Autoxidation of Insect Lipids: Inhibition on the Cuticle of the American Cockroach

Abstract. *The major hydrocarbon, cis,cis-6,9-heptacosadiene, of* Periplaneta americana *cuticles and oothecae solidifies in air by autoxidation. The reaction resembles the autoxidation of other dienes by known free radical mechanisms and yields stearic acid, stearal, hexanoic acid, and hexanal. On the cuticle, autoxidation is inhibited by polyhydric phenols.*

The epicuticular lipids of different insects range in physical properties from hard waxes to soft greases. The preservation of an intact lipid layer which reduces water loss is essential to the survival of insects in many environments. The cuticular lipid of *Periplaneta americana* (L.) is a soft grease, whether derived from fresh cuticles or from cast skins up to 1 year old. This is in contrast to the hardening which occurs in isolated wax as it ages (*1*). Beament (*2*) suggested that volatile paraffins and alcohols are secreted continuously in vivo. However, Gilby (*3*) failed to distill solvents from cockroaches. Gilby and Cox (*1*) accounted for the fluidity of the grease by the high proportion of nonvolatile liquid constituents present in the hydrocarbon and free acid fractions. They suggested that hardening of the wax may be due to unspecified chemical reactions which, on the cuticle, are controlled by inhibitors or physical factors. We now present evidence that the hardening of isolated cockroach wax is due to autoxidation of unsaturated lipid. On the cuticle and the ootheca, autoxidation is inhibited by polyhydric phenols, compounds whose known functions previously were limited to the tanning of cuticular proteins.

The major component (49 to 51 percent) of the cuticular wax of *P. americana is cis,cis*-6,9-heptacosadiene (*4*). When exposed to air at room temperature, the liquid hydrocarbon begins to harden within a few days. After 1 month the original diene comprises only about 20 percent of the solidified sample. The products of degradation are mainly hexanal, hexanoic acid, stearal, and stearic acid. These products indicate autoxidative cleavage of the diene. The mechanism appears to be similar to the autoxidation of methyl linoleate which has an identical diene system and is known to undergo a corresponding cleavage of double bonds (*5*) by a free radical mechanism (*6*). The autoxidation of linoleate is characterized by the production of conjugation and the occurrence of a prolonged induction period before significant reaction occurs. The rates of autoxidation of methyl linoleate and of the C_{27}-diene, as determined by gas chromatography, are similar. Ultraviolet absorption spectroscopy indicates the production of conjugation in the C_{27}-diene. Addition of 0.05 percent gallic acid, a known inhibitor of free radicals, causes a 20-fold increase in length of the induction period. The hardening of extracted cuticular wax is thus readily explained by autoxidation and the production of solid stearal and stearic acid from *cis,cis*-6,9-heptacosadiene. Minor oxygenated compounds, intermediate hydroperoxides, and polymers formed by free radicals may also contribute to hardening.

A similar situation exists in the oothecae of *P. americana*. The major hydrocarbon of the oothecal wax is *cis, cis*-6,9-heptacosadiene. Similar proportions of the diene are observed in fresh and in old oothecae.

Materials extracted with ethanol or water from the cast skins of *P. americana* protect the C_{27}-diene against autoxidation. The extracts when tested with ferric chloride produce a green color that becomes red upon the addition of sodium carbonate, which indicates that they contain *o*-dihydricphenols. The probable sclerotization percursor of the cockroach cuticle is *N*-acetyldopamine (*7*) which is an *o*-dihydricphenol soluble in ethanol and in water, insoluble in chloroform, and capable of free radical inhibition. Hackman and Goldberg (*8*) have shown that oothecae contain free protocatechuic acid (3,4-dihydroxybenzoic acid), which is believed to be the primary source of *o*-quinone involved in tanning (*7*). Experiments demonstrate that this *o*-dihydricphenol is an effective inhibitor to autoxidation of the C_{27}-diene.

It is suggested therefore that, in addition to their known function in sclerotization, the polyhydric phenols of the insect cuticle and ootheca serve to inhibit formation of free radicals in cuticular and oothecal waxes. The lipids in their natural environment are thereby protected against oxidative degradation.

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Coevolution of Escherichia coli and Bacteriophages in Chemostat Culture

The continuous culture of bacteria, yeasts, and algae in chemostats provides a convenient means of studying large populations under closely defined environmental conditions in a system that permits energy flow. A study in which T series coliphages are grown on populations of *Escherichia coli* demonstrates the use of a chemostat culture in following long-term genetic interactions which model in many ways predator-prey or host-parasite systems.

Many coliphages show "lysis inhibition" (*1*) where an infection of a host cell in which phage reproduction is taking place by a second phage may delay or prevent the release of the progeny of the first phage. A mutant of T4, in which the rII chromosome segment responsible for this phenomenon is deleted, was selected for this work and is designated T4r.

Cultures of *E. coli* B were grown in chemostats at 37°C in bacterial minimal medium in which the nitrogen source, 0.005 percent ammonium sulfate, limits the bacterial population (*2*). The bacterial numbers are allowed to equilibrate, at 2.2×10^8 cell/ml at a flow rate of 0.04 hr^{-1}, and the population is then inoculated with bacteriophage to a concentration of ap-