Chromatography of the digested complex on Sephadex indicated a decrease in the amount of high-molecular-weight, colored material.

The pigment-protein complex from *Gonyaulax* did not catalyze the photoreduction of ferricyanide or 2,6-dichlorophenol indophenol. No enhancement of photoreduction by spinach chloroplasts was observed in the presence of the complex.

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References and Notes

1. A. Stoll and E. Wiedemann, *Fortschr. Chem. Org. Naturstoffe* **1**, 159 (1938); E. L. Smith, *J. Gen. Physiol.* **24**, 565 (1941).
2. E. L. Smith and E. G. Pickels, *J. Gen. Physiol.* **24**, 753 (1941); M. B. Allen and J. C. Murchio, in *Photosynthetic Mechanisms in Green Plants*, B. Kok and A. T. Jagendorf, Eds. (Publication No. 1145, National Academy of Sciences–National Research Council, Washington, D.C., 1963), pp. 486–495; J. S. Kahn, *ibid.*, pp. 496–503.
3. V. C. Bode, thesis, University of Illinois (1961).
4. B. M. Sweeney and J. W. Hastings, *J. Cell. Comp. Physiol.* **49**, 115 (1957).
5. O. H. Andrews, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
6. J. J. Wolken, *Brookhaven Symp. Biol.* **11**, 87 (1958).
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Ethylene Formation in Rat Liver Microsomes

Abstract. *Reduced triphosphopyridine nucleotide and pyrophosphate-dependent peroxidation of lipids in rat liver microsomes were coupled to the generation of ethylene in the presence of cuprous ions. This system suggests a model for the biogenesis of ethylene in cells.*

The almost ubiquitous presence of endogenous ethylene in minute amounts in plant cells and the profound physiological effects of exogenous ethylene on plant tissue have raised the question of the origin of ethylene in plant metabolism (*1*). There is no known metabolic pathway to which the formation of ethylene can be assigned. Lieberman and Mapson (*2*) described a model system in which ethylene was produced during the cuprous-catalyzed degradation of peroxidized linolenic acid. This model suggests that the origin of ethylene may be associated with peroxidation of lipids in the plant cell. It also raises the possibility that ethylene may be formed by a similar process in animal cells.

In this report we describe experiments which demonstrate that ethylene formation can be coupled to the reduced triphosphopyridine nucleotide ($NADPH_2$) and pyrophosphate-dependent peroxidation of lipids in rat liver microsomes (*3*, *4*) with a cuprous-generating system.

Suspensions of rat liver microsomes incubated with $NADPH_2$ and adenosine triphosphate (ATP) produced lipid peroxides but no ethylene. However, addition of a cuprous-generating system (5 μmole of cupric ions and 50 μmole of ascorbate) resulted in formation of physiologically significant amounts of ethylene (0.1 to 0.5 nmole/hr per milligram of protein). The amount of ethylene formed was approximately the same in systems containing the cuprous-generating system from the start of the reaction and in those to which the cuprous-generating system was added after peroxidation was virtually complete. Rate of ethylene production in these systems and its relation to lipid peroxidation are shown in Fig. 1. The course of ethylene production is similar to malonaldehyde formation, which is a measure of lipid peroxidation. However, malonaldehyde is not the precursor of ethylene, since there is a much greater increase in the production of ethylene (about 45 percent) than of malonaldehyde (about 25 percent) following a further addition of ascorbate 2 hours after starting the reaction. We have also found that malonaldehyde is a very inefficient source of ethylene in the cuprous-catalyzed system when compared directly with peroxidated linolenate. An intermediate that appears earlier in the lipid peroxidation sequence must be the precursor of ethylene.

Suppression of lipid peroxidation by incorporating vitamin E into the reaction system sharply reduced ethylene production and malonaldehyde formation (Table 1). When vitamin E and the cuprous-generating system were added to the lipid peroxidation system after a 1-hour incubation, during which malonaldehyde accumulated, there was relatively little ethylene formed on further incubation for 1 hour. This indicates that vitamin E can inhibit the formation of ethylene not only by preventing peroxidation but also during the cuprous-catalyzed reaction. Since vitamin E acts as a free-radical terminator (*5*), the data suggest that a free-radical mechanism may be involved in the formation of ethylene in these systems.

Table 1. Effect of vitamin E on lipid peroxidation and ethylene formation in $NADPH_2$-induced lipid peroxidation of rat liver microsomes coupled to a cuprous-generating system. The lipid peroxidation and cuprous-generating systems were as described in the legend to Fig. 1. C_2H_4, ethylene; MA, malonaldehyde.

Addition	Product (nmole/hr per milligram of protein)	
	C_2H_4	MA
None	0.210	630
Vitamin E, 1 mg	.045	220
Vitamin E, 1 mg, and cuprous system (after lipid peroxidation)*	.066	356

* Lipid peroxidation system minus cuprous system incubated for 1 hour, followed by addition of vitamin E and the cuprous-generating system, as indicated, and again incubated for 1 hour.

When the concentration of microsomes was increased above 6 mg of protein per flask in these reactions a decrease in ethylene formation resulted. No ethylene production occurred with about 50 mg of protein in the incubation mixture. The explanation of this phenomenon may lie in the copper-sequestering capability of the microsomes. The proteins of these microsomes contain many sulfhydryl groups which tie up the copper ions by forming –S–Cu–S– moieties. Consequently, no cuprous ions remain to catalyze the formation of ethylene from peroxidated fatty acids. It is also possible that redox reactions, with higher concentrations of microsomes, oxidize ascorbate and thus leave little or no reducing agent for the formation of cuprous ions.

Production of ethylene by homogenates of rat livers was recently reported by Ram Chandra and Spencer (*6*). Whether or not ethylene can be formed by intact animal cells is speculative. However, the system reported here presents a model which provides a mechanism for ethylene formation in microsomes by way of lipid peroxidation, followed by reaction with a hypothetical copper enzyme.

The physiological significance of ethylene in animal cells is not known, but the fact that it has for many years served as an anesthetic in surgery (*7*) attests to its physiological

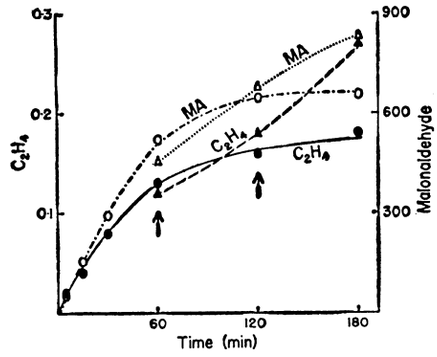

Fig. 1. Rate (in nanomoles per milligram of protein) of ethylene (C_2H_4) and malonaldehyde formation by rat liver microsomes. The reaction system contained in a total volume of 5 ml (in μmole): tris buffer (*p*H 7.5), 50; KCl, 300; $NADPH_2$, 0.6; ATP, 2.0; cupric ions, 5; and ascorbate, 50; in addition it contained 6 mg of microsomal protein. Microsomes were prepared as described by Ernster *et al.* (*8*). Arrows indicate further addition of 50 μmole of ascorbate at times indicated. Malonaldehyde was determined by the thiobarbituric acid reaction (*9*); a molar extinction coefficient, at 535 mμ, of 1.56×10^5 was used to determine malonaldehyde formed in the reaction (*10*). Ethylene was determined as previously described by Meigh *et al.* (*11*).

activity in animal systems. The dictates of comparative biochemistry suggest that if ethylene, in minute concentrations, possesses physiological activities in plant cells (*1*), it is feasible to consider that it may also affect animal cells.

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References and Notes

1. S. Burg, *Ann. Rev. Plant Physiol.* **13**, 265 (1962).
2. M. Lieberman and L. W. Mapson, *Nature* **204**, 343 (1964).
3. P. Hochstein and L. Ernster, *Biochem. Biophys. Res. Commun.* **12**, 388 (1963).
4. P. Hochstein, K. Nordenbrand, L. Ernster, *ibid.* **14**, 323 (1964).
5. L. A. Witting, *Federation Proc.* **24**, 912 (1965).
6. G. Ram Chandra and M. Spencer, *Nature* **197**, 366 (1963).
7. C. N. Chipman, *Curr. Res. Anesth. Analg.* **10**, 206 (1931).
8. L. Ernster, P. Siekevitz, G. Palade, *J. Cell Biol.* **15**, 541 (1962).
9. F. Bernheim, M. L. Bernheim, K. M. Wilbur, *J. Biol. Chem.* **174**, 257 (1948).
10. R. O. Sinnhuber and T. C. Yo, *Food Res.* **23**, 626 (1958).
11. D. F. Meigh, K. H. Norris, C. C. Craft, M. Lieberman, *Nature* **186**, 902 (1960).
12. Supported in part by grants from NSF GB-1094 and GB-1416 and by PHS career award to P.H.

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Nature of the Packing of Ribosomes within Chromatoid Bodies

Abstract. *Optical Fourier transforms, made of electron micrographs of the crystalline array of ribonucleoprotein known as chromatoid bodies of* Entamoeba invadens, *have been interpreted as the transforms of a helix of 12 nodes in five turns. A model shows that this helix may be built of spheres 180 angstroms in diameter, placed at a radius of 150 angstroms. The optical transform of the model is very similar to the transform of the original electron micrograph.*

Chromatoid bodies, so named because they stain with basic dyes, have been known to parasitologists as diagnostic features of several species of *Entamoeba* for 70 years. In *Entamoeba invadens*, the species we have studied, the bodies occur within the cysts as refractile rods, about $3 \times 3 \times 8$ μ in size. Their nature was unknown until recently. In 1955 Pan and Gieman (*1*) were able to demonstrate the presence of RNA, but not DNA, within the bodies by histochemical tests. In 1958 Barker and Deutsch (*2*) reported that, in electron micrographs, these bodies consisted of crystalline arrays of particles ~200 Å in diameter. In further studies (*3*), Barker and Svihla were able to demonstrate that the ultraviolet absorption of the chromatoid bodies was consistent with a high RNA content, and they suggested the probable ribosomal nature of the ~200-Å particles. In 1963 Siddiqui and Rudzinska (*4*) published electron micrographs showing a helical arrangement of the ribosomes in chromatoid bodies, but the details of the helix were not resolved. These authors pointed out that the hexagonally packed crystalline array seen in some sections must be the appearance of the chromatoid body when sectioned at right angles (cross sections) to the planes in which the helical arrays are disclosed (longitudinal sections).

Below we interpret fortunately oriented electron micrographs of sections through chromatoid bodies by the use of optical diffraction techniques. The combined use of these techniques was first described by Klug and Berger (*5*). From such data, we have been able to deduce the helical parameters which describe the packing of the ribosome particles within the chromatoid bodies.

Amoebas were cultivated as described by Morgan and Terkelsen (*6*). Cysts were fixed for microscopy by the addition of glutaraldehyde (50-percent solution, Fisher) directly to the culture fluid. After being washed and postfixed in Dalton's chrome-osmium solution, the cells were stained with 1 percent uranyl acetate in 10 percent formalin. The preparations were then dehydrated in acetone, followed by immersion in propylene oxide and embedding in an Epon-Araldite mixture. Sections were cut with glass knives on LKB ultramicrotomes, stained with lead citrate, and examined in a Philips model 200 electron microscope. An example of a longitudinal section is shown in Fig. 1.

From such micrographs, various measurements (such as those of particle diameter and chain separation) may be made directly. Although a helical packing is strongly suggested, it is virtually impossible to follow the exact course of any one helix in such a picture. An objective method of analyzing all the regularities seen here involves taking the Fourier transform of the picture, and this may be done by using the micrograph as the "mask" (or "specimen") of the optical diffractometer.

Table 1. Layer line measurements from optical transform of Fig. 1.

Layer line assignments	Observed spacing* (Å)	Calculated spacing for 900-Å period
2	432	450
3	308	300
5	180	180
7	127	129

* Spacing calculated from measurements on the diffractogram, the optical constants of diffractometer, and the magnification of electron micrograph. The accuracy of these and other spacings given in the text is about ±5 percent.

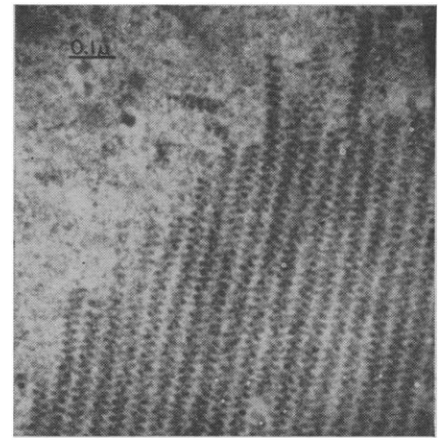

Fig. 1. Electron micrograph of a section through a chromatoid body of *Entamoeba invadens*. The distance between neighboring chains is 440 Å.