LOCALISATION OF CHLOROPHYLL WITHIN THE CHLOROPLAST

by

J. B. THOMAS*, L. C. POST AND N. VERTREGT

Biophysical Research Group Utrecht/Delft under the Direction of A. J. Kluyver, Delft, and of J. M. W. Milatz, Utrecht (Netherlands)

INTRODUCTION

For a detailed study of the way of functioning of the photosynthetic apparatus it is necessary to penetrate into the micro-localisation of the photosynthetic pigments. At present it seems most likely that, in chloroplasts containing grana embedded in a stroma substance, the chlorophylls are concentrated in the grana. This conception is based on light microscopical examination of various chloroplasts. HEITZ[1] observed coloured grana surrounded by a colourless stroma within chloroplasts of many plant species. According to HEITZ[2], METZNER[3], and STRUGGER[4] chlorophyll fluorescence is restricted to the grana.

However, in many cases the dimensions of the grana are below light microscopical resolution. This fact may account for contradictory statements concerning the occurrence of grana in chloroplasts, and, consequently, with regard to the distribution of the photosynthetic pigments. As to this, it may suffice to refer the reader to the following pertaining reviews: WEIER[5], RABINOWITCH[6], FREY-WYSSLING[7], GRANICK[8], and WEIER AND STOCKING[9].

The electron microscope revealed the presence of grana in various chloroplasts that looked homogeneous under the light microscope. In fact, these observations rendered it highly probable that all chlorophyll-bearing plastids contain grana. Even in chloroplast-free organisms, such as photosynthesizing bacteria and various algae, grana were found—CALVIN AND LYNCH[10], THOMAS[11].

However, WOLKEN AND PALADE[12] published electron micrographs of cross-sections through chlorophyll-containing plastids in two flagellates, which clearly demonstrate a regularly lamellate structure free from the "usual" grana. STEINMANN[13] showed in the same way that this also holds for plastids of *Spirogyra* and *Mougeotia*.

Moreover, STEINMANN[14] and FREY-WYSSLING AND STEINMANN[15] published a micrograph of a cross-section through a tulip chloroplast in which lamellate grana are shown to be suspended in the stroma.

So, some chloroplasts contain grana, while other ones do not. However, the structure of the grana-free chlorophyll-bearing plastids strikingly resembles that of a granum itself. If one compares them *e.g.* with the bacterial and algal grana, one may ask whether there is any objection to denoting the latter as grana-free chloroplasts too, in spite of

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their much smaller size. In that case it may be preferable to define as follows: in green cells chlorophyll is concentrated in grana; the grana may occur either individually or combined in chloroplasts. Anyhow, one can state that, in all cases studied up till now, the occurrence of chlorophyll seems to be restricted to lamellate structures.

It is the aim of the present investigation to render support to this conception by giving further evidence that lamellae are the bearers of chlorophyll. Since to this purpose the light microscope is no longer an adequate tool, it was necessary to introduce an indicator for chlorophyll activity, which could be observed under the electron microscope. For this the MOLISCH[16] reaction seemed most suitable. This reaction consists of a blackening of illuminated chloroplasts in the presence of silver nitrate due to the reduction of the salt to metallic silver.

Many investigations concerning this reduction have been carried out: these have been reviewed by WEBER[17] and METZNER[18]. However, the mechanism of the MOLISCH reaction has not yet been fully elucidated. Originally the silver nitrate reduction was believed to occur in living cells only. METZNER[18], however, demonstrated that the reaction took place in killed cells as well. The MOLISCH reaction also proceeds in the dark; light, however, increases its rate considerably. METZNER showed that the pH of the medium determines which cell constituents are blackened by the metallic silver; at pH 3–4 the chloroplasts—their surface and the grana in particular—show the reaction, while at pH 7–8 the precipitate is mainly found in the cytoplasm. In the pH range in which the chloroplasts react this author examined the wavelength dependency of the MOLISCH reaction. This was done by counting the silver precipitates in the cells and by statistically evaluating the figures obtained. The action spectrum established in living cells shows some features in common with the chlorophyll absorption spectrum. However, that obtained from killed cells hardly admits any conclusion.

In order to examine whether chlorophyll occurs in isolated lamellae it was necessary to use grana suspensions instead of tissues. Therefore, it was necessary to study whether the MOLISCH reaction can be established in such a suspension. Consequently the present study deals with:

1. demonstration of a light-induced silver nitrate reduction in grana suspensions,
2. demonstration of the dependency of the MOLISCH reaction sensitization on the presence of excited chlorophyll molecules by establishing an action spectrum of this reaction,
3. localisation of the chlorophyll.

MATERIAL AND METHODS

Preparation. Grana suspensions were prepared from fresh leaves of *Hibiscus rosa sinensis.* Usually about 4 g of washed leaves were ground in a Waring blendor in 75 ml of a pre-cooled 0.2 *M* glucose or, mostly, sucrose solution in glass-distilled water for 2 minutes. The brei was filtered through cotton wool and centrifuged at 1200 *g* for 5 minutes. The supernatant was diluted with an equal volume of a 0.4 *M* sodium acetate buffer in 0.2 *M* sucrose. The pH values will be mentioned together with the experiments. This suspension will be referred to as "grana suspension".

Lamellae suspensions were prepared by weakly disintegrating the grana suspensions with the aid of a magnetostriction oscillator. The energy delivered to the magnetostrictive tube was about 300 watts at a frequency of 7000 Hz. Approximately 10% of the energy was effective. The grana were treated for 1 minute under nitrogen. During operation the vibration cup was cooled with tap water. Our thanks are due to Dr J. A. NIEMEIJER, who kindly placed the oscillator of the Laboratory for Physiological Chemistry, Utrecht, at our disposal.

Throughout the preparation the suspensions were kept at low temperature as much as possible. Occasionally short-lasting storage occurred in a refrigerator. Care was taken to keep the suspensions

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in the dark. The preparation was performed in weak green light. Before starting the experiments the suspension was stirred vigorously for 5 minutes at least. The experiments were performed at room temperature.

Quantitative estimation of the MOLISCH *reaction*. Samples for illumination were drawn from the suspension. In each experiment control aliquots were kept in the dark under otherwise equal conditions.

Though determinations of the amount of metallic silver with the aid of the "dead stop end-point" titration, modified after DELAHAYE[19], were unsuccessful, they may be briefly mentioned here since they reveal a feature of the MOLISCH reaction. After addition of silver nitrate solutions of different concentrations at various pH, the grana suspension was illuminated. Then it was centrifuged. The silver content of the supernatant as well as of the sediment, washed with distilled water and destroyed with nitric acid was determined by titration. In both illuminated and dark suspensions, even in highly blackened ones, equal amounts of silver were found to occur in the sediment. This result, which was repeatedly obtained, seems to indicate that part of the silver nitrate is strongly adsorbed by the organic matter; it is not noticeably removed by two washings. Moreover, since the titration in question is rather sensitive this phenomenon may suggest that the metallic silver merely is produced by reduction of primarily adsorbed silver nitrate.

For the quantitative determination of the effect of the MOLISCH reaction the decreased light transmission of the suspension, owing to blackening by metallic silver, was measured photometrically. 10 ml of the grana suspension were pipetted into a plane-parallel 5.5 mm glass cuvette. The cuvette was mounted in the photometer and a vibrating stirrer was adjusted in order to prevent sedimentation of the suspended particles. Then 1 ml of a 10% silver nitrate solution in glass-distilled water was added carefully. Initially the light transmission, determined in weak green light, decreased owing to the formation of nearly white insoluble silver salts by reactions with some anions, adhering to the organic matter or diffusing from it. After a few minutes the light transmission reached a steady state. This value was called 100%. Next the cuvette was illuminated and the light transmission was redetermined. Except for the illumination period, a sample, serving as a control, was treated in the same way. With time the light absorption of the controls proved to increase to a small amount only: the highest value obtained was 13% for 4 hours.

The photometric equipment allowed the projection of a parallel green beam through the suspension cuvette on to a selenium barrier layer cell connected to a Zernike D mirror galvanometer. A 12 V 100 w incandescent lamp was used as a light source. A glass filter combination of Schott BG 17 and VG 9 was placed in front of it. The readings were executed as quickly as possible. Check experiments pointed out that, in this way, no reaction due to this procedure resulted.

Illumination equipment. The continuously stirred suspensions were illuminated either by sodium light or by light of various wavelengths obtained from a 30 V 900 w Philips E 40 projection lamp with the aid of combinations of a Christiansen-Weichert dispersion monochromator and Schott glass filters. Measurements of incident light intensity were carried out with a standardized thermopile.

Electron microscopy. Electron microscopic preparations of both grana and lamellae were made as previously[20] described. Mounting and washing occurred in weak green light. Full light was admitted only after thoroughly drying of the preparations. They were examined in a Philips electron microscope.

RESULTS

Demonstration of light-induced silver nitrate reduction in grana suspensions

Time factor. As an example out of three experiments Fig. 1 shows an evident increase of absorption, owing to the formation of metallic silver, with time at an illuminated *Hibiscus* grana suspension. Apparently the rate of increase declines with time. This phenomenon may be due to three causes. First, the amount of black metallic silver increases with time. Thus, within the suspension an increasing part of the light is both screened of and absorbed. Consequently, the intensity of the effective light is decreased. Secondly, the available quantity of silver nitrate may become exhausted. Titration experiments showed that the amount of dissolved silver nitrate is not noticeably decreased. However, as suggested above, if merely adsorbed salt is reduced, exhaustion phenomena may enter the picture. Thirdly, the activity of the grana suspension may decrease. This was checked by drawing samples from the same suspension at various intervals. These samples were kept at room temperature and their absorption increase per unit of time—mostly 30 minutes—was measured at equal light intensity. Only

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a very slight reduction of the reaction was observed. As a mean of 6 experiments, this value amounted to 0.8 ± 0.4% per hour. The question remains, whether the activity of the suspension is decreased in light. However, because of the continuous change of

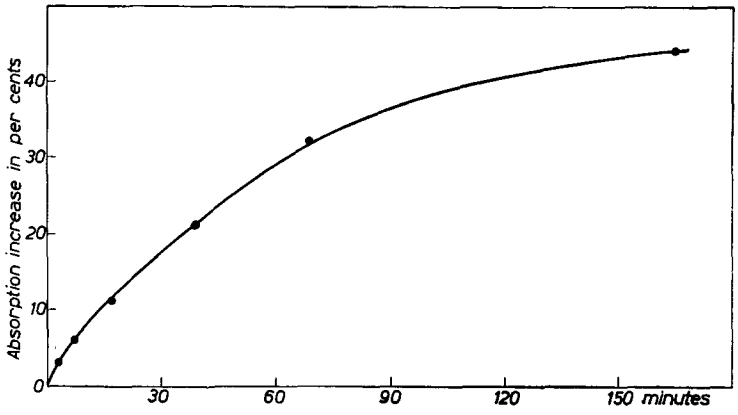

Fig. 1. Time course of silver nitrate reduction, measured as absorption increase of a *Hibiscus* grana suspension in sodium light. Incident intensity: $4.4 \cdot 10^4$ ergs cm^{-2} sec^{-1}. pH: 6.6.

light condition within the suspension, owing to the formation of black silver, this problem can hardly be solved.

One may ask whether the reaction, once started, proceeds autocatalytically, the primary silver precipitates functioning as condensation nuclei. This question may be answered by interrupting the exposure to light of a reacting suspension. Such an experiment is shown in Fig. 2. Evidently no after-effect occurs. Moreover two experiments were carried out, in which the absorption increase of a suspension, pre-illuminated for

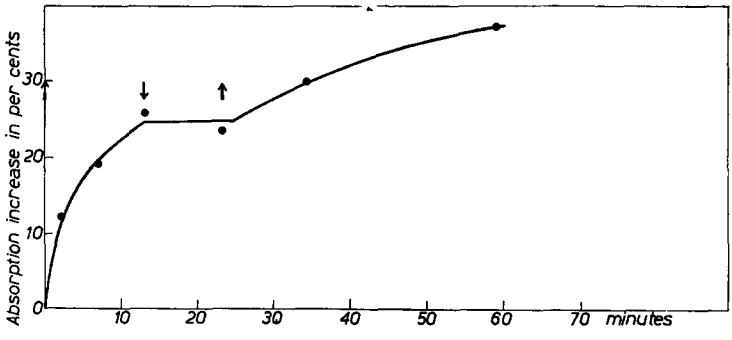

Fig. 2. Effect of darkening, indicated by the arrows, on the silver nitrate reduction, measured as absorption increase, of a *Hibiscus* grana suspension. Incident sodium light intensity: $4.4 \cdot 10^4$ ergs cm^{-2} sec^{-1}. pH: 6.6.

5 minutes, was compared with that of a control sample. The experiments lasted 75 and 230 minutes, respectively. The corresponding absorption increase values of the controls were 2 and 13%; those of both pre-illuminated suspensions amounted to 9%. Thus, in these prolonged experiments no after-effect of illumination was observed.

pH sensitivity of the MOLISCH *reaction.* According to METZNER[18], chloroplasts within

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the cell show this reaction optimally at pH 3–4 of the surrounding solution. In a suspension, however, the conditions are markedly different, and, with them, the optimal pH may be shifted. Table I demonstrates that the pH dependency is changed considerably indeed. Since in the alkaline pH range spontaneous blackening is apt to occur, acid suspensions were used only.

TABLE I

INFLUENCE OF pH ON THE MOLISCH REACTION RATE

Incident sodium light intensity $4.4 \cdot 10^4$ ergs cm^{-2} sec^{-1}.

Exp.	*Duration of illumination in minutes*	*Absorption increase in per cent of control at pH*		
		4.6	*5.6*	*6.6*
1	75	3	11	19
2	230	23	25	44

Light factor. The relation between MOLISCH reaction and incident light intensity is presented in Fig. 3. Evidently no light saturation is reached at intensities about 4 times higher than those at which it occurs in photosynthesis of green cells in suspensions of comparable density. So it is rendered doubtful whether enzymes are involved in the MOLISCH reaction. METZNER[18] stated that this reaction proceeds in cells that were killed by pretreatment with silver nitrate. However, though it seems highly improbable, some enzymes might be left unaffected by this reagent. For this reason some experiments were performed with suspensions "killed" by heating them in a boiling water bath. After cooling down to room temperature the silver nitrate was added. The results are given in Table II.

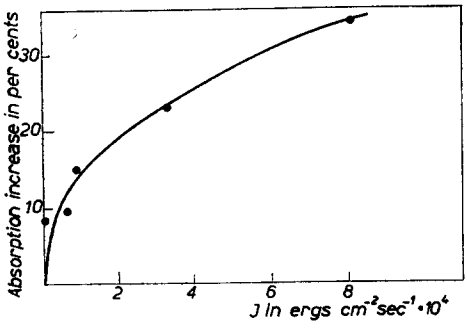

Fig. 3. Relation between light intensity and rate of silver nitrate reduction, measured as absorption increase per 25 minutes of a *Hibiscus* grana suspension in sodiumlight. pH: 6.6.

TABLE II

MOLISCH REACTION OF GRANA SUSPENSIONS PRE-HEATED IN A BOILING WATER BATH. pH: 6.5. ILLUMINATION WITH SODIUM LIGHT

Grana suspension	*Heating period in minutes*	*Illumination period in minutes*	*Incident light intensity in ergs cm^{-2} sec^{-1}*	*Absorption increase of illuminated suspensions in %*	*Absorption increase of dark control in %*
a	—	25	$8.12 \cdot 10^4$	34	—
a	10	25	$8.12 \cdot 10^4$	36	3
b	15	30	$9.35 \cdot 10^3$	19	8
b	20	30	$9.35 \cdot 10^3$	20	8
b*	60	30	$9.35 \cdot 10^3$	11	—

* At the end of the heating period this suspension showed a brownish-green colour.

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The values, obtained in the "a" series show that the MOLISCH reaction is not influenced by a 10-minute heating period. In the "b" series, at lower incident intensity, an evident light reaction of the samples heated for 15 and 20 minutes is shown. These data seem to rule out the occurrence of any enzymic character of the studied reaction. After a heat treatment of one hour the rate of this reaction is decreased. The colour of this sample had turned from fresh green to brownish-green. This phenomenon may suggest that partly destroying the chlorophyll results in a reduction of the MOLISCH reaction rate. This would mean that excited chlorophyll itself is the reducing agent in this reaction.

Some additional data on the mechanism of the MOLISCH *reaction.* The idea that excited chlorophyll may be responsible for the reduction of the silver nitrate was already tested by GAUTHERET[21]. This author prepared active alcoholic chlorophyll extracts from spinach leaves. However, after washing out the chlorophyll with petrol ether the alcoholic phase proved to have retained its activity. GAUTHERET concluded that the reducing power responsible for the MOLISCH reaction, instead of being due to the chlorophyll, depends on the presence of a substance soluble in ethanol, but insoluble in petrol ether. In this connection a few additional experiments will be briefly reported. However, though they may yield some suggestive information, they are not essential for the present study.

A colloidal solution of chromatographically pure chlorophyll, obtained from Sandoz, Basel, was prepared by dissolving 1 mg of this pigment in 2 ml acetone. Under rapid stirring this solution was added to 20 ml of a 0.1 *M* sucrose containing 0.1 *M* buffer solution of pH 6.5. In this way a —rather unstable—hydrophobic sol was obtained. Then 1 ml of a 10% silver nitrate solution was added. The light absorption of the mixture was determined. Next a sample was kept in the dark, whilst an aliquot was exposed to sodium light—incident intensity: $3.26 \cdot 10^4$ ergs cm^{-2} sec^{-1}—for 20 minutes. After this period the light absorption was determined again. The absorption increase of control and illuminated suspension amounted to 1 and 13% respectively. So this experiment indicates that the MOLISCH reaction directly depends on the reducing power of excited chlorophyll. Two attempts to reproduce this result, however, were unsuccessful; the colloidal chlorophyll solutions at once showed a flocculation at the addition of the silver nitrate. These flocculated suspensions did not show any light reaction. This difference in behaviour is not clear since preparation as well as experimental conditions did not noticeably deviate from those in the first experiment. It may be advantageous to stabilise the chlorophyll sol with the aid of a protective colloid. Since, as already remarked, this study does not aim at an investigation of the mechanism of the MOLISCH reaction, these experiments were not extended any further.

Finally, a single experiment with a chlorophyll-free alga, *Prototheca Zopfii*, may also be mentioned. After addition of silver nitrate up to a final concentration of 1% an algal suspension of 2 g fresh weight in 100 ml acetate buffer of pH 6.5 was illuminated with sodium light of $9.35 \cdot 10^3$ ergs cm^{-2} sec^{-1} for 30 minutes. After this period the absorption was increased by 6%, whilst the corresponding value at a dark control sample amounted to 4%. So these figures may suggest that the MOLISCH reaction does not proceed in the absence of chlorophyll.

The results mentioned are contrary to those of GAUTHERET. This divergency can be explained by assuming that GAUTHERET may have obtained "inactive" chlorophyll suspensions, just like the "inactive" chlorophyll preparations described above. The activity of GAUTHERET's chlorophyll-free alcoholic leaf extract may be due to some reducing substance soluble in ethanol. In this respect it may be mentioned that, in literature, ascorbic acid is often considered to be responsible for the reducing power needed in the MOLISCH reaction. METZNER[22] remarks that, at the present, it cannot yet be excluded that the MOLISCH reaction in illuminated chloroplasts may proceed without the intermediary of ascorbic acid. This statement seems to be confirmed by the above results. However, it may be remarked explicitly that for further confirmation additional experiments are needed.

Summarising the main results it can be stated that grana suspensions are able to show the MOLISCH reaction. Light is an essential factor. The reaction is pH-sensitive. It is non-enzymic.

Action spectrum of the MOLISCH *reaction.* Direct proof of the dependency of this reaction in grana suspensions on the presence of chlorophyll may be obtained by determining an action spectrum. This was done by measuring the reaction rate at various wavelengths and computing it in per cents of this rate at sodium light. The resulting action spectrum is shown in Fig. 4. Since the selectivity of the Christiansen-Weichert

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filter in the long wavelengths region of the spectrum is relatively poor, this monochromator was used in combination with Schott glass filters as indicated in the legends. For this reason the action spectrum is drawn in three parts. Especially at 650 mμ the values diverge. This may be expected to occur since, when determining the relative efficiency without the RG filters, some highly active 680 mμ light is also transmitted. Because of the very low light intensity at wavelengths below 490 mμ the results in this spectral region are rather inaccurate. Nevertheless they are represented too, the divergency clearly demonstrating their unreliability.

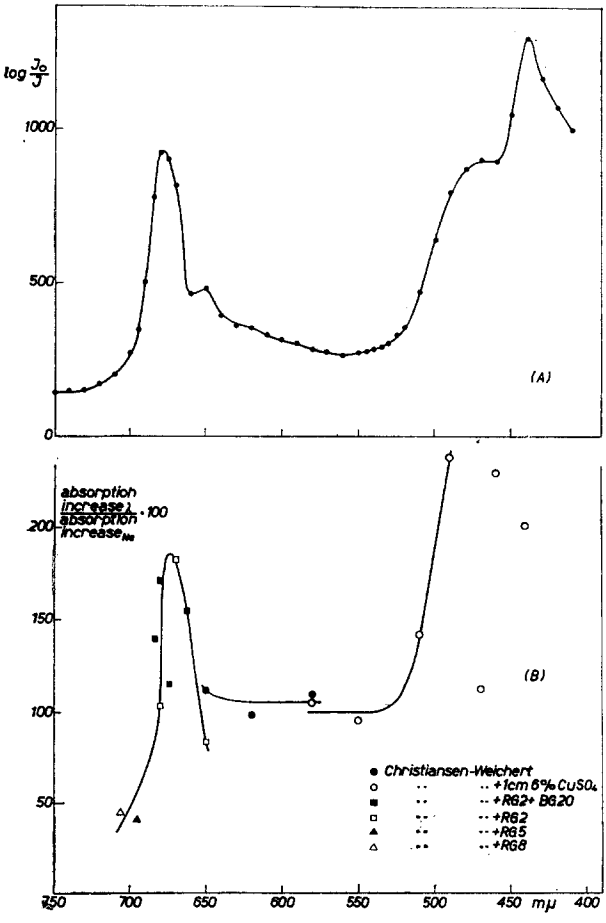

Fig. 4. Action spectrum of the MOLISCH reaction as well as absorption spectrum established at *Hibiscus* grana suspensions.

Despite these inconveniences it may be evident that the MOLISCH reaction action spectrum closely resembles the absorption spectrum of a grana suspension. For the establishing of the latter spectrum our thanks are due to Miss L. J. BARTELS. The result proves that the MOLISCH reaction depends on the presence of chlorophyll. It may be remarked in addition that the accuracy of the determinations does not allow to draw any conclusions concerning a possible carotenoid activity in this reaction.

Localisation of the MOLISCH *reaction*

Since it is shown that, under the chosen experimental conditions, silver nitrate reduction is coupled with the presence of excited chlorophyll, the MOLISCH reaction may be used as a tool in localising these pigment molecules under the electron microscope. The precipitated metallic silver causes a strong electron scattering; in this way it is easily recognised. Since it is necessary to remove buffer salts as well as excessive silver nitrate from preparations of illuminated suspensions, washing is required. Though this washing with glass-distilled water was carried out very cautiously, it could not be prevented that part of the silver deposits were ruptured. These broken fragments were always observed in the neighbourhood of silver covered chloroplasts. Apart from the fact that these strongly electron scattering layers were absent in non-illuminated suspensions it was proved that they consisted of silver by establishing electron diffraction diagrams. Fig. 5 shows that the pattern of silver occurs in the diagrams of "blackened" grana.

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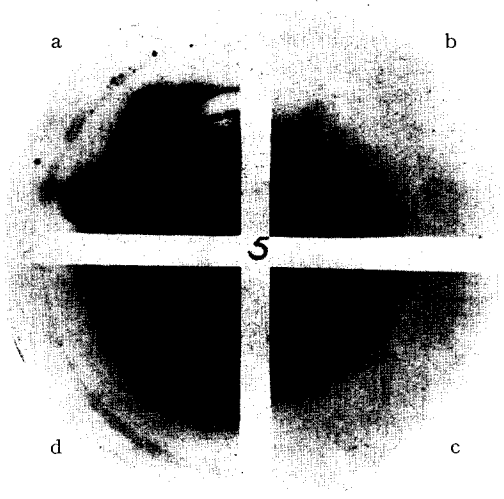

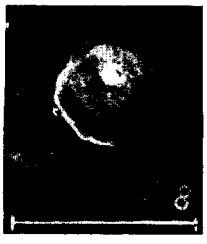

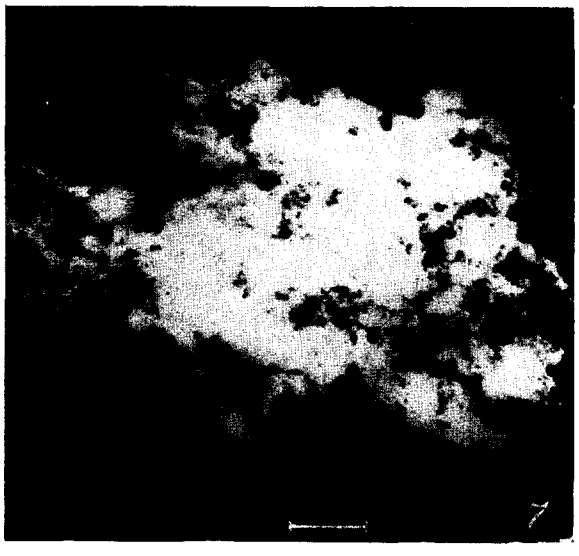

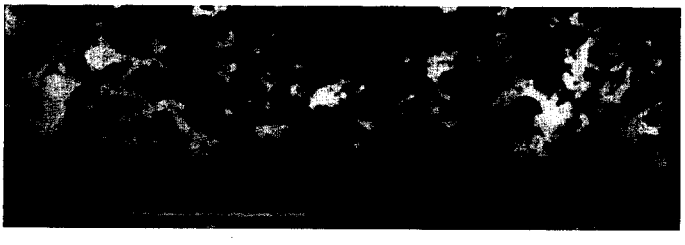

Fig. 5. Electron diffraction diagrams of a: silver-coated granum, b: control granum, c: blank collodion film, d: silver precipitate. Fig. 6. *Hibiscus* granum. Fig. 7. Silver deposits on *Hibiscus* chloroplast. Advanced MOLISCH reaction. Fig. 8. Initial stage of MOLISCH reaction at a *Hibiscus* granum. Fig. 9. Advanced MOLISCH reaction at a *Hibiscus* granum. Fig. 10. Silver protrusions from heavily coated *Hibiscus* grana. Partly shadowed. Fig. 11. Silver protrusions from *Hibiscus* grana and lamellae.

By way of comparison Fig. 6 shows an untreated granum. Fig. 7 represents a chloroplast fragment densely coated with silver as a result of the MOLISCH reaction. Still, the shape of the precipitate partly indicates contours of grana. In order to draw more detailed conclusions it is required to interrupt the silver nitrate reduction at an earlier stage. This was done by illuminating the suspensions at a distance of 15 cm from a 60 w incandescent lamp, with a ground glass plate adjusted in front of it, for 10 minutes.

Fig. 8 shows an initial stage of the reaction at a granum. As is demonstrated by the enhanced contrast the silver is deposited in a homogeneous layer. The granum is

Fig. 12. Supersonically disintegrated *Hibiscus* granum. Heavily silver-coated lamellae, arranged in a fan-like way.

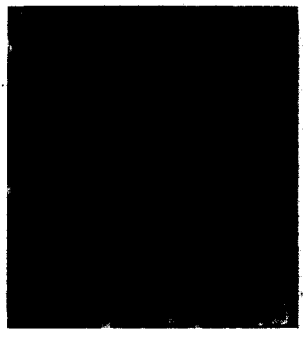

Fig. 13. Initial stage of the MOLISCH reaction at a single *Hibiscus* lamella.

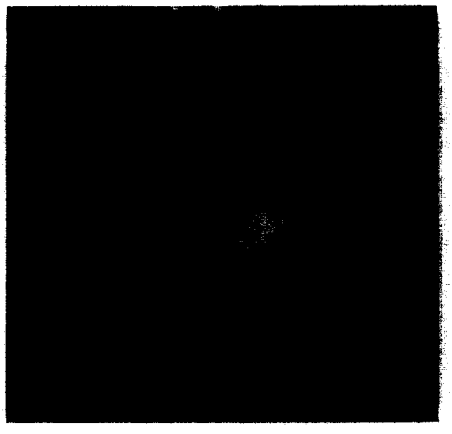

Fig. 14. Advanced MOLISCH reaction at a single *Hibiscus* lamella.

damaged; lipids are protruding in myelin figures. Since the latter do not show increase of electron scattering, it seems probable that silver deposits are restricted to the proteinaceous parts of the granum.

A more advanced reaction is represented in Fig. 9. Apart from the homogeneous coating, typical silver protrusions are shown. In Fig. 10 such-like structures are more pronounced. It may be noted that, in this figure, the grana are situated at a boundary between a shadowed and a non-shadowed region. In this way it is possible to get an impression of the high electron scattering power of the silver coating of a single granum as well as of its three-dimensional shape.

Possibly the above description may suggest that, at first, the silver is deposited homogeneously and, next, the protrusions arise. This, however, is not true. At initial

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stages of the MOLISCH reaction either homogeneous blackening or a few protrusions are observed. We did not succeed in influencing the formation of one of these types deliberately. Various authors—*cf.* METZNER[18]—noticed the irregularity of silver precipitates in plant cells too. In advanced reactions protrusions were always observed.

With the aid of supersonic treatment of grana, lamellae suspensions were prepared and provided with the MOLISCH reagent. Fig. 11 represents a preparation of such an illuminated suspension. Judging from the shadow length, and apart from three thickly coated grana, four lamellae are shown. Apparently the latter are partly covered with silver precipitates. At prolonged silver nitrate reduction it is often difficult to recognise the lamellae. Sometimes, however, identification remains possible even then. This may be demonstrated by Fig. 12. Weak supersonic treatment often causes a granum to disintegrate into a fan-like cluster of lamellae. Such a cluster, heavily coated with silver, is shown here. The contours of the lamellae are slightly visible. Figs. 13 and 14 clearly demonstrate the occurrence of silver deposits on single lamellae.

In conclusion it may be stated that lamellae are able to bring about the MOLISCH reaction.

DISCUSSION

In a preceding paper[23] a correlation between activity in the HILL reaction and size of grana fragments, prepared by supersonic disintegration, was shown to occur. These experiments suggested that the photochemically active fragments originated from lamellae. Consequently, it seemed most likely that chlorophyll is concentrated in these structures.

The present investigation confirms this deduction in a more direct way. The MOLISCH reaction is shown to depend on the presence of excited chlorophyll. Since this reaction is demonstrated to occur in lamellae, it seems justified to conclude that lamellae are the bearers of chlorophyll.

A few remarks may be made concerning the MOLISCH reaction as it is observed in the above experiments. METZNER[18] stated that silver nitrate reduction by chloroplasts in "intact" cells occurred within 35 seconds in monochromatic light, the intensity of which amounted to, probably, about 100 times that used in the present investigation. The considerable divergence in both exposure time and light intensity may be understood by assuming that, owing to the presence of reducing substances—such as ascorbic acid—within the cell, this reaction with chloroplasts *in situ* proceeds more rapidly than that with isolated grana. In fact, a few additional experiments showed that the MOLISCH reaction in grana suspensions could be considerably increased by adding ascorbic acid. This seems to indicate that excited chlorophyll may act either as a catalyst of a reaction between silver nitrate and a reducing agent, or as the reducing substance itself. Since, however, the present investigation does not aim at an elucidation of the mechanism of the MOLISCH reaction, the validity of this suggestion was examined no further.

SUMMARY

Silver nitrate reduction was shown to occur in illuminated suspensions of *Hibiscus* grana.

The action spectrum of this reduction, the MOLISCH reaction, proved to coincide satisfactorily with the chlorophyll absorption spectrum.

Electron micrographs reveal that this reaction occurs in single lamellae. From this it is concluded that lamellae are the bearers of chlorophyll.

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RÉSUMÉ

Il est montré que dans des suspensions de grana illuminées de *Hibiscus* la nitrate d'argent est réduite.

Le spectre d'action de cette réduction, la réaction de MOLISCH, ressemble le spectre d'absorption chlorophyllienne.

La microscope électronique démontre, que des lamelles isolées sont capables d'effectuer cette réaction. La conclusion semble justifiée que les lamelles contiennent la chlorophylle.

ZUSAMMENFASSUNG

Es wurde gezeigt, dass beleuchtete Suspensionen von *Hibiscus*-granen im Stande sind Silbernitrat zu reduzieren.

Das Wirkungsspektrum dieser Reduktion, die MOLISCH Reaktion, ist dem Absorptionsspektrum des Chlorophylls ähnlich.

Beobachtungen mittels des Elektronenmikroskops zeigten, dass diese Reaktion von isolierten Lamellen veranlasst werden kann. Es wird hieraus geschlossen, dass die Lamelle Träger des Chlorophylls sind.

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