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*.

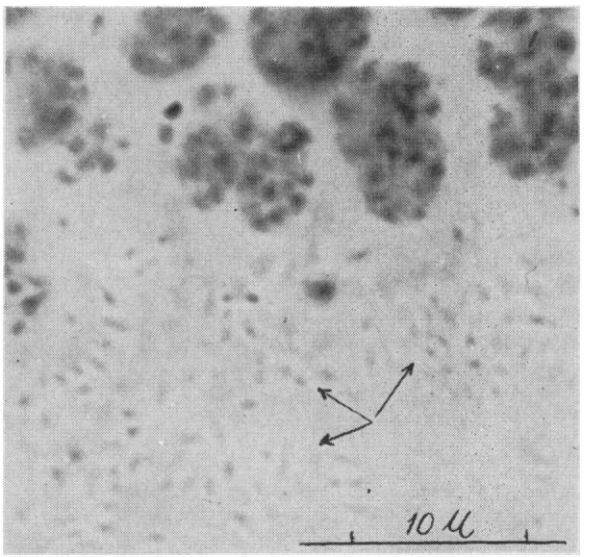

Fig. 1. Photomicrograph of a tangential section of a developing oocyte showing the Feulgen-stained symbiotes and the nuclei of the follicle cells.

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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Table 1. Effect of various treatments on the Feulgen staining of symbiotes and nuclei of follicle cells.*

1st treatment	2nd treatment	Feulgen reaction	
		Follicle nuclei	Symbiotes
None	DNAse	−	+
	DNAse control	+	+
1*N* HCl at 60°C; 10 min	DNAse	−	+
	DNAse control	+	+
RNAse; 0.2 mg/ml; 56°C for 2 hr	DNAse	−	+
	DNAse control	+	+
	None	+	+
RNAse control	None	+	+
Saliva; 30 min; room temp.	DNAse	−	+
	DNAse control	+	+
2X crystalline pepsin; 2.0 mg/ml in 0.02*N* HCl; 37°C; 45 min	DNAse	−	−
	DNAse control	+	+
	None	+	+
Pepsin controls	None	+	+

* DNAse: 0.1 mg/ml in phosphate buffer *p*H 7.5 with gelatin and magnesium sulfate at room temperature for 45 min.
Controls: same conditions as respective enzyme solutions except absence of the enzyme.
DNAse and RNAse obtained from the Worthington Biochemical Laboratory, Freehold, N.J.; pepsin obtained from the Nutritional Biochemical Corp., Cleveland, Ohio.

oxyribonuclease (DNAse) (*12*). A concentration of 0.1 mg/ml of DNAse in phosphate buffer completely removed all Feulgen-positive material from the nuclei of the follicle cells and other cockroach tissues within 30 min, but the symbiotes remained Feulgen-positive even after 4 hr exposure to the enzyme. To eliminate the possibility of interfering substances staining with Feulgen, free aldehydes and acetals were blocked by hydroxylamine (*13*). The results indicate that the staining of the microorganisms is not due to either of these groups.

Perhaps the failure of DNAse to remove the Feulgen-positive material is a result of the inaccessibility of the DNA which is protected or masked in some way by other cellular constituents. This possibility has been explored by pretreatment of sections with ribonuclease (RNAse) (*14*), acid hydrolysis, saliva and pepsin followed by DNAse. These experiments and the results are summarized in Table 1. The resistance of the DNA of the microorganisms to DNAse was unaffected by predigestion with RNAse, saliva, and acid hydrolysis. However, pretreatment of sections with 2.0 mg/ml of pepsin for 45 min at 37°C was sufficient to allow removal of the Feulgen-positive material from the microorganisms by DNAse. Sections in the DNAse control solution (that is, the same solution and conditions minus the enzyme) and sections in the pepsin control were hydrolyzed and stained simultaneously with the DNAse-treated slides. The nuclei of the follicle cells were considered the control for the activity of DNAse and the other enzymes and reagents.

In conclusion, the symbiotic microorganisms of the cockroach contain DNA as revealed by the Feulgen reaction. Unlike the nuclei of the cockroach tissues the DNA of the symbiotes in the oocytes and mycetocytes is resistant to digestion by DNAse without previous exposure to the proteolytic enzyme pepsin.

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A Fungus Disease in Clam and Oyster Larvae

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The improved method for growing lamellibranch larvae developed at the U.S. Fish and Wildlife Service laboratory, Milford, Conn., has offered many new possibilities and approaches for studying the behavior and physiological and ecological requirements of these organisms (*1, 2*). Using this method, the larvae of more than 15 species have been successfully cultured through metamorphosis at our laboratory. Of these, however, the larvae of the common clam, *Venus mercenaria,* and of the American oyster, *Crassostrea virginica,* have been most widely studied (*3, 4*).

Clam larvae are especially suitable for experimental work, and usually their cultures are carried through metamorphosis without experiencing undue difficulties. On several occasions, however, some cultures of an experimental series would show a heavy mortality unrelated to the experimental treatment. Recently, while examining such a culture of clam larvae, Davis noticed an organism, which was tentatively identified as a fungus, in many of the dying and dead larvae. Loosanoff and Davis later observed the same or a related fungus in larvae of *C. virginica, Venus mortoni,* and the hybrids of *V. mortoni* ♀ crossed with *V. mercenaria* ♂ and *V. mercenaria* ♀ *crossed* with *V. mortoni* ♂.

The presence of fungus in our larval cultures appears to be of endemic nature. Usually there are only