ciated with the iron of the heme groups or, as suggested by Boeri and Bonnischen for the oxidation of thiol group (*2*), may be a new property of the catalase molecule unrelated to the catalytic properties heretofore ascribed to it. A few preliminary studies of the absorption curve of the protein moiety at the end of the reaction show a rather marked increase at 2800 A. As yet we have not been able to rule out an artifact as a possible cause of this increase.

It appears that sulfide may inhibit catalase in two ways. First, it may form an inactive compound with the primary catalase-peroxide complex in a manner similar to that postulated for monovalent anions (*1, 7*). Second, it may react directly with either the protein moiety or with the heme. Both processes are reversible to a degree depending on the experimental conditions.

References and Notes

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The Role of Magnesium in Photosynthesis

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The obvious connection between the presence of an Fe atom and the oxidation-reduction properties of certain biologically important porphyrin compounds, such as the heme pigments, catalase, and the cytochromes, has long been recognized, whereas, in the important phenomenon of photosynthesis, which also is an oxidation-reduction process, the presence of the Mg atom and its role in the reaction has been overlooked, if not studiously ignored.

From the simple fact that the Mg-containing molecules of chlorophyll *a* and *b* are effective in photosynthesis, whereas the Mg-free pheophytins are without effect, it is apparent that the Mg atom plays an important, and indeed, an indispensable role in the process. It might be thought that the presence of Mg merely endows the chlorophyll molecule with the particular absorption spectrum which is the peculiar requisite for the energy requirements of photosynthesis, and that other than this, the Mg atom takes no direct part in the reaction. That such is not the case may be seen from the fact that pheophytin has an absorption spectrum quite similar in many respects to that of the chlorophylls, and yet is inert in photosynthesis (*1*). It may be concluded that the Mg atom is inexorably bound up with the entire phenomenon, as well as with the chlorophyll molecule.

According to the photoelectric theory of photosynthesis (*2*), in which it is postulated that light-activated chlorophyll transfers electrons to an oxidant (presumably, the disulfide group of pyruvic oxidase (*3*), which will be abbreviated $\overline{\text{S—R—S}}$) and abstracts electrons from water, it is believed that the chlorophyll might be converted intermediately to a positively charged ionic species, representing an oxidized state. Direct evidence in support of this thesis will now be presented; and in addition, it will be shown that the specific micro-site of the positive charge is at the Mg atom.

In the ground state the chlorophyll molecule contains Mg covalently linked to any two of the four pyrrole nitrogen atoms. From the standpoint of resonance the Mg atom may be thought of as linked simultaneously by one-half a covalent bond to each of the four N atoms. Extreme forms in which the Mg and N atoms have, respectively, one or more positive and negative formal charges and vice-versa, also contribute, undoubtedly, to the resonance hybrid; but on the whole the Mg atom may be considered neutral. It is here postulated that in photosynthesis it is this essentially neutral Mg atom which absorbs the photon with resultant activation of one of its 3*s* electrons to a higher energy state:

$$\text{Chl–Mg} + h\nu \rightarrow \text{Chl–Mg}^{*} \qquad (1a)$$

Subsequent loss of the excited electron to the weak oxidant results in formation of oxidized chlorophyll, characterized by unipositive Mg:

$$\text{Chl–Mg}^{*} + \overline{\text{S—R—S}} \rightarrow \text{Chl–Mg}^{+} + \cdot\,\text{S—R—S}:^{-} \qquad (1b)$$

Alternatively, it would appear that absorption of a quantum of sufficiently high energy could result in the immediate ejection of a photoelectron and its direct capture by the oxidant:

$$\text{Chl–Mg} + h\nu \rightarrow \text{Chl–Mg}^{+} + e^{-}, \qquad (2a)$$

$$\overline{\text{S—R—S}} + e^{-} \rightarrow \cdot\,\text{S—R—S}:^{-} \qquad (2b)$$

In either case (Eqs. 1 and 2), positively ionized chlorophyll should result, and it may be supposed that during active photosynthesis in ordinary sunlight, the bombardment of chlorophyll by photons is of such intensity as to produce and maintain a considerable concentration of the unipositive oxidized form. If an oxidized chlorophyll molecule should fail to recapture an electron from a water molecule before being struck by a second photon, its remaining 3*s* electron may, presumably, absorb the energy of the incident photon and be excited to a higher quantum level, and then be removed by the oxidant (*4*). Such a sequence may be represented by

$$\text{Chl–Mg}^{+} + h\nu \rightarrow \text{Chl–Mg}^{+*}, \qquad (3a)$$

$$\text{Chl–Mg}^{+*} + \cdot\,\text{S—R—S}:^{-} \rightarrow \text{Chl–Mg}^{++} + :\text{-S—R—S}:^{-} \qquad (3b)$$

which would be followed immediately by

$$\text{Chl–Mg}^{++} + \text{H}_2\text{O} \rightarrow \text{Chl–Mg} + 2\text{H}^{+} + \tfrac{1}{2}\,\text{O}_2. \qquad (3c)$$

Only extremely small amounts of the dipositive oxi-

Table 1. Comparison of visible absorption bands* of ether solutions of chlorophyll with emission lines of Mg^0 and Mg^+. Wavelengths are in millicrons. Principal bands of the chlorophylls are in italics.

Mg^0	Mg^+	Chl *a*	Chl *b*	Chl *c*
	655	*660*	655†‡	
	635	613	*642*	*635*§
553		574	594 567 553 540	
517		530 511		
501		498	501	
457		464	457	
	448		*452*	*450*
	439	440‡		
435		*430*	435‡ 428	
406		409		

* Complete bibliography available in Rabinowitch (*6*), pp. 606, 668, 736, 801, 826.
† Fluorescence value.
‡ *In vivo.*
§ Value for both absorption and fluorescence.

dized chlorophyll could be expected from a process such as the one represented by Eqs. 3, and any further electronic excitation of this species would be improbable kinetically because of its low concentration, and quite impossible energetically because of the unavailability of sufficiently short wavelengths in white light to effect activation of the $2p$ or $2s$ electrons of Mg^{++}.

In view of the foregoing considerations one might expect, to some extent at least, a correspondence of certain of the absorption and fluorescence bands of the chlorophylls with the emission spectra of electronically excited states of the Mg^0 atom (according to Eq. 1*a*), and of the Mg^+ ion (according to Eq. 3*a*), but not of the Mg^{++} ion.

In Table 1 are presented, for purposes of comparison, all of the usually observed lines of the visible emission spectra (400 to 700 mμ) of Mg^0 and Mg^+ (*5*) together with all of the absorption maxima of chlorophylls *a*, *b* and *c* in the visible range (*6*).

It is immediately apparent that there is almost a complete line-for-line correspondence of the chlorophylls with magnesium. In view of the many factors which could be expected, in as complicated a structure as chlorophyll, to distort and mask the spectrum due to magnesium, it can hardly be denied that the agreement is little short of remarkable. That the correspondence is not just fortuitous can be ascertained from the fact that for other singly ionized metallic atoms closely related to Mg^+ there is an almost universal noncorrespondence with the chlorophyll lines. For example, among the other rare earth elements and other third period metals, Na^+ and Sr^+ exhibit a complete absence of lines in the visible range; Ca^+ has only two lines (at 422 and 408 mμ); Ba^+ has 18 (with none above 598 mμ); and Al^+ has some 24 (from 624 to 400 mμ), few of which correspond to those of chlorophyll. A further correlation is obtained if the spectral range under consideration be extended to the ultraviolet region. Table 2 gives all of the known ultraviolet absorption peaks (*6*) of chlorophylls *a* and *b*, and the corresponding emission lines (*5*) of Mg^0 and Mg^+.

Table 2. Comparison of ultraviolet absorption bands of chlorophyll and magnesium. Wavelengths in millimicrons.

Mg^0	Mg^+	Chl *a*	Chl *b*
384	385	375	
334			335
323		325	
309	310		310
	245	250	250

Additional evidence that the Mg atom is directly involved in the primary photochemical process is the fact that all Mg-containing porphyrins and phthalocyanins can be oxidized much more readily than is the case when Mg is absent from the molecule (*7*).

Even more conclusive is the fact that "photochemically oxidized" chlorophyll has an absorption spectrum identical with that of pheophytin (*7*). This shows unequivocally that it is the Mg atom, and it alone, which is oxidized in this process. It seems evident from the foregoing facts that the site of photochemical reaction is at the Mg atom of chlorophyll, and it would appear that excited electronic states of both Mg^0 and Mg^+ (*8*) are involved (the lowest excited state of Mg^{++} is far in the ultraviolet, and as previously explained, would not be expected to appear). The rather startling conclusion may be reached that the Mg atom in chlorophyll is bound in such a manner as to be nearly equivalent to the free atom in the elemental state, at least insofar as its electronic excitation energies are concerned.

The expulsion of a photoelectron by Chl–Mg (Eq. 2*a*) apparently would require energy equivalent to the first ionization potential of Mg, which is 7.61 v. This corresponds in energy to an incident photon of wavelength 162 mμ, which is far in the ultraviolet and hence not available ordinarily for photosynthesis. Thus, the process represented by Eq. 2 can be ruled out, leaving us with schemes 1 and 3. It is important to note that five of the six principal lines of the visible spectra of the three chlorophylls (Table 1) correspond to the Mg^+ ion. This fact at once suggests that electronic excitation of Chl-Mg^+ (Eq. 3*a*) is a process of considerable importance in photosynthesis. From the spectroscopic data available, then, we may surmise that Eqs. 1, followed by Eqs. 3, comprise the main sequence of reactions taking place.

An interesting fact which remains to be explained is the similarity of the absorption spectrum of Mg-free pheophytin to that of the chlorophylls. We may speculate that the pheophytin molecule was evolved (*9, 10*) in such a fashion as to specifically accommodate, and simulate as closely as possible, the electronic excitation characteristics of the Mg atom. In this way,

the entire molecule is in "energy resonance" with the Mg atom (that is, in resonance with nearly the same wavelengths), thus effectively increasing many fold the cross-sectional area for absorption of a photon. A rapid shift of electrons to or from the Mg atom is greatly facilitated by the many resonance forms (in the ordinary chemical sense) of which the conjugated bond system is capable, and it may be supposed that these factors, too, are important in photosynthesis.

References and Notes

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Desoxyribose Nucleic Acid in the Symbiotic Microorganisms of the Cockroach, *Blattella germanica*

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The existence of intracellular microorganisms in insects has recently been questioned on the basis of negative staining results with the Feulgen reaction and acid-Giemsa technique (*1, 2*). A further objection raised by Lanham is the failure of many investigators to cultivate the symbiotes *in vitro*. Peklo (*3*) and Trager (*4*) have responded to the latter objection, reviewing cases of successful cultivation as well as experimental evidence favoring the reality of these intracellular particles as microorganisms. The occurrence of symbiotes in cockroaches is well known (*5*). Glaser (*6, 7*) claimed isolation of bacterial symbiotes from *Periplaneta americana* and *Blattella germanica* and named them *Corynebacterium periplanetae* and *C. blattellae*, respectively. Gier (*8, 9*), who investigated the distribution of these symbiotes during the life-cycle of the cockroach, reported failure of cultivation *in vitro*. More recently Keller (*10*) has cultured symbiotes of *Periplaneta orientalis*.

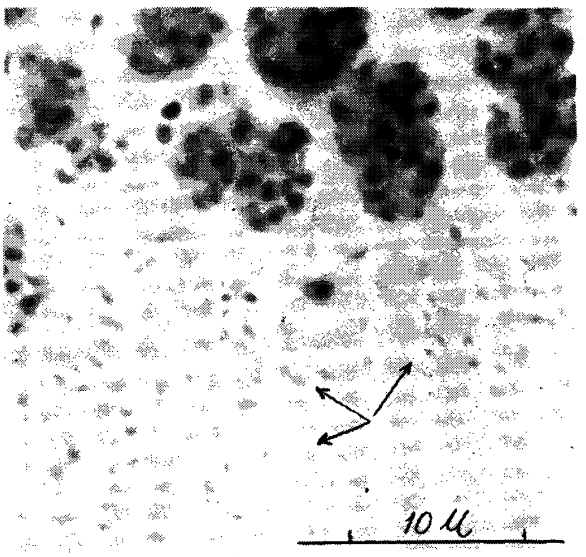

Fig. 1. Photomicrograph of a tangential section of a developing oocyte showing the Feulgen-stained symbiotes and the nuclei of the follicle cells.

The Feulgen reaction is specific for desoxyribose nucleic acid (DNA) and has been used extensively for the cytochemical demonstration of DNA in plant and animal nuclei. The intracellular symbiotes of cockroaches are Feulgen-positive; therefore, the conclusion of Lanham that symbiotes lack DNA (based on a study of microorganisms of aphids) cannot be extended to other cases.

The Feulgen-positive symbiotes in the mycetocytes and ovaries of cockroaches (two species) were first observed 4 yr ago. The fixative used at that time was Sanfelice. To reexamine this problem, ovaries and fat-bodies of adults and various ages of embryos of *B. germanica* were fixed in Carnoy (3 absolute alcohol: 1 acetic acid) and paraffin sections were cut at 4 to 6 μ. Feulgen stain was prepared and used according to Stowell's method (*11*). A preliminary hydrolysis of sections in 1*N* HCl at 60°C for 1 to 24 min revealed optimal staining of the microorganisms with the Feulgen reagent at 8 to 10 min (Fig. 1); however, both the follicle cells of the developing oocytes and the symbiotes remain unstained in unhydrolyzed control sections. The microorganisms in the mycetocytes and at the periphery of the oocytes are rod-shaped (approximately 1 by 3 μ), as described by Glaser and Gier. The banded appearance of the microorganisms as reported by these authors has been observed in Feulgen-stained material, but these bands are visible in unstained preparations as well. It is difficult to localize the Feulgen-positive material to any particular region of these rods.

For further confirmation of the specificity of the Feulgen reaction for DNA in the microorganisms, sections of embryos and ovaries were treated with des-

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