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.

P. W. Atkinson
A. R. Gilby
Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Canberra, Australian Capital Territory, 2601

References

1. A. R. Gilby and M. E. Cox, *J. Insect Physiol.* **9**, 671 (1963).
2. J. W. L. Beament, *J. Exp. Biol.* **32**, 514 (1955).
3. A. R. Gilby, *Nature* **195**, 729 (1962).
4. I. M. Beatty and A. R. Gilby, *Naturwissenschaften* **56**, 373 (1969).
5. R. J. Horvat, W. H. McFadden, H. Ng, W. G. Lane, A. Lee, R. E. Lundin, J. R. Scherer, A. D. Shepherd, *J. Amer. Oil Chem. Soc.* **46**, 94 (1969); A. M. Gaddis, R. Ellis, G. T. Currie, *ibid.* **38**, 371 (1961); E. N. Frankel, J. Nowakowska, C. D. Evans, *ibid.*, p. 161.
6. W. O. Lundberg and P. Järvi, *Progr. Chem. Fats* **9**, 377 (1968).
7. P. C. J. Brunet, *Endeavour* **26**, 68 (1967).
8. R. H. Hackman and M. Goldberg, *Biochim. Biophys. Acta* **71**, 738 (1963).

20 February 1970

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-

proximately 10^5 particles per milliliter. Viable counts are made of the bacteria by plating on nutrient agar and of the phage by counting plaques of the total infective particles formed on a lawn of *E. coli* B (*3*). Infection of a chemostat population in this way is followed by a period of considerable instability in numbers both of bacteria and phage in which no regular pattern has been detected. However 20 to 40 hours after infection the numbers of bacteria increase until the population reaches the same, stable value as that maintained by control populations without phage (within the limits of experimental error). Fluctuations continue in the numbers of phage present but, in this phase, are of low frequency and amplitude. A vessel containing T4r phage has been maintained in this state for 52 weeks, suggesting that a relatively stable relationship becomes established.

Phage taken from vessels after stabilization and plated onto lawns of control *Escherichia coli* B characteristically produce minute plaques visible only after an extended incubation time. It is still sensitive to antiserum to T4r and adsorbs to but fails to grow on *E coli* K 12(λ), both being characteristics of control T4r. The latent period is considerably extended and the burst size is reduced when these are measured with control bacteria as host.

Of the factors that affect plaque size (*4*), the evolution of phage in chemostat populations has involved changes in the latent period. The relative effectiveness of generation time in altering growth rate has been argued by Lewontin (*5*), "small absolute changes in developmental rates of the order of 10 per cent are roughly equivalent to large increases in fertility of the order of 100 per cent." The same feature, the length of time which the virus spends in its host, is important in stabilizing myxomatosis epidemics in rabbit populations (*6*). The resistance of the rabbits and the virulence of the virus both change so that, in areas with a long history of myxomatosis, the majority of virus strains isolated have a mean survival time in the host which is longer than in areas where outbreaks of the disease are more recent.

A similar study has been made with phage T3 which differs from T4 in showing "pseudolysogeny" (*7*), that is, infected bacteria may undergo one or more divisions before releasing progeny phage. The results are very similar except that the period of instability after infection lasts for 500 to 700

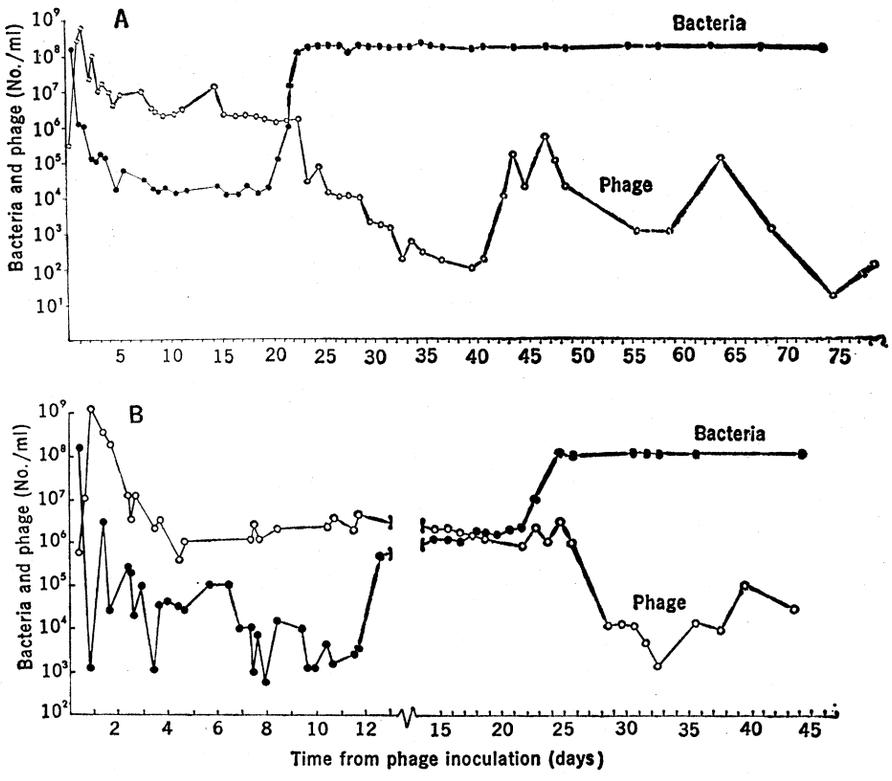

Fig. 1. The history of population changes of *Escherichia coli* B and bacteriophage T3 in two replicate chemostat cultures. There is a change in time scale after 13 days in (B).

hours (Fig. 1, A and B), considerably longer than with T4r. Populations of T3 and *E. coli* B have been maintained in the more stable phase for 80 weeks. The plaques formed on control bacteria are again much reduced in size and the latent period is extended.

Coevolution of bacterial resistance and phage virulence may involve changes in both bacteria and phage (*8*). A study of this has been made with the T3–*E. coli* B system. From the time of infection of a population with phage until the bacterial numbers increase again to the environmentally determined level, bacteria susceptible to both control and co-occurring phage are present in a majority in the population. This suggests that in a predator-prey or host-parasite system in which there is no element of search, dispersal may permit the survival of some nonresistant individuals. After the increase, the majority of the bacteria are resistant to stock and co-occurring phage.

The variety of mechanisms by which natural populations maintain a constant size in the face of continuing individual reproduction is represented in a chemostat by loss of individuals through the overflow.

Fluctuations in phage (predator) numbers only rarely have a measurable effect on the number of bacteria (prey) after the bacterial population has become largely resistant. By exchanging one cause of mortality (the overflow) for another (increased loss by phage attack), the population is buffered against substantial changes in pressure from its predator (*9*). A degree of coevolution of the two components of the population may be a prerequisite to the establishment of such a stable relationship.

M. T. Horne
School of Plant Biology, University College of North Wales, Bangor

References and Notes

1. A. H. Doermann, *J. Bacteriol.* **55**, 257 (1948).
2. D. Herbert, *Recent Progr. Microbiol. Proc. Int. Congr. Microbiol. 7th* (1958), p. 381.
3. A. Gratia, *Ann. Inst. Pasteur* **57**, 652 (1936).
4. M. H. Adams, *The Bacteriophages* (Interscience, New York, 1959).
5. R. C. Lewontin, in *The Genetics of Colonising Species*, H. G. Baker and G. L. Stebbins, Eds. (Academic Press, New York, 1965), p. 77.
6. D. Marshall and F. Fenner, *J. Hyg.* **58**, 485 (1960); D. Marshall and G. W. Douglas, *ibid.* **59**, 117 (1961); R. Mykytowycz, *Nature* **172**, 448 (1953).
7. D. K. Fraser, *Virology* **3**, 527 (1957).
8. G. S. Stent, *Molecular Biology of Bacterial Viruses* (Freeman, San Francisco, Calif., 1963).
9. B. Wallace, in *Population Biology and Evolution*, R. C. Lewontin, Ed. (Syracuse Univ. Press, Syracuse, N.Y., 1967).
10. Supported by a grant from the Science Research Council to Professor J. L. Harper. I thank Professor Harper and Dr. D. S. Shaw for guidance at various stages in the work.

19 January 1970; revised 13 March 1970