Light Metabolism and Chloroplast Structure in Chlorophyll-Deficient Tobacco Mutants

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ABSTRACT In tobacco mutants which contain $\frac{1}{8}$ to $\frac{1}{30}$ of the normal chlorophyll content per leaf area the content of yellow pigments (carotenoids) is also diminished but less in proportion to the chlorophyll content. The pale yellow-green mutant grows and matures provided that light intensity and temperature make up for the chlorophyll deficiency. In most green plants and algae light saturation of photosynthesis is reached between 5000 and 12,000 ergs/sec·cm^2. The mutants continue to give higher photosynthetic rates until the incident intensity reaches 50,000 ergs/sec·cm^2. While often unable to compensate their respiration at intensities at which the normal green plant approaches saturation, the pale yellow-green leaves are able to provide the mutant plant with two to three times the absolute amount of carbon dioxide assimilation per hour and leaf area at 50,000 ergs/sec·cm^2 and 20° to 25°C. These observations are valid for red light $\lambda > 600$ mμ. In blue light $\lambda < 575$ mμ (below saturation levels) the mutants separate into two classes, one in which absorption by some carotenoid enhances the photosynthetic rate and the other in which the absorbing pigments are inactive and therefore depress the rate strongly. The unusual kinetics of photosynthesis in these chlorophyll-deficient tobacco mutants is reflected in the structure of their chloroplasts which we found to be of a kind thus far not described for healthy, normally growing, higher plants.

INTRODUCTION

At the Tobacco Research Institute at Forchheim, Germany, we saw a 7 ft tall, mature, blooming, tobacco plant that was the color of yellow parchment from top to bottom. Wondering how it could possibly have collected sufficient light energy to grow so well, and remembering Willstätter and Stoll's experiments with the chlorophyll-deficient leaves of *Sambucus nigra* var. aurea, we decided to look again into the old problem of pigment concentration and photosynthetic rate. The literature on this topic is enormous (6 *b*, 12), simply because there are so many different plant species which have been investigated during the past half century. Nothing essentially new has been added to this chapter during the last two decades. The over-all kinetic aspects of the relation be-

tween light, temperature, pigment concentration, and photosynthetic rates are well known and constitute part of our basic information.

The chlorophyll content of mature green leaves of deciduous higher plants varies only by the factor two, the ratio of chlorophyll *a* to chlorophyll *b* is fairly constant, and so is the ratio of chlorophyll to carotenoids. The number of light quanta required for the evolution of 1 molecule of oxygen ranges from 8 to 12. No wonder then, that the light saturation rate in leaves and algae stays also within narrow limits and is reached between 5000 and 12,000 ergs/sec·cm^2 of incident light intensity. The very few investigations of a natural chlorophyll deficiency, for example those made by Willstätter and Stoll (19), and 30 years later again by Gabrielsen (6 *a*, 6 *b*) have shown that less chlorophyll need not spell a lower saturation rate. On the other hand, photosynthetic rates are nearly always lower than normal in plants which have been made chlorophyll-deficient by artificial means. Failure to recognize that not all chlorophyll deficiencies lead to the same metabolic consequences makes a perusal of the literature rather unrewarding.

In this paper we shall describe a chlorophyll deficiency which can be easily compensated for by increasing the light intensity. The maximal rate of carbon dioxide fixation in our chlorophyll-deficient plants (the saturation rate) defined on the basis of illuminated leaf area, may go twice or three times as high as that found in the normal green leaf. This happens in red light where no auxiliary yellow pigment can contribute to useful light absorption. The light curves for the yellow-green mutants run below the norm at low intensities but rise considerably above it at intensities several times those which usually suffice for saturation. In other words the mutation has produced an extreme case of what is referred to in the literature as a sun plant (1).

With blue light at low intensities we found that absorption by yellow pigments may either be quite advantageous to the chlorophyll-deficient plant or detrimental by just shading what chlorophyll there is without contributing to the useful photochemistry.

These peculiar photosynthetic traits of our viable pale mutants acquire a greater importance by their correlation with structural changes in the chloroplasts. As the many published micrographs show, all green leaves contain in their chloroplasts numerous dense stacks of short lamellae, the grana. Our electron microscopic studies, (15) of which we give only two examples here, revealed unexpected structural changes. We found no fewer chloroplasts per cell, and no true grana. The lamellae are few, widely separated with occasional triple folding, mostly at their ends. These chloroplasts resemble an algal cell more than they do their grana-packed closest relatives.

METHODS

Growing of Tobacco Plants We grew and investigated a number of tobacco variants. The plants selected on the basis of reproducibility of results were:

(*a*) *Nicotiana tabacum*, Connecticut cigar variety, John Williams Broadleaf, as the parent strain. An 18 yr old seed lot gave spontaneous yellow mutants. The yellow mutants were selfed and yielded a strain designated as su/su ♂ × su/su ♀. They will be designated in the text as JWB and JWB mut., respectively; (*b*) *Nicotiana tabacum* Kentucky 151 and an aurea mutant from Kentucky 151, abbreviated K 151 and K 151 mut.; (*c*) leaves of other species for comparison.

All tobacco plants were grown under the same greenhouse conditions without shading. The lowest night temperature was 20°C, the lowest day temperature 25°C. Temperatures above 36°C for several hours caused heat damage. In the high light intensity of the Floridian spring and summer the pale mutants grew well. They grew slowly or not at all during the dark winter months. The seeds germinated approximately 1 wk after sowing. 3 to 4 wk later the plants were transplanted into sterilized soil in 14 in. saucers. Another 4 wk later the plants were ready for experiments. They were fertilized 2 wk before use. 1 liter of fertilizer solution contained the following quantities of inorganic nutrients: 40 mg N; 40 mg P_2O_5; 10 mg MgO; 0.04 mg B; 0.1 mg Fe; 0.1 mg CaO.

If a plant had to be grown to maturity it was transplanted into big pots containing approximately 10 kg of sterilized soil. A month later 2 g of N; 3.5 g P; 4.5 g K; and 0.25 g Mg were added as fertilizer. Special trace elements were given when symptoms of lack of trace elements appeared. All plants grew to maturity within 100 days after transplantation. Some of the very pale tobacco plants took a longer time to produce blossoms or did not flower at all. For the experiments proper we used only perfectly healthy middle leaves while they were still expanding.

Extraction of Pigments 10 g of green or mutant tobacco was extracted for half an hour with 50 ml 98% methanol at 60 to 70°C. The solution was filtered and filled up with methanol and water to give 100 ml of a chlorophyll solution in 90% methanol. In the classical way the xanthophylls were separated from the chlorophyll by fractionation between petroleum ether and 90% methanol, their extinction measured at λ 430 mμ, and compared with our standard solutions.

5 cc of concentrated petroleum ether extract containing the chlorophylls and carotenes was passed through a carefully dried sugar column. The pigments were eluted with petroleum ether, followed by petroleum ether/benzene mixtures, gradually increasing in their volume ratio from 9:1 to 5:3. The carotenes ran through the column with the first pure petroleum ether washing. Chlorophylls *a* and *b* were separated by the petroleum ether/benzene treatment. Total carotenes were measured in petroleum ether at λ 430 mμ and the extinction compared to a standard curve. Chlorophylls *a* and *b* were measured in 90% methanol at λ 663 and 645 mμ.

There are good reasons to believe that the discrepancies between our chlorophyll determinations and those of Willstätter and Stoll (19) are due to incomplete extraction of pigments in the earlier classical work (12). Thus activity of chlorophyll per milligram has a tendency to come out too high in the tables of Willstätter and Stoll, particularly for those plants in which the chlorophyll content is low. Gabrielsen repeated some of their experiments with the same material, *Ulmus* and *Sambucus* (6 *b*). He too found a discrepancy between their values and his own, but blamed faulty light measurements.

Measuring Gas Exchange and CO_2 Fixation Cutouts from leaves were made with a razor blade along the edges of a superimposed rectangle of 2.1 × 3.9 cm = 8.2 cm^2. The cuts were floated face down on 3 ml of 0.1 M bicarbonate-carbonate buffer, pH 8.9. The photosynthetic rates were measured either manometrically as gas exchanges or as counts $^{14}CO_2$.

The reliability of the method is attested by the observation that JWB tissue often gave the same rate of respiration for 7 or 8 consecutive hours. The more sensitive K 151 lasted for about 4 hr, after which it rather quickly died. The absolute values of photosynthetic rates found in our normal green controls check quantitatively with the values reported in the literature (12, 13, 17).

The results of many single experiments were directly comparable only when the chlorophyll content per leaf area happened to be the same. Depending on slight differences in environmental growth conditions, the chlorophyll content varied with time, and the area of each cut by accident. We decided to make chlorophyll determinations for each Warburg vessel. In this way the small errors due to variations in the cut area of leaf samples could be easily corrected. If the metabolic rates were expressed in terms of milligrams of chlorophyll, widely separated experimental series yielded quite reproducible figures.

We concur with Gabrielsen (6 *b*) in that it makes more sense from a plant physiological point of view to compare rates on the basis of either illuminated leaf area or of chlorophyll content instead of dry weight. In particular, the dry weight of leaves or leaf sections changes too rapidly with the storage or utilization of metabolic products, such as starch, fat, or protein (see Table II). Therefore we have not given rates per dry weight.

For obvious methodological reasons it was necessary to investigate the nonphotochemical gas exchange, i.e. respiration, when rates of photosynthesis were determined manometrically, and dark fixation of $^{14}CO_2$ when they were determined by fixation of radiocarbon. The yellow mutant deviated conspicuously from the normal green plants also in the dark metabolism. The dark fixation of CO_2 was much smaller. And just as the dry weights did, the rates of respiration varied from experiment to experiment, or even in the course of one experiment. In order to obtain accurate intensity curves, it was necessary in some cases to determine respiration separately for each point on the curve and to correct the light effect accordingly. When this was done the maximal rates of photosynthesis on a chlorophyll basis became almost identical for all experiments with normal green leaves from several types of plants.

Another approach to the problem of the variable respiration was to abolish the light effect by poisoning the leaf tissue with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea). This method works easily with suspensions of unicellular algae, but we found it erratic with leaf cuts. Penetration of DCMU from the buffer into the leaf cuts or from the stems into the leaf required about 80 min and even then the inhibition was often incomplete at higher light intensities. Thus our experiments with poisoned leaf sections remain under suspicion. We mention them nevertheless because some new observations point to an effect of light on respiration (10).

The experiments with radioactive carbon were done during a steady-state run of photosynthesis by tipping 1 μc of $Na^{14}CO_3$ (0.02 μmole) into 2.9 ml of the 0.1 M bicarbonate-carbonate buffer. The tissue was fixed a few minutes later by washing

the leaf sections with 10% acetic acid and subsequent heating. After drying the leaf sections (4 cm^2 in this case) were counted in an automatic planchet counter (Nuclear-Chicago Corp., Model C-110-B).

Effect of Stomatal Opening on Photosynthesis Zelitch (20) has lately discussed the influence of the stomatal movements on photosynthesis in tobacco leaves. He reports that the stomata are mostly closed in air with 1.8% CO_2. Because we thought it possible that genetic differences in the yellow and green tobacco might affect also the control of those movements and thereby introduce an unexpected error, we checked the dependence of photosynthesis on CO_2 concentration. The tobacco was the Connecticut cigar variety JWB. We chose the photosynthetic rate at 12,000 ergs/sec·cm^2, red light at 25°C, a light intensity at which with 0.45% CO_2 in air the photosynthetic rates of yellow and green tobacco are almost the same.

TABLE I

DEPENDENCE OF PHOTOSYNTHESIS ON CO_2 CONCENTRATION

Rate of photosynthesis: μl O_2/hr 8.2 cm^2. Red light 12,000 ergs/sec.cm^2. T = 25°C

Volume % CO_2 in the gas phase	JWB mut.	JWB
0.03	32	32
0.075	54	60
0.275	168	143
0.45*	180	195
1.00	168	216
3.5	180	210

12 individual leaf sections varied in area and chlorophyll content not more than ±10%.

* Usual buffer.

With buffers providing different partial CO_2 pressures and therefore giving different rates, we found that the rates of green and yellow leaf sections did not change in their relation to each other. The highest rate was reached with a CO_2 concentration in equilibrium with the buffer we used throughout. Therefore any selective influence of carbon dioxide on the movement of the stomata in yellow as compared with green leaf sections could be ruled out. In experiments with varying leaf areas the gas exchange between gas and liquid was not limiting under our conditions.

Light Measurements As a light source we used either floodlight bulbs of 150 or 300 W for "red" light, or Sylvania 300 W mercury lamps for "blue" light. Relative light intensities were measured with a selenium photocell in place of the Warburg vessel in the thermostat. The spectral distribution behind red or blue plastic filters was determined with an ISCO Spectroradiometer, model SR (Instruments Specialties Co., Lincoln, Neb.). The spectral section dividing red and blue was λ 575–600

mμ. The sole purpose of this spectral division was to distinguish between pure chlorophyll and mixed pigment absorption.

RESULTS

All experiments were run at 25°C unless other temperatures are especially mentioned. The majority of experiments were done with JWB and Kentucky 151. There are two sets of experiments, in red light and in blue light. The figures are plotted from single experiments because in this way interesting

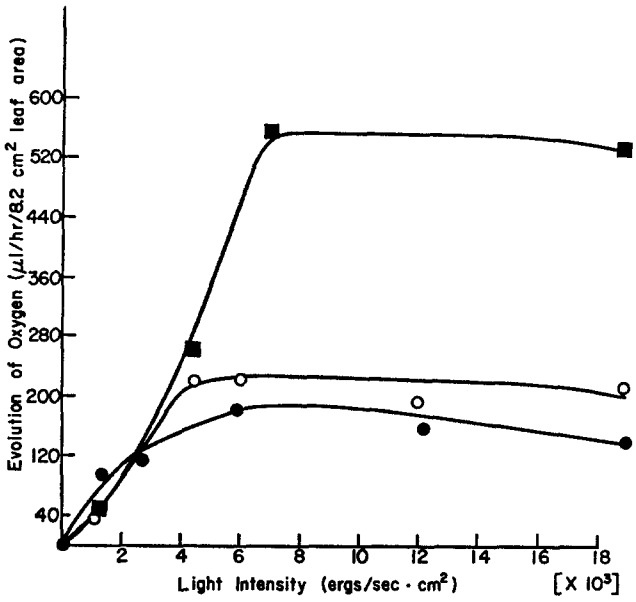

FIGURE 1. Light saturation curves plotted on the basis of 8.2 cm² illuminated area. Red light, 575 mμ < λ < 700. Normal dark green leaves of *Nicotiana tabacum* var. Florida 15 with 110 γ chlorophyll (filled circles); *Nicotiana tabacum* var JWB with 152 γ chlorophyll (open circles); *Cassia obtrusifolia* with 340 γ chlorophyll (filled squares).

details show better. In the course of a year each experiment was repeated 10 to 20 times with identical results.

Saturation in Red Light The saturation rates of normal green leaves are roughly proportional to their chlorophyll content per area, in other words, despite large variations of chlorophyll content the saturation rate per milligram of chlorophyll in a higher plant has the aspect of a constant. To clarify the points we want to make in this paper it is necessary to compare the plot per area with that per chlorophyll. Figs. 1 and 2 are typical light curves for normal leaves plotted in both ways. Our absolute figures for rates and saturation in red light check very well with those given in the literature for white light (12, 13).

In 1931 Stöcker found in *Cassia fistula* a rate of photosynthesis of 13.5 expressed as mg CO_2/hr/100 cm² (12). We found 13.2 as the maximal rate for *Cassia obtrusifolia*. Giltay (1898 Java) investigated *Nicotiana rustica* in air and found a value of 9 (12) which compares with our values in the range of 6 to 8. Values which deviate strongly have aroused suspicion (see Rabinowitch, 2(1): 1000). Roberts and Corbeit (13) measured the photosynthesis of tobacco leaf sections using the same manometric method and a comparable buffer system. They found for leaf sections of healthy green tobacco a rate of photosynthesis of 1.44 μl O_2 evolution per μg chlorophyll per hr which corresponds to our value of 24 μl/mg chlorophyll min.

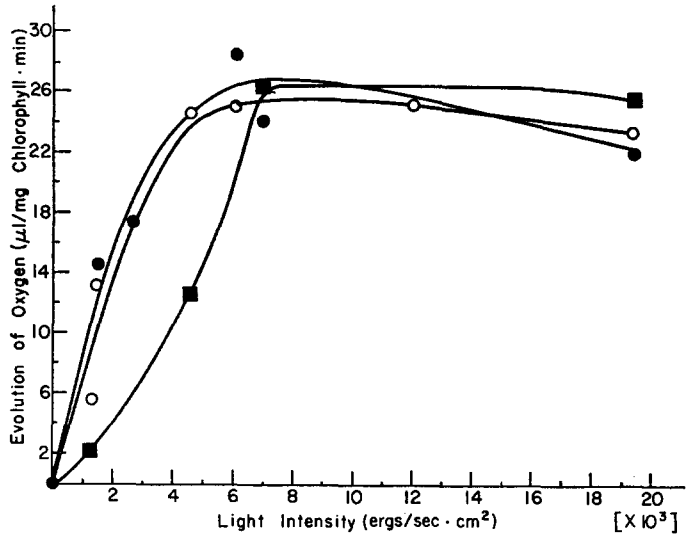

FIGURE 2. Same as Fig. 1 but plotted on the basis of chlorophyll content.

Whenever we changed from the dark green, grana-filled leaf to the pale green, yellow, or even whitish mutants, the typical low saturation rate gave way to a higher rate at much higher incident intensities. Table II gives the pigment content for the various plants we have investigated.

The dependence of growth on the season is shown in Figs. 3 *a* and *b*. In May in the greenhouse, seedlings of the normal green and of the pale variety grew equally well; in November the pigment-deficient plants did not and had to be transferred into growth chambers. The loss of pigments through mutation is general. It includes carotenoids as well as chlorophyll, except that the decline in chlorophyll content is steeper. Thus the ratio chlorophyll: carotenoids changes in favor of the latter.

The aurea mutation of JWB has on the average $\frac{1}{8}$ of the normal chlorophyll content per cm² leaf area. This amount corresponds to $\frac{1}{4}$ of the normal chlorophyll content in per cent of dry weight. Light curves for this plant in

the red half of the visible spectrum are shown in Figs. 4 and 5. Higher light intensity makes up for the missing chlorophyll. The initially much smaller rates in the aurea leaves approach those of the chlorophyll-rich leaves (as seen by Willstätter and Stoll 50 years ago). The new and important observation is that the curves cross at a steep angle. In Fig. 5 the rate continues to rise with intensity in a straight line even at four times the normal saturation intensity. At this point the photosynthetic capacity of the yellow leaf was on the average ⅓ higher than that of the green one. 19,000 ergs/sec·cm² was the highest available intensity in a set of 20 similar experiments. In another experiment with red light, the energy reached 37,500 ergs/sec·cm². Here the maximal rate was 2.2 times higher than that of the green control. Yet there was no

TABLE II

PIGMENT CONTENT OF 10 cm² LEAF AREA

Averages of 20 separate determinations.

Tobacco strain*	Dry weight‡	Chlorophyll		Carotenoids		Ratio total chlorophyll: total carotene
		Total μg	Ratio a:b	Total μg	Ratio xanthophyll: carotene	
I	34±15	264	2.2	65	3.2	4
II	33±10	225	2.0	55	4.2	3.7
III	19±7	100	2.0	30	5.5	3.2
IV	19±7	34	2.9	18	3.3	1.9
V	17±5	15.5	1.9	17	—	1
VI	17±5	11	2.4	9.7	—	1.1

* I, Connecticut cigar variety from John Williams Broadleaf, 6 wk after germination; II, Dixie Shade green, 10 wk after germination; III, Dixie Shade derivative w. yellow-green character 525–10–9y, 10 wk after germination; IV, Aurea from J. W. Broadleaf and back-cross su/su ♂ × su/su ♀, 6 wk after germination; V, Aurea mutant from Ky 151, 12 wk after germination; VI, Aurea from Japanese Bright Yellow, adult.

‡ The dry weight varied greatly according to season.

indication that saturation was near. With a spotlight the intensity obtained was 44,000 ergs/sec·cm² of red light, and the rate of photosynthesis in the mutant was 2.5 times higher than in the chlorophyll-rich control. The chlorophyll itself was at least 20 times more effective. In Fig. 5 the light curve for the mutant is composed of points taken from several experiments. At low light intensities where the gas exchange was difficult to measure accurately, we used $^{14}CO_2$ fixation instead of manometry. Saturation apparently occurs between 44,000 and 56,000 ergs/sec·cm² at a rate of photosynthesis approaching 13 mg CO_2/hr 100 cm² or about 200–250 μl O_2/mg chlorophyll min.

Effect of Temperature With our regular lamps, which had always been adequate to produce saturation in algae or ordinary leaves, saturation in the aurea variation was out of reach at 25°C, the temperature at which most

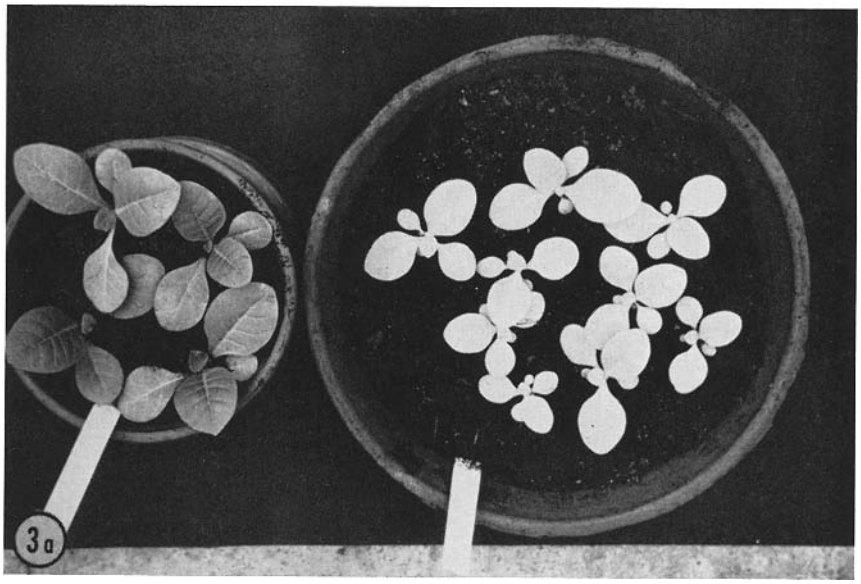

FIGURE 3 *a*. 7 wk old plants of *Nicotiana tabacum*, var. JWB, normal green and mutant, grown in April–May.

FIGURE 3 *b*. Same plants grown in November–December.

experiments were done. We therefore lowered the temperature in order to depress the saturation level of photosynthesis in the well known manner.

Temperatures were varied between 32° and 7°C in numerous single experiments. Figs. 6–8 are a summary of the results. For the normal green leaves

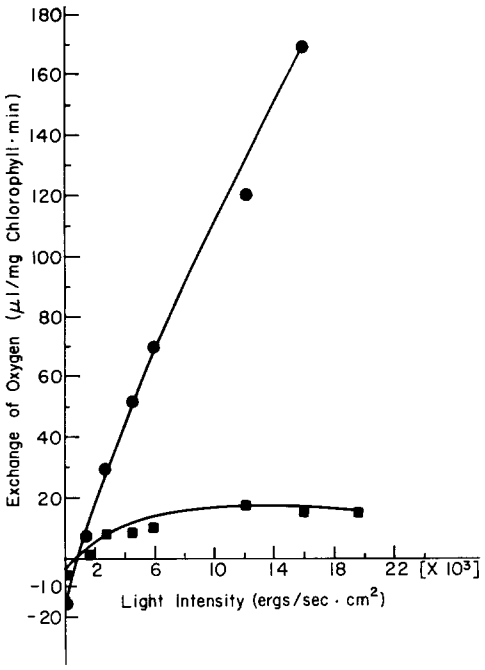

FIGURE 4. Light intensity curve for oxygen gas exchange. Red light. Rates on the basis of chlorophyll. Normal JWB (filled squares); JWB mut. (filled circles); JWB with 224 γ chlorophyll, JWB mut. with 19.4 γ chlorophyll per 8.2 cm^2 leaf area.

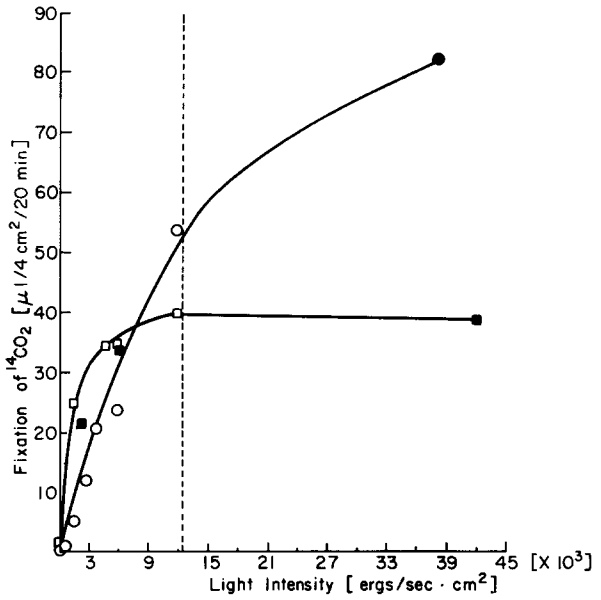

FIGURE 5. Light intensity curve for $^{14}CO_2$ fixation on the basis of illuminated leaf area. Red light. JWB (open squares); JWB mut. (open circles). The highest point was determined by O_2 exchange instead of $^{14}CO_2$ fixation. JWB (filled squares); JWB mut. (filled circle).

(Fig. 6) they check exactly with the results described in the textbooks (12), namely a steady decline of the maximum rate of photosynthesis with temperatures from 32°C on down.

The data obtained with the yellow leaves were at first surprising. There was no influence of temperature in the chlorophyll-deficient mutant between 16° and 32°C and up to 19,000 ergs/sec·cm². This confirms what was said above, namely that with this mutant the highest photosynthetic rates we measured routinely still fall into the "linear" part of the respective light curves (Fig. 7).

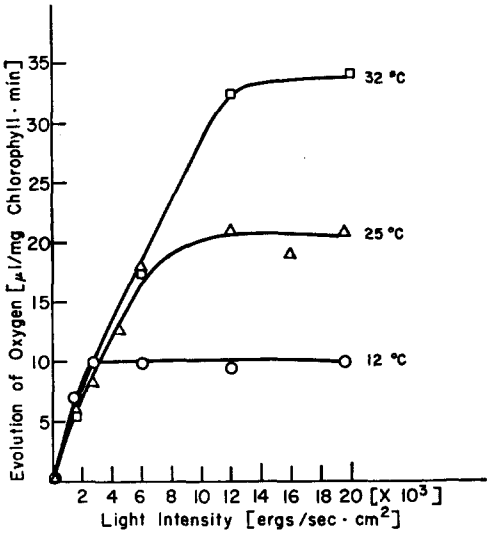

FIGURE 6. Light intensity curves at different temperatures in red light on the basis of chlorophyll for normal green JWB (corrected for respiration).

There is, however, a rather sudden break, a steep decline in metabolic rates, when the temperature falls below 16°C. At 12°C it is already half of the maximum and at 7°C the saturation rate is down to one-sixth of that found at 16°C (Fig. 8).

Fig. 8 is an experiment designed to check a corresponding one shown on page 155 of Willstätter and Stoll's book. The intensity was 6,000 ergs/sec·cm² and was kept constant. While the rate of the normal plant continued to rise with temperature, the rate increase in the mutant suddenly stopped, it had become independent of temperature at about 16°C. The old figure referred to looks identical and this proves that Willstätter and Stoll did not have light saturation in their *Ulmus* aurea leaves above 20°C.

Plants with Greater Chlorophyll Deficiencies What has been said above about the strain JWB mut. with $\frac{1}{8}$ the normal chlorophyll content is true, in a more impressive way, with other aurea or albino varieties which contain only $\frac{1}{20}$ to $\frac{1}{30}$ of the normal amount of chlorophyll (Table II).

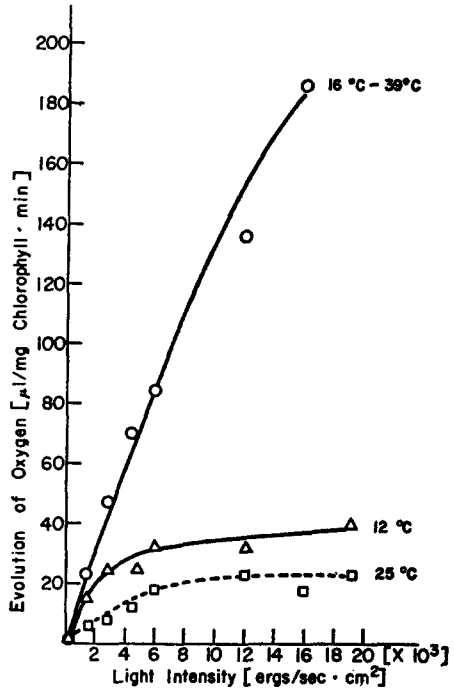

FIGURE 7. Light intensity curves at different temperatures in red light on the basis of chlorophyll for JWB mut. (corrected for respiration). The dashed curve shows the light intensity curve for the green control at 25°C.

Fig. 9 gives the light intensity rate curve per milligram chlorophyll and Fig. 10 per unit leaf area. In Fig. 10 the rate for the whitish yellow variety eventually reaches that for the control at four times the saturation intensity

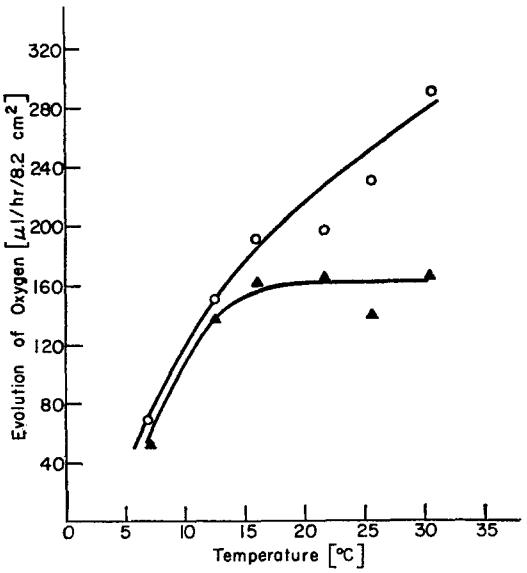

FIGURE 8. Temperature dependence of photosynthesis at 6000 ergs/sec.cm². Rates plotted on the basis of illuminated leaf area and corrected for respiration. Green Ky 151 (open circles); JWB mut. (filled triangles).

for the latter. Again the curve crosses the control curve at an angle which does not permit us to believe that a true saturation for the very pale variety is near.

Respiration and Dark CO_2 Fixation The mutant plants seem to be equipped with an especially effective respiratory system to handle the large amounts of photosynthetic products which are formed at high intensities and high temperatures. Paradoxically the dark fixation of carbon dioxide ($^{14}CO_2$) due to exchange and biosynthetic reactions, which we thought ought to parallel the rates of respiration, was astonishingly low in the yellow mutant, while in the

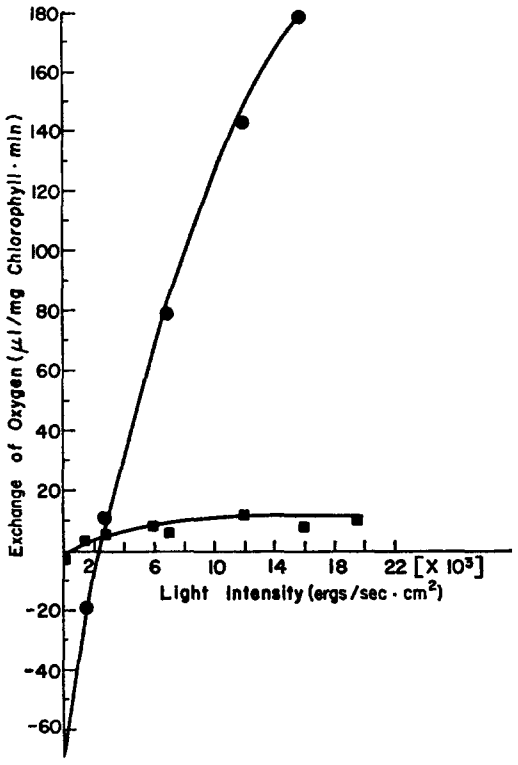

FIGURE 9. Light intensity curve in red light ($575 < \lambda < 700$ mμ) for Ky 151 on the basis of chlorophyll. Ky 151 with 220 γ chl and 58 γ carotenoids per 8.2 cm² (filled squares); Ky 151 mut. with 11 γ chlorophyll and 14 γ total carotenoids per 8.2 cm² (filled circles).

green controls it checked again with the values given in the literature. See Table III. Whether the constancy of dark fixation rates on the basis of chlorophyll content is just a coincidence or in some way meaningful remains to be seen.

Because of the rapid and large variations of respiration with experimental and earlier environmental conditions, the photosynthetic rates had to be corrected in many experiments for each intensity and temperature range. Figs. 4 and 10 show uncorrected plots of oxygen gas exchange. At an intensity at which the normal plant is reaching half-saturation the yellow Ky 151 has just compensated respiration (Fig. 10).

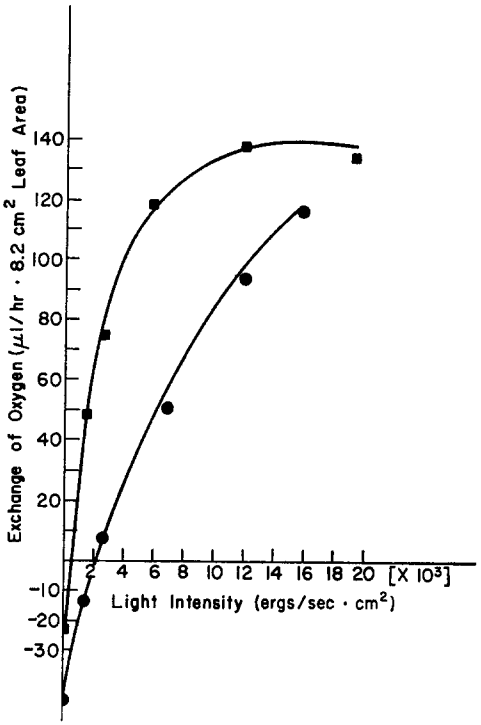

FIGURE 10. Light intensity curves with same data as in Fig. 9 on the basis of illuminated leaf area. Ky 151 (filled squares); Ky 151 mut. (filled circles). Note high rate of respiration.

There is a further complication connected with respiration, the Kok effect (9, 18). Any light intensity vs. rate curve begins with a light action which is strictly speaking not photosynthesis but a reversal of the carbon dioxide evolution and oxygen uptake. In this region below and about respiratory compensation, i.e. at the intensity at which the gas exchange balances, the light curve may show a change in slope. With *Nicotiana tabacum* Kok effects are common.

TABLE III

AEROBIC DARK FIXATION μl $^{14}CO_2$ PER HOUR

Temperature 25°C

	Fixation in μl $^{14}CO_2$/hr			
	Per 10 cm²		Per mg chlorophyll	
Tobacco John Williams Broadleaf	Average	Maximum	Average	Maximum
JWB	4.6	7.2	16.8	20.2
JWB mut.	0.465	0.86	14.5	24.6

Averages of six determinations. Actual times, 60 min.
Range of counts per 10 min, 600 to 6000.

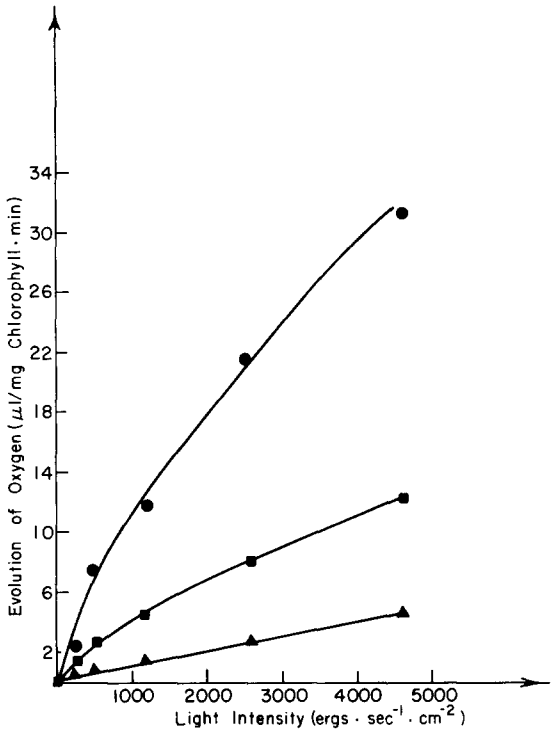

FIGURE 11. Light intensity curves in blue light (380 < λ < 575 mμ). Rates of oxygen evolution (corrected) on the basis of chlorophyll per 8.2 cm². *Cassia obtrusifolia* 340 γ chlorophyll (filled triangles); JWB, 152 γ chlorophyll (filled squares); JWB mut., 20 γ chlorophyll (filled circles). The initial bend in the curves for tobacco is introduced by the correction for respiration.

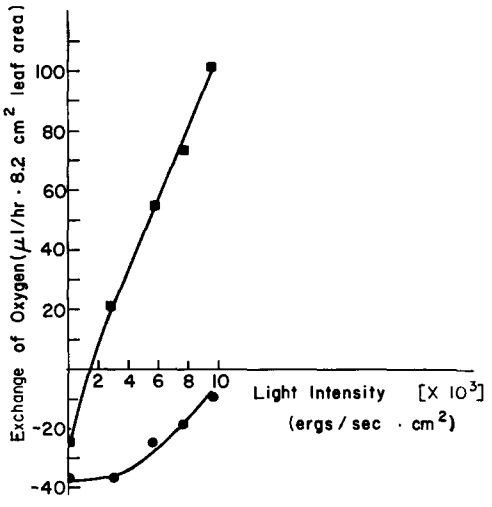

FIGURE 12. Light intensity curves in blue light (λ 380 < λ < 575 mμ). Observed rates of oxygen exchange on the basis of 8.2 cm² illuminated leaf area. Ky 151 (220 γ chlorophyll, 58 γ carotenoids) (filled squares); Ky 151 mut. (11 γ chlorophyll; 14 γ carotenoids) (filled circles).

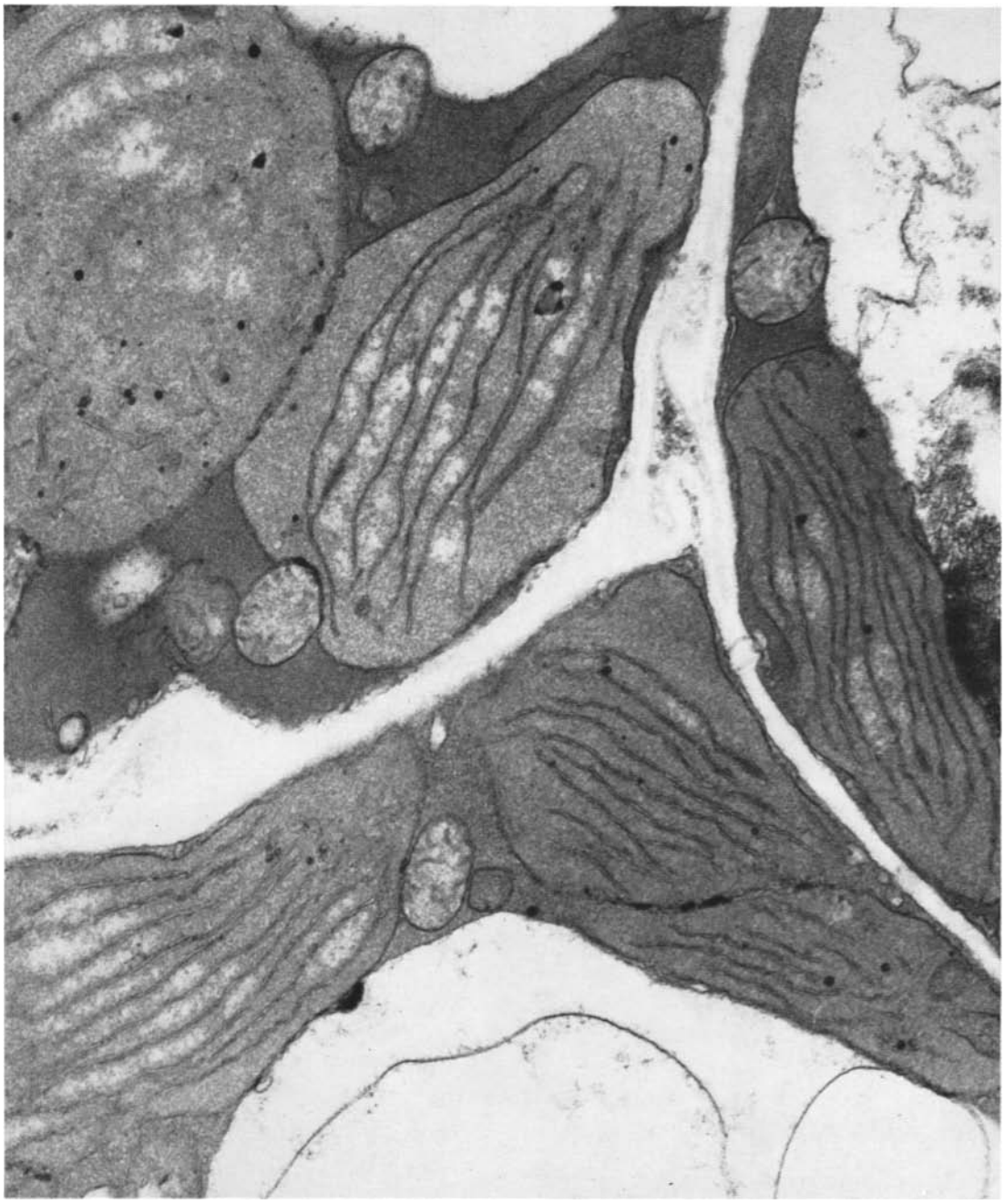

FIGURE 13. Electron micrograph of chloroplasts of JWB mut. Glutaraldehyde fixation; postfixed with OsO_4.
FIGURE 13 *a*. Cotyledon, age 8 days after sowing. × 18,000.

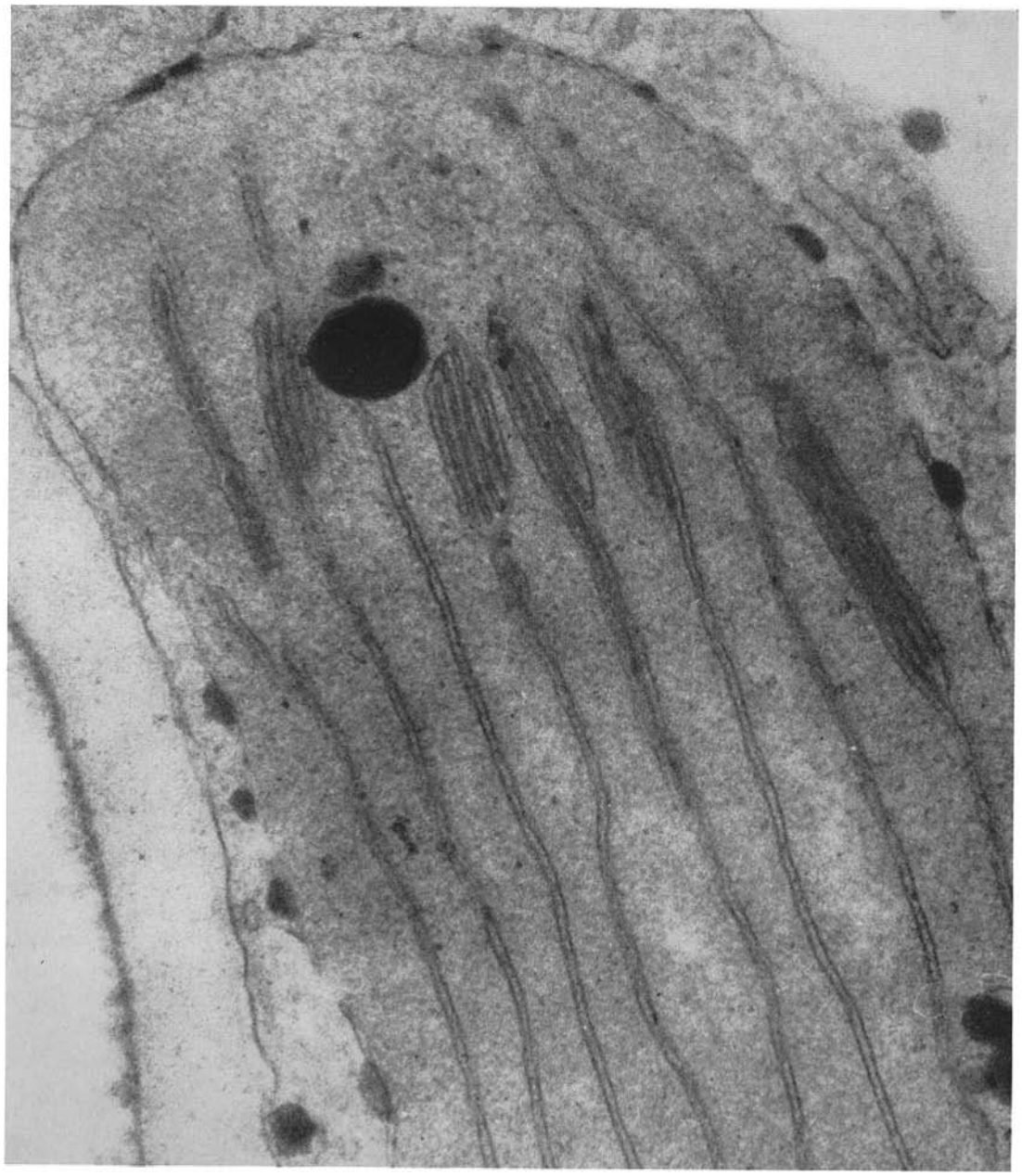

FIGURE 13 *b*. Leaf from an 8 wk old plant. × 65,500.

Blue Light and Accessory Pigments The preceding measurements were done with red light to avoid the problem of variations in energy transfer from light-absorbing yellow pigments (3).

Initially we had expected that all yellow varieties would show a better utilization of light absorbed by some yellow "accessory" pigment than the green plant. Figs. 11 and 12 show that this assumption turned out to be correct for one line of tobacco (JWB mut.) but not for another (K 151 mut.).

The use of blue light in place of red light in the experiment of Fig. 11 with JWB reversed the relative positions of the light curves for normal and chlorophyll-deficient tissue in red light (see Fig. 5). We are now speaking of low and medium light intensity, for true photosynthetic saturation must be independent of color, and indeed has been found again to be so by Pickett and Myers (11). With $\frac{1}{8}$ of the chlorophyll and $\frac{1}{3}$ of the carotene JWB mut. has a greater ratio of yellow to green pigments than the normal plant. All through the linear region of the light curve the mutant gives three times as much photosynthesis per incident blue ergs on a chlorophyll basis, though a shading effect of the carotenoids is felt and thus the absolute rates of photosynthesis are lower than in the corresponding red light. K 151 mut. with $\frac{1}{20}$ of the chlorophyll, but $\frac{1}{4}$ of the carotenoid present in the control, derives no advantage whatever from the greatly increased carotene to chlorophyll ratio (see Fig. 12). Up to 10,000 ergs of blue light (corresponding in efficiency to about 6000 ergs of red light) the absorbed energy barely suffices to compensate respiration.

Chloroplast Structure Electron microscopy is still considered a specialty. We have, therefore, shown and discussed the details of the differences in the structure of normal and pale chloroplasts in another publication (15). In addition, electron micrographs of chlorophyll grana are now to be seen in many textbooks, apart from the papers dealing with their nature and development. For this reason we bring, in Fig. 13, only two other examples of the abnormal lamellar arrangements we discovered in JWB mut. chloroplasts. The lamellae are few and single, extend across the length of the chloroplast, lie rather far apart, and sometimes end in short double and triple folds. Remarkable are the number of mitochondria in proximity to the chloroplasts.

DISCUSSION

The botanical literature contains no example which shows that the maximum rate of carbon dioxide fixation in a given green cell may increase when its pigment content decreases. The experiments reported above establish this as a puzzling fact. For obvious reasons, dark green, chlorophyll-dense tissues or concentrated cell suspensions require more light to become light-saturated. Experimentally this has been found to be true so often that it did not occur to Willstätter and Stoll, for instance, that intensities high enough to saturate dark green *Sambucus* might have been insufficient to saturate photosynthesis in their aurea variety. They discovered, however, the first part of the phenomenon, the higher efficiency on the basis of chlorophyll content of the aurea

variety at the same intensity. These classical experiments are well known, but after their confirmation and discussion by Gabrielsen (1, 6 *a*, 6 *b*), they have been neglected.

It looks as if the light curves and the electron micrographs of our tobacco mutants provide a sequence to the story of the photosynthetic unit (2, 4, 5, 7, 8, 14, 16).

Compared with the dark green plant, the aurea variety may contain more chlorophyll action centers in relation to total light-absorbing pigment. Either the units are smaller or the enzyme content (or its turnover) is higher, or both. We shall not proceed here with theoretical elaborations because we are engaged in the very obvious experiments which the results obtained so far call for. One point, however, needs no further discussion: the role of the structure called "grana." These lamellar stacks are only a device to pack more chlorophyll into the chloroplast, but as such are not essential for the photosynthetic mechanism. Actually this was evident from the moment electron micrographs permitted a comparison between the structures of algae and of chloroplasts. In this respect we must remember that the concept of the chlorophyll unit arose from experiments with unicellular algae. If the unit has any structural identity, such structure must become visible in a single lamella.

The question as to what extent accessory pigments, in particular the yellow ones, are in useful contact with the chlorophyll system seems to be independent of the problem of variability of units in green plants. Nevertheless it may be worthwhile to investigate further the very efficient utilization of blue light in JWB mut.

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