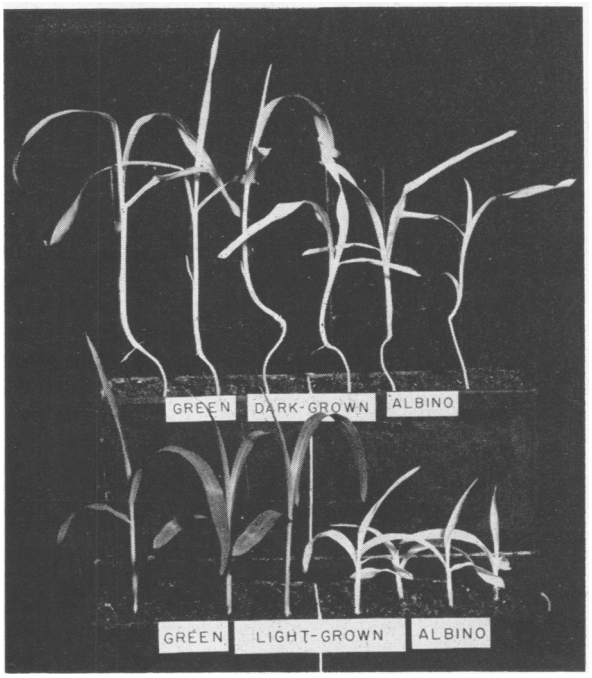

FIG. 1. Light- and dark-grown green and albino corn seedlings 9 days old.

amounts of asparagine found during early seedling development probably were synthesized from NH_4^+ absorbed from the soil in the presence of large amounts of carbohydrates from the endosperm (11). However, the accompanying decrease of glutamine N may suggest it as a source of N for the early asparagine synthesis. In the studies reported here, asparagine decreased after 6 days of growth and then increased slowly in the green seedlings, suggesting asparagine storage. After 6 days of growth, the level of asparagine in the albino remained considerably higher than that in green seedlings. The amino N per gm DW of the albino seedlings increased above that of the green and remained relatively constant throughout the period of study. Glutamine per gm DW was essentially the same in green and albino epicotyls from 4 to 20 days of growth. Asparagine and ammonia levels in albino seedlings rose sharply after 12 days of growth. The asparagine level became constant after 15 days of growth, but the ammonia level continued to rise. Thus, it appeared that during the 12 to 15 day period, ammonia released as a byproduct of protein hydrolysis and deamination, formed asparagine through amination of α-ketoglutaric acid presumably supplied via the tricarboxylic acid cycle from respirable substrate with subsequent amidation of the resulting aspartic acid. After 15 days, however, it could be that α-ketoglutaric acid was no longer available for amination. As a result, ammonia accumulated. Accordingly, asparagine formation, for a time, probably served as a detoxifying mechanism removing ammonia from the tissues (11).

There was little difference between etiolated green

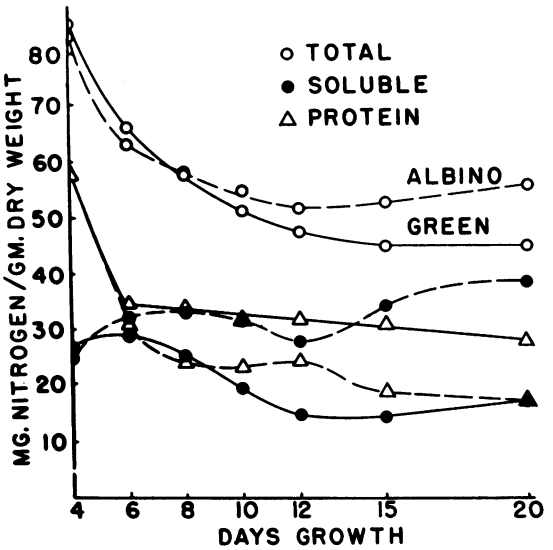

FIGURE 2

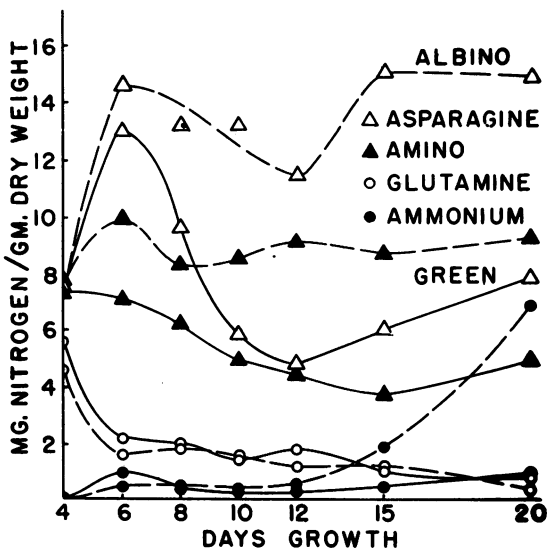

FIGURE 3

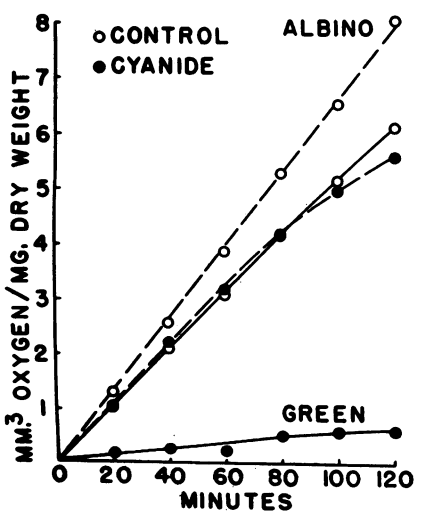

FIGURE 4

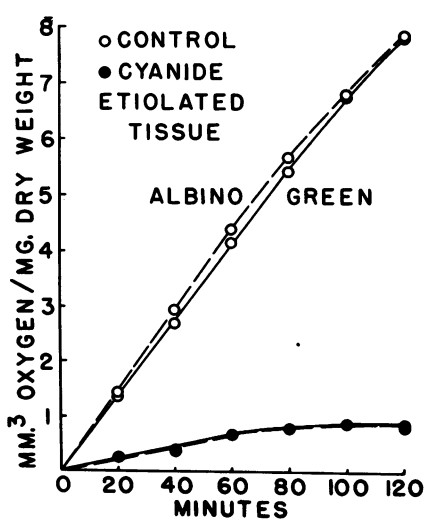

FIGURE 5

FIG. 2. Changes in total, soluble, and protein N of epicotyls of light-grown green (solid lines) and albino (broken lines) corn seedlings during the first 20 days of growth.

FIG. 3. Changes in asparagine, α-amino, glutamine, and ammonium N of epicotyls of light-grown green (solid lines) and albino (broken lines) corn seedlings during the first 20 days of growth.

FIG. 4. Effect of 0.005 M cyanide upon oxygen uptake of light-grown green (solid lines) and albino (broken lines) 9-day-old leaf tissues at pH 5.5 in the presence of 0.1 M glucose.

FIG. 5. Effect of 0.005 M cyanide upon oxygen uptake of dark-grown green (solid lines) and albino (broken lines) 9-day-old leaf tissues at pH 5.5 in the presence of 0.1 M glucose.

and albino seedlings with respect to total, protein, soluble, asparagine, glutamine, α-amino, and ammonium N per gm DW from 6 to 12 days of growth (table II). These tissues resembled the light-grown albino tissues in these particulars. Consequently, the distribution of nitrogenous compounds in light-grown albino and dark-grown green and albino tissues can probably be attributed to the lack of carbohydrate.

Asparagine or glutamine (0.005 M, pH 4.0) stimulated oxygen uptake in albino, but not in green tissues (table III). This stimulation may be indicative of asparagine and glutamine respiratory utilization in

the albino. Casein amino acids (5 mg/ml, pH 4.0) stimulated oxygen uptake in both tissues. Alanine, glycine, aspartate or glutamate (0.005 M, pH 4.0) used separately did not stimulate the oxygen uptake of either tissue. Tissue penetration of these substances was not determined.

INTERMEDIARY METABOLISM: The endogenous Q_{O_2} of 9-day-old, light-grown green leaf tissues at pH 5.5 was 2.5 to 3.0 with an RQ of 1.1. The Q_{O_2} of light-grown albino tissue was 3.0 to 3.5 (20 to 30 % higher than comparable green tissue) with an RQ of 1.0. In etiolated tissues, however, no difference in rates of oxygen uptake was evident between "green" and "albino" leaves (endogenous Q_{O_2} was 3.0 to 3.5). The same relationships occurred between green and albino tissues either light- or dark-grown in the presence of 0.1 M glucose (see green and albino controls in figs 4 and 5). Apparently green and albino seedlings differed metabolically when grown in light but not when grown in darkness.

The endogenous $Q_{CO_2}{}^{N_2}$ in 0.01 M phthalate buffer, pH 5.0, with N/CO_2 (95/5) as the gas phase, was 0.65 and 0.90, respectively, for light-grown green and albino tissues. It appeared that the metabolic dissimilarities in light-grown green and albino tissues were evident also under anaerobic conditions. Dark-grown tissues were not investigated anaerobically. It is interesting to point out the absence of a Pasteur effect in both green and albino light-grown tissues as treated here (green tissue—fermentation CO_2/respiration $CO_2 = 0.65/2.5 = 0.26$; albino tissue—fermentation CO_2/respiration $CO_2 = 0.90/3.0 = 0.30$) (Marsh and Goddard, 9).

Glucose, sucrose or maltose (0.1 M, pH 6.0) stimulated oxygen uptake in both green and albino light-grown tissues (table III). Pyruvic, citric, α-ketoglutaric and succinic acids increased oxygen uptake in the green tissues, but pyruvic and α-ketoglutaric acids were not effective in increasing respiration in the albino. Tissue penetration of these substances was not determined. Glycolic acid increased oxygen uptake in both green and albino light-grown tissues (table III) (Tolbert et al, 17).

Using malonate (0.01 M, pH 4.0) inhibition of oxygen uptake was 25 and 26 %, respectively, in green and albino tissues. The inhibition was completely reversible with 0.05 M succinate. This inhibition and its reversal may be considered as evidence for the presence of succinic dehydrogenase in both tissues. Upon addition of fluoride (0.005 M, pH 4.0) in phthalate-phosphate buffer, oxygen uptake was 51 % of controls in both green and albino tissues. Under anaerobic conditions and in the presence of fluoride (0.005 M, pH 5.0) fermentation was 37 and 31 %, respectively, of controls in green and albino tissues. The effect of fluoride upon both light-grown tissues was the same.

Light-grown green and albino tissues differed in their behavior in the presence of 2,4-dinitrophenol (DNP) (10^{-5} M, pH 5.0). Considering endogenous oxygen uptake as 100 % in green and albino tissues, respectively, oxygen uptake was 169 % in the green and 110 % in the albino tissues. With the same concentration of DNP oxygen uptake of etiolated green and albino tissues was 170 and 155 %, respectively. Thus it appeared that the respiration of light-grown albino seedlings was not stimulated by the phosphate uncoupling action of DNP (16), suggesting that they had primarily non-phosphorylative mechanisms operating.

TERMINAL OXIDATION: Azide (0.001 M, pH 4.5) inhibited oxygen uptake of green tissues 61 %, but inhibited oxygen uptake of the albino only 30 %. With cyanide (0.005 M, pH 5.5; 1.0 M cyanide in

TABLE II

NITROGEN FRACTIONS IN DARK-GROWN GREEN AND ALBINO TISSUES EXPRESSED AS MILLIGRAMS OF NITROGEN PER GRAM OF DRY WEIGHT

NITROGEN FRACTIONS	TISSUE	DAYS GROWTH		
		6	9	12
Total	Green	58.5	51.4	48.8
	Albino	59.3	57.8	52.3
Protein *	Green	26.2	20.9	22.2
	Albino	26.7	27.1	26.9
Total soluble	Green	32.3	30.5	26.6
	Albino	32.6	30.7	25.4
Asparagine	Green	14.8	13.6	11.2
	Albino	16.4	12.2	9.8
Glutamine	Green	1.8	1.6	1.4
	Albino	1.4	1.4	1.0
α-Amino	Green	7.5	8.9	8.5
	Albino	6.8	7.1	9.1
Ammonium	Green	1.5	0.7	0.7
	Albino	0.8	0.7	0.6

* By subtraction.

TABLE III

OXYGEN UPTAKE AS PERCENTAGE OF ENDOGENOUS RESPIRATION OF GREEN AND ALBINO CORN LEAVES FROM 9-DAY-OLD PLANTS, USING VARIOUS SUBSTRATES *

SUBSTRATE	CONC M	pH	GREEN %	ALBINO %	NO. OF DETERMINATIONS
Glucose	0.1	6.0	164	147	3
Sucrose	0.1	6.0	155	137	3
Maltose	0.1	6.0	149	131	2
Pyruvic acid	0.005	4.0	137	100	3
Citric acid	0.1	4.0	227	189	1
α-Ketoglutaric acid	0.005	4.5	135	100	1
Succinic acid	0.05	4.0	168	170	2
Glycolic acid	0.005	5.5	128	120	2
Asparagine	0.005	4.0	100	112	1
Glutamine	0.005	4.0	100	112	1
Casein amino acids	5 mg/ml	5.0	117	116	2

* Computed from data having less than 10 % variability.

the center well) there was 80 and 30 % inhibition, respectively, of oxygen uptake of green and albino tissues. The addition of 0.1 M glucose had no effect on this inhibition (fig 4). Studies in the dark using carbon monoxide ($CO : O_2 :: 95 : 5$) resulted in 75 and 30 % inhibition of the oxygen uptake of leaves of light-grown green and albino seedlings, respectively. Light reversal of the carbon monoxide inhibition was possible only in the albino tissues. Three attempts to demonstrate light reversal in the green tissues were unsuccessful.

Since azide, cyanide, and carbon monoxide were presumably inhibiting enzymes containing heavy-metal prosthetic groups, these data indicated that most of the oxygen uptake in the light-grown green plant proceeded via pathways mediated by such enzymes and that most of the oxygen uptake in the light-grown albino plant did not. In contrast, cyanide inhibited oxygen uptake 89 % in both green and albino dark-grown tissues under endogenous conditions or in the presence of glucose (fig 5). Azide and carbon monoxide were not tried on either of the dark-grown tissues.

LIGHT ACTIVATION: It has been demonstrated that differences in rates of and cyanide inhibition of oxygen uptakes were not evident when the tissues were grown in darkness. To determine which wavelengths of light were operative in inducing these differences, green and albino seedlings were grown in sunlight filtered through each of three colored gelatin filters with transmissions of 445 to 490 $m\mu$ (purple), 452 to 536 $m\mu$ (blue), and 556 to 660 $m\mu$ (yellow). Differences in oxygen uptake and cyanide inhibitions, typical of light-grown tissues, occurred in the 9-day-old seedlings when the purple and blue filters were used. When the tissues were grown under the yellow filter, these differences were not observed. It would appear that the observed differences were activated by the blue-green portion of the visible spectrum. Differences in oxygen uptake between green and albino tissues were also manifested when dark-grown tissues were exposed to 24 hours of white light from a 60-watt tungsten lamp (200 fc at pot level).

DISCUSSION

The results reported here confirm earlier findings (6) that albino corn seedlings have a higher soluble N and a lower protein N content than do green seedlings. In etiolated individuals of the higher green plant where proteolysis occurs after soluble carbohydrates become limiting, some of the amino acids formed through proteolysis may be metabolized by deamination and subsequent oxidation (1). The released ammonia can be synthesized into glutamine and asparagine so long as glutamate and aspartate are supplied via metabolic pathways. Certain amino acids and other nitrogen substances that cannot be so metabolized then accumulate. Since in this study there is little difference in the content of various nitrogenous substances in etiolated green and albino seedlings, and since these results are similar to those obtained from light-grown albino seedlings, the proteolysis hypothesis suggested by Groner for albino corn seedlings seems valid. Further evidence supporting her hypothesis is the failure of added keto acids to stimulate oxygen uptake in the albino tissues, for if large amounts of nitrogenous substances were released during proteolysis, many of these may enter into paths of carbohydrate metabolism. Where these substances are available in the albino corn tissues, additions of "physiological" concentrations of added keto acids may well have no measurable effect, for the enzyme systems are saturated by the products of proteolysis. Differential penetration of these keto acids into the tissues should not be discounted.

In the studies with inhibitors, notably cyanide and DNP, the light-grown albino seedlings show metabolic differences from the light-grown green and the dark-grown green and albino tissues. Cyanide inhibition suggests the presence of the same heavy-metal-mediated enzyme systems in all tissues. Carbon monoxide inhibition indicates the presence of the cytochrome system in both tissues. However, the role these systems play in light-grown albino seedlings is different from that in the light-grown green and dark-grown green and albino tissues. The terminal oxidation in the light-grown albino seems to be carried on by cyanide- and carbon monoxide-insensitive enzymes, probably flavo-protein in character. Further evidence is given by Eyster (2) who reports that in the albino corn seedling the activity of catalase, an iron porphyrin enzyme, is approximately one-third that of the light-grown green seedlings. When, however, the seedlings are grown in the dark, the catalase activity is the same in both green and albino tissues, but slightly higher than the light-grown green tissues. The results with DNP suggest that the respiration in the light-grown albino is primarily non-phosphorylative in character.

Because of the lack of a gene for plastid development, the albino individuals of the strain studied here lack the mechanism for development of chlorophyll and for carbohydrate synthesis. Consequently, proteolysis occurs in the light-grown albino seedlings. However, it also occurs in the dark-grown seedlings, yet their respiratory patterns differ. Therefore, the type of respiration, non-phosphorylative and cyanide insensitive, in the light-grown albino is not a consequence of proteolysis. Being unprotected by the screening action of chlorophyll the "normal" respiration, i.e., the type found in the green or dark-grown green and albino tissue, is probably destroyed or inactivated by light. The proteolysis, on the other hand, eventuates in necrosis of the tissues, possibly by the accumulation of toxic substances like ammonia released during oxidation of amides and amino acids. Spoehr (15) and Gorham (4) reported that the fatal terminus could be prevented by supplying sucrose to their albino corn seedlings. Their method apparently prevented excessive proteolysis, oxidation of its products, and accumulation of toxic byproducts.

Summary

A study of the course of changes in total, protein, soluble, asparagine, glutamine, α-amino, and ammonium N was made on light-grown and dark-grown (etiolated) green and albino corn seedlings. Soluble, asparagine, and amino N content was higher in the albino tissues. Ammonium N was the same in both green and albino tissues, but its level increased rapidly after 12 days of growth in the albino with a parallel increase in asparagine. Approximately the same values for the nitrogenous fractions, as analyzed in the light-grown seedlings, were found in dark-grown green and albino tissues.

Oxygen uptake per gram dry weight in the albino leaf tissues was 20 to 30 % higher than in the green tissues. This difference in oxygen uptake was not evident in etiolated tissues. Fermentation showed the same pattern as respiration with light-grown tissues. These results indicate the absence of a Pasteur effect in either tissue. The oxygen uptake of light-grown albino tissues is mediated by some cyanide-insensitive enzyme system, probably flavo-protein in nature, which is present when the tissues receive 24 hours of light or are grown in the blue-green portion of the spectrum. Results with DNP suggest that the metabolism of the light-grown albino corn seedlings is primarily non-phosphorylative.

The results reported are in accordance with the study reported by Groner that proteolysis occurs in the albino corn seedling.

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