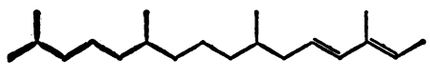
1,3-phytadiene with the *cis* structure being assigned to the lower-boiling compound II.

The infrared spectrum of compound IV shows no terminal unsaturation but rather a disubstituted ethylene (*trans*) structure conjugated with a trisubstituted ethylene. This is in agreement with the position of the ultraviolet absorption peak and the production upon ozonolysis of a C_{15} aldehyde. Thus IV



IV

is one of four possible geometric isomers of 2,4-phytadiene.

The biochemical source for the phytadienes in zooplankton has to be sought in the phytol of the phytoplankton diet of the animals. Phytol is a labile compound which is dehydrated by mild catalytic action of acids and bases to mixtures of phytadienes. Dehydration easily takes place on chromatographic adsorbents (alumina, silica gel). The zooplankton phytadienes are not laboratory artifacts from phytol present in the animals; their presence in crude zooplankton extracts or distillates which have not been in contact with chromatographic adsorbents can be shown by gas chromatography. A mixture of phytadienes very similar to that encountered in the zooplankton is formed by catalytic dehydration of phytol with oxalic acid (*3*). This might suggest their formation by acid catalysis in the digestive tract of the animals.

Pristane (*1*), the phytadienes, and the related mono- and polyolefins constitute a group of closely related compounds of graded chemical resistance to nonbiological and biological degradation. We intend to study the spread of these compounds from their common source through the marine biosphere and hydrosphere.

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References and Notes

1. M. Blumer, M. M. Mullin, D. W. Thomas, *Science* **140**, 974 (1963).
2. ———, *Helgolaender Wiss. Meeresberichte*, **10**, 187 (1964).
3. R. A. W. Johnstone and P. M. Quan, *J. Chem. Soc.* **1963**, 5706 (1963).
4. We thank Professor K. Biemann for the use of his mass spectrometric facilities. Supported by a research contract Nonr-2196(00) with the USONR and by grants from NSF, 3250 and 23472. Contribution No. 1589 of the Woods Hole Oceanographic Institution.

19 January 1965

Protein Synthesis Inhibition: Mechanism for the Production of Impaired Fat Absorption

Abstract. *Treatment of rats with puromycin and acetoxycycloheximide results in a defect in intestinal lipid transport. Under these conditions rats given corn oil accumulate triglyceride within the intestinal cells and fail to develop the normal postprandial hyperlipemia. The observed interference in lipid transport appears to be a consequence of impaired chylomicron formation.*

The absorption of dietary triglyceride from the intestine involves the formation of specific lipid particles, chylomicrons, which are secreted from the mucosal cells and appear predominantly in the lymphatic system (*1*).

The chylomicron is a complex structure consisting of a central core of triglyceride, additional lipid components (phospholipids, cholesterol), and an outer protein surface (*2*).

Although the protein moiety is very small (0.5 to 2 percent), this component is probably essential for the movement of lipid out of the mucosal cell, especially since lipoproteins are required for the transport of lipids in the systemic circulation. Support for such a concept was obtained from observations that a rare hereditary disorder in which there is a deficiency of β-lipoproteins is associated with a striking defect in the movement of lipid out of the intestinal mucosal cells (*3*). We have now shown that the inhibition of protein synthesis in the rat by puromycin and acetoxycycloheximide is associated with interference in the absorption and transport of lipid from the intestine.

Female Sprague-Dawley rats (180 to 200 g) were fasted for 24 hours and then injected intraperitoneally with puromycin (15 mg) (*4*) dissolved in buffered salt solution (0.04*M* phosphate buffer, *p*H 7.4 in 0.154*M* NaCl); the drug was administered in a series of hourly injections of 2.5 mg for 4 hours followed by five injections of 1 mg each. One hour after the fourth injection the animals were given 1.5 ml of corn oil by intubation, and killed 2, 4, and 6 hours later. Plasma triglycerides were determined at intervals (see *5*) and multiple jejunal sections were examined with light and electron microscopes.

In the normal animal lipid droplets appear rapidly within intestinal villous epithelial cells after corn oil administration. Four hours after such administration microscopy of intestinal biopsies shows most of the stainable lipid within villous and submucosal lymphatics with relatively little lipid remaining within epithelial cells; however, the lymphatics of the mucosa and submucosa show an abundance of stainable lipid droplets. By 6 hours lipid droplets in the mucosa are generally sparse, even within lymphatics.

In contrast, rats treated with puromycin show progressive accumulation of fat within the intestinal epithelial cells. Six hours after corn oil administration the villous epithelial cells are laden with lipid droplets of varying size (Fig. 1). The lipid is distributed rather uniformly throughout the cells and appears morphologically unremarkable except for the very large size of some particles, which suggests that a coalescence of retained droplets has occurred. In comparison with normal controls, relatively little lipid is seen in the villous lymphatics at any time after corn oil administration.

The accumulation of fat within the mucosal cells of the puromycin-treated rats is reflected by a failure of the plasma triglycerides to rise after a corn oil "load." Normal rats given 1.5 ml corn oil exhibit a progressive elevation of plasma triglycerides with the mean of the maximum values being 380 mg per 100 milliliters after 6 hours. In contrast, the means of the values for plasma triglycerides in the puromycin-treated animals barely rise above those of fasting animals (10 mg per 100 ml), and at 6 hours after administration of corn oil are only 37 mg per 100 ml, or 10 percent of those of the normal group (Fig. 2).

A similar defect in mucosal lipid

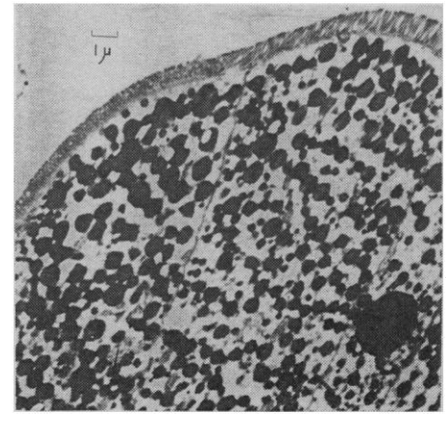

Fig. 1. Electron micrograph of small intestinal villous epithelial cells 6 hours after administration of corn oil (1.5 ml) in a rat treated with 15 mg of puromycin.

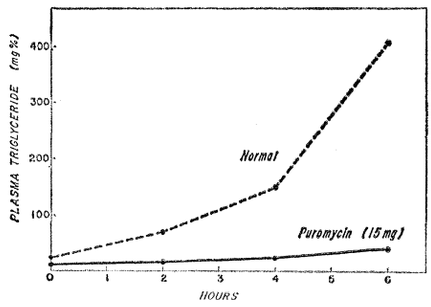

Fig. 2. Plasma triglycerides after oral administration of corn oil (1.5 ml) in the normal fasted rat and after treatment with 15 mg of puromycin. The values represent mean determinations.

transport may be produced in rats when they are treated with acetoxycyclohexiimide, another potent inhibitor of protein synthesis (*6*). The administration of acetoxycycloheximide (0.2 mg per kg) and, 3 hours later, 1.5 ml corn oil also results in pronounced intestinal mucosal lipid accumulation together with low concentrations of plasma triglycerides. Six hours after administration of corn oil the means of the plasma triglyceride concentrations are only 13 mg per 100 ml.

This defect in the absorption of lipid produced by inhibitors of protein synthesis did not depress other transport processes. Thus the oral administration of glucose (400 mg) to rats resulted in a normal rise of blood glucose. Similarly, the doses of puromycin did not depress the uptake of C^{14}-alanine by intestinal slices. There was no evidence that the inhibition of fat transport by puromycin was secondary to cell damage. Ultrastructural abnormalities were not observed in the intestinal epithelial cells of animals treated with as much as 60 mg puromycin over 8 hours; in fact the intracytoplasmic lipid droplets were surrounded by well-formed membranes similar to those described by Palay and Karlin (*7*).

Table 1. Effect of puromycin on C^{14}-palmitate esterification to glycerides and on palmitate thiokinase by microsomes of rat intestinal mucosa. For assay of C^{14}-palmitate esterification microsomes were isolated (*10*) and incubated as described (*11*). Thiokinase activity was assayed by the formation of palmitate hydroxamate (*9*).

Group	C^{14}-palmitate esterified to glycerides* (mμmole/mg)	Palmitate thiokinase† (mμmole/mg)
Normal	59	280
Puromycin (20 mg)	52	250

* 70 mμmole C^{14}-palmitate present in incubation system. † 2 μmole palmitate in 1 percent albumin present in incubation system.

In normal absorption of fat from the intestine at least three processes can be defined; (i) uptake of lipid from the lumen into the mucosal cells; (ii) esterification of the absorbed fatty acids (or monoglycerides) to triglycerides; and (iii) formation of chylomicrons and their entry into the lymphatic system.

In considering which of these three mechanisms might take part in the lipid blockade produced by inhibition of protein synthesis, a defect in uptake seemed unlikely as a primary event since accumulation was in fact the most prominent feature. If the lipid accumulation was due to a decrease in the enzymes necessary to convert fatty acids to triglycerides, one would expect the lipid retained within the cells to consist mostly of fatty acid. This, however, was not the case; the analysis of the mucosal lipid by thin-layer chromatography revealed that it was predominantly triglyceride. Further evidence for normal esterification by mucosal enzymes was obtained by studies in vivo.

Rats were fasted for 24 hours and then given puromycin intraperitoneally every hour for 4 hours. They were then anesthetized and given 5 μc of C^{14}-palmitate in 10 percent Tween 80 directly into the duodenum. The animals were killed 15 minutes later, and the mucosal lipids were extracted (*8*) and isolated by thin-layer chromatography. In the normal mucosa 78 percent of the C^{14} was in triglyceride and in the puromycin-treated animals 76 percent of the label was also in this fraction.

Final evidence for normal lipid esterification was obtained with experiments in vitro. Esterification of fatty acids to triglycerides is catalyzed by enzymes in the microsomal fraction of the mucosal cell (*9*). The initial step in this process includes the activation of the fatty acids to their acyl-coenzyme A derivatives by a thiokinase. Both the conversion of palmitate to palmityl coenzyme A and the overall esterification of palmitate to triglyceride were measured in microsomes of intestinal mucosa. In the animals treated with puromycin (20 mg) there was no significant reduction in either the overall esterification of C^{14}-palmitate to triglyceride or the conversion of palmitate to palmityl-coenzyme A (Table 1). Thus the block in intestinal lipid transport produced by puromycin is probably in the final phase of absorption—namely, in the assembly of the chylomicron particle.

The intestinal mucosa can synthesize lipoproteins, including those in chylomicrons (*12*), and puromycin effectively inhibits mucosal lipoprotein formation (*13*). There is still considerable debate, however, as to whether the proteins in lymph chylomicrons belong to the alpha or beta group of lipoproteins (*12*). In the present experiments electrophoresis (*14*) showed a marked decrease in β-lipoproteins of the plasma at a dose of puromycin (10 to 15 mg) which did not produce demonstrable changes in plasma α-lipoproteins. Thus the turnover of β-lipoproteins may be more rapid, and, in conjunction with the mucosal lipid accumulation, would support the concept that β-lipoproteins are more intimately related to fat absorption than are α-lipoproteins. Further evidence is the fact that patients with a congenital deficiency of α-lipoproteins have no impairment of fat absorption (*15*).

Puromycin influences the accumulation of lipid in other organs, especially liver. Robinson and Seakins produced a fatty liver in rats given this agent and noted a reduction in the synthesis of plasma lipoproteins by liver slices from puromycin-treated rats (*16*). They reasoned that the lipid accumulation was secondary to defective lipoprotein synthesis.

Finally, as mentioned already, the experimental observations cited resemble findings in the human disorder known as congenital or hereditary β-lipoprotein deficiency. In this disease there is a reduction in all plasma lipids, impaired fat absorption, and pronounced accumulation of lipid within mucosal epithelial cells, even when the patient is in the fasting state. The morphologic appearance of the intestinal mucosa in the human disease and under the conditions of puromycin or acetoxycycloheximide administration in our experiments is almost identical with both the light and electron microscopes. Thus it would appear that proteins are not only essential for the transport of lipids in the vascular system but also for the movement of lipids within the mucosal wall and for their normal entry into the lymphatic system.

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References and Notes

1. B. Borgstrom and B. Borgstrom, in *Progress in the Chemistry of Fats and Other Lipids*, R. T. Holman, W. O. Lundberg, T. Malkin, Eds. (Pergamon, London, 1955), vol. 3, p. 351.
2. J. H. Bragdon, *Ann. N.Y. Acad. Sci.* **72**, 845 (1959).
3. H. Salt, H. Wolff, J. Lloyd, A. Fosbrooke, A. Cameron, D. Hubble, *Lancet* **1960-II**, 325 (1960); K. J. Isselbacher, R. Scheig, G. R. Plotkin, J. B. Caulfield, *Medicine* **43**, 347 (1964).
4. M. Yarmolinsky and G. de la Haba, *Proc. Natl. Acad. Sci. U.S.* **45**, 1721 (1959).
5. E. Van Handel and D. B. Zilversmit, *J. Lab. Clin. Med.* **50**, 152 (1957).
6. C. Young, P. Robinson, B. Sacktor, *Biochem. Pharmacol.* **12**, 855 (1963).
7. S. Palay and L. Karlin, *J. Biophys. Biochem. Cytol.* **5**, 373 (1959).
8. J. Folch, M. Lees, C. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
9. J. Senior and K. J. Isselbacher, *Biochim. Biophys. Acta* **44**, 399 (1960).
10. ———, *J. Biol. Chem.* **237**, 1454 (1962).
11. A. M. Dawson and K. J. Isselbacher, *J. Clin. Invest.* **39**, 150 (1960).
12. M. Rodbell, D. Fredrickson, K. Ono, *J. Biol. Chem.* **234**, 567 (1959).
13. K. J. Isselbacher and D. M. Budz, *Nature* **200**, 364 (1963).
14. Electrophoretic studies were performed by Dr. F. Hatch, Department of Medicine, Massachusetts General Hospital.
15. D. S. Fredrickson, *Ann. Intern. Med.* **55**, 1016 (1961).
16. D. Robinson and A. Seakins, *Biochim. Biophys. Acta* **62**, 163 (1962).

17. We thank G. Drummey and D. Budz for technical assistance. Supported by grants from the John A. Hartford Foundation, Inc., and NIH (AM-01392 and AM-03014).

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Bacteria on Leaf Surfaces and in Intercellular Leaf Spaces

Abstract. *Ultraviolet irradiation kills bacteria on the leaf surface but not those in the intercellular leaf spaces.*

Plant leaves provide habitats for saprophytic microorganisms and infection courts for various plant pathogens. This ecologically neglected environment has been called "the phyllosphere" (*1*). One of the problems in a study of this environment is the determination of which microbes occur on the surface of the leaf and which occur in the intercellular spaces, such as the substomatal chambers.

The number of viable cells of *Xanthomonas phaseoli* var. *sojensis* recovered from intact soybean leaves steadily decreased for 12 hours after inoculation and washing (Fig. 1*A*). How many of the original number of viable cells were on the surface, and how many were in the intercellular spaces of the leaf? Perhaps the decrease in number for the first 12 hours was a reflection of the death rate of those bacteria on the surface, particularly since *X. phaseoli* var. *sojensis* has a rapid death rate on drying and exposure to air (Fig. 1*C*). When leaves were treated with ultraviolet light (UV) for 15 minutes, there was an initial decrease in the number of viable cells that was followed by a continuous increase (Fig. 1*B*).

Cultures of *X. phaseoli* var. *sojensis*, a pathogen of soybean, and *Serratia marcescens*, a saprophyte in soil and water, were cultured on nutrient agar. Soybean plants (*Glycine max* var. Blackhawk) were grown in a greenhouse. Leaves were inoculated by spraying a suspension (10^8 cells/ml) on the undersurface which had first been sprayed with water until the intercellular spaces were filled (water-soaked). After 5 minutes the inoculated leaves were thoroughly rinsed with running water.

Numbers of viable bacteria were determined by grinding a disk (12 mm) of leaf tissue against the side of a test tube with a stirring rod and making culture plates at appropriate dilutions. Colonies representing the number of viable cells were well separated from fragments of leaf disks.

Ultraviolet light was applied with a General Electric G30T8 germicidal lamp at a distance of 46 cm (a dosage of approximately 3000 ergs sec^{-1} cm^{-1}).

Ultraviolet light has little penetrating capacity and should kill microorganisms on the surface of the leaf before killing those in the intercellular spaces. With increasing exposure to UV the total number of viable bacteria should decrease for a time (indicating the death rate for those on the surface) and then remain constant with prolonged exposure (indicating the number of bacteria in intercellular spaces and protected from the UV). The number of viable cells of *X. phaseoli* var. *sojensis* and *S. marcescens* applied by inoculation to soybean leaves decreased rapidly with exposure to UV for 15 minutes and then remained constant with prolonged exposure (Fig. 2, *B* and *C*). Viable cells of the naturally occurring bacterial flora in uninoculated but water-soaked leaves responded in a like manner (Fig. 2*E*).

The cells surviving prolonged treatment do not represent bacteria resistant to UV, since cells of *X. phaseoli* var. *sojensis* suspended in water to a depth of 1 mm are rapidly killed by exposure to UV (Fig. 2*D*). And that the leaf does provide protection from UV is indicated by the survival of *X. phaseoli* var. *sojensis* when the side of the leaf opposite to that which has been inocu-

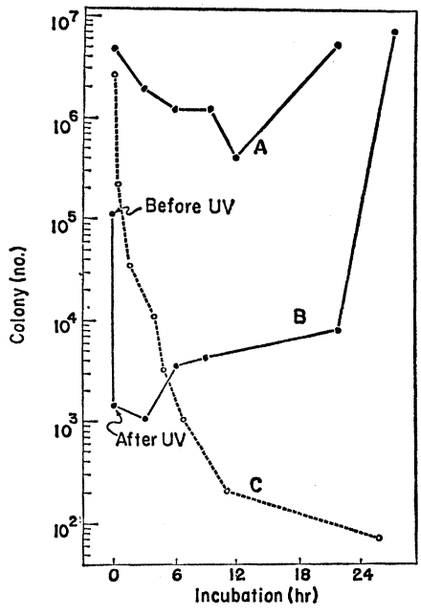

Fig. 1. Number of colonies of *X. phaseoli* var. *sojensis* from intact soybean leaves after inoculation and incubation. *A*, Without exposure to UV; *B*, with 15 minutes exposure to UV immediately prior to counting; *C*, the death rate of the organism after drying and exposure to air on disks on aluminum foil.

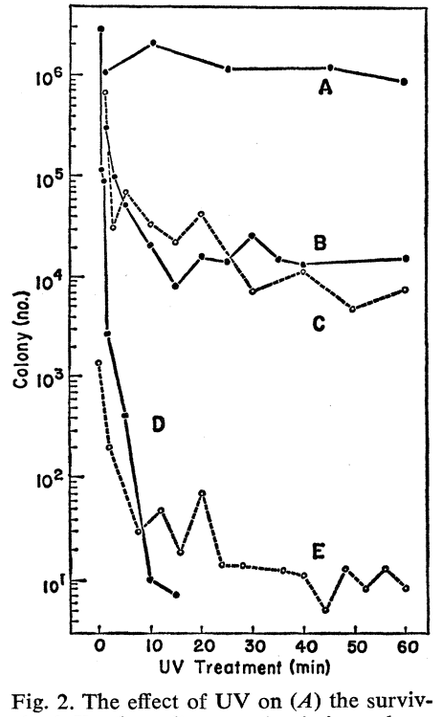

Fig. 2. The effect of UV on (*A*) the survival of *X. phaseoli* var. *sojensis* in soybean leaves when the side opposite to that which is inoculated is irradiated and when (*B*) the inoculated side is irradiated. (*C*) Survival of *S. marcescens* in soybean leaves when the inoculated side is irradiated. (*D*) Survival of cells of *X. phaseoli* var. *sojensis* when suspended in water and irradiated. (*E*) Survival of naturally occurring bacterial flora in soybean leaves when both sides of the leaf are irradiated.