(1972)] during the interval 1959 to 1971. These have been cited as displaying an inverse correlation between sunspot cycle and ionospheric potential [R. Markson, *NASA Spec. Publ. SP-366* (1975), p. 174]. Although we tested this negative correlation and found it to be at less than the generally accepted 5 percent confidence level, there appears to be an inverse relationship. Error would have been introduced into these data because the U.T. diurnal variation was not removed, as it was to some extent with Olson's data through stratification, depending on the time of the measurement. Recently, W. Gringel (University of Wyoming, Laramie; personal communication) provided information showing that the average of 80 ionospheric potential measurements made by Mühleisen and Fischer during the sunspot minimum periods (1962 to 1966 and 1967 to 1972) was 301 kV; while the average for 142 measurements made during the sunspot maximum periods (1959 to 1961 and 1967 to 1972) was 234 kV. These data thus display a 25 percent peak-to-peak variation around a mean of 267 kV (unadjusted for the U.T. variation), with ionospheric potential inversely correlated with solar activity through a sunspot cycle period.
71. R. Shapiro, *J. Atmos. Sci.* **36**, 1105 (1979).
72. This is because (i) the solar wind does not penetrate the magnetosphere to the ionosphere and (ii) the ionospheric potential is maintained by global thunderstorm currents.
73. J. H. King, *Interplanetary Medium Data Book Appendix* (National Space Science Data Center, NASA-Goddard Space Flight Center, Greenbelt, Md., 1977).
74. R. Markson, thesis, State University of New York, Albany (1974).
75. H. A. Panofsky and G. W. Brier, *Some Applications of Statistics to Meteorology* (College of Mineral Industries, Pennsylvania State University, University Park, 1963), pp. 126–161.
76. R. Shapiro, personal communication.
77. The data were obtained from the Space Science Center, University of New Hampshire, Durham.
78. Y. Tanaka, T. Ogawa, M. Kodama, *J. Atmos. Terr. Phys.* **39**, 523 (1977).
79. The dawn-to-dusk potential difference in the ionosphere across the auroral oval is at the most +125 to −125 kV (*13*), superimposed on the ionospheric potential of about +250 kV. The ionospheric electric field intensity decreases with latitude so that by mid-latitude it is 10 percent of the intensity in the auroral zone [T. Kikuchi, T. Araki, H. Maeda, K. Maekawa, *Nature (London)* **273**, 650 (1978)], thus a 5 percent maximum perturbation of the ionospheric potential would be expected at mid-latitude.
80. R. V. Anderson, *J. Geophys. Res.* **74**, 1697 (1969).
81. The variation of ionospheric potential cannot be measured reliably from the earth's surface but must be obtained well above the exchange layer (*5*).
82. The constant-altitude measurements in which ionospheric potential variations were recorded were obtained at 3.5 km (*5*).
83. Besides removing the tethered balloon or kite from the airspace over land, where it could be a hazard to aviation, another advantage of this approach is that the ship's velocity could be used to counteract the adverse affects of wind on a tethered balloon, or to provide lift for a kite.
84. A workshop jointly sponsored by the Space Institute of the University of Tennessee and NASA-Marshall Space Flight Center was held in February 1979 to discuss the requirements for lightning observations from space [L. S. Christensen, W. Frost, W. W. Vaughan, Eds., *NASA Contract. Rep. CP-2095* (1979)].
85. J. W. Warwick, C. O. Hayenga, J. W. Brosnahan, *J. Geophys. Res.* **84**, 2457 (1979).
86. Geostationary satellites must be stationed over the equator and because of the earth's curvature cannot view latitudes higher than about 60° very well. However, there is little thunderstorm activity in these high-latitude regions.
87. Aircraft measurements in the troposphere over Maine 15 days after thermonuclear explosions over the Pacific Ocean in 1956 indicated a conductivity increase of up to 75 percent due to the cloud of radioactivity carried by the westerly winds [R. V. Anderson and G. P. Serbu, *J. Geophys. Res.* **65**, 223 (1960)]. Secular decreases of as much as 50 percent in electric field intensity at ground level apparently were caused by increased ionization due to radioactive fallout following the initiation of thermonuclear testing in 1952 [E. T. Pierce, *ibid.* 77, 482 (1972)]. If future atmospheric releases of krypton-85, an inert radioactive gas with a half-life of 10 years, takes place according to the scheduled introduction of nuclear fission reactors, it could ionize the atmosphere over the oceans (71 percent of the earth's surface) at a rate comparable to that due to the natural background cosmic radiation. It has been estimated that this condition would significantly modulate the atmospheric electric global circuit by lowering the total resistance of the atmosphere [W. L. Boeck, *Science* **193**, 195 (1976)].
88. E. N. Parker, *NASA Spec. Publ. SP-366* (1975), p. 243.
89. J. A. Eddy, *Clim. Change* **1**, 173 (1977).
90. We thank J. H. King, Goddard Space Flight Center, for providing daily averages of solar wind velocity. W. Roberts, K. Schatten, and B. Vonnegut offered valuable suggestions during revision of the manuscript. This work has been funded by the Atmospheric Science Program, Office of Naval Research, under contracts N00014-74-C-0336 and N00014-79-C-0399. One of us (M.M.) received additional support from the South African Council for Scientific and Industrial Research.

Biopolyester Membranes of Plants: Cutin and Suberin

P. E. Kolattukudy

Living organisms are packaged in envelopes that consist of polymeric structural components; in terrestrial organisms a waterproofing mixture of lipids, collectively called waxes, are associated with this outer layer. The structural component in animals is either an amino acid polymer (protein) or a carbohydrate polymer (chitin), whereas in higher plants it is a biopolyester, cutin, in the aerial parts, and suberin, a polymer containing polyester domains, in the underground parts and at wound surfaces. Such polyesters not only constitute the major protective barrier between the plant and its environment but also function as a rather permanent biological barrier within a variety of organs so that diffusion of molecules can be controlled, a role essential to plant life. During the past decade considerable progress has been made in our understanding of the composition, biosynthesis, and biodegradation of these phytopolymers. In this article I summarize these recent findings (*1–4*).

Location and Ultrastructure

Cutin is the structural component of the plant cuticle, which is attached to the outside of the epidermal cell wall in the aerial parts of both angiosperms (*1*) and gymnosperms (*5*). Even primitive plants such as liverworts (*6*) and the moss *Mnium cuspidatum* (*7*) have cutin. Cutin is present on virtually every aerial organ of plants such as stem (except possibly the bark of woody plants), petiole (*8*), leaf, including substomatal cavities (*1*), flower parts (*8, 9*), fruits (*1*), seed coats (*8*), and even internal parts such as juice sacs of citrus (*7*). The thickness of the polymer layer varies among the different species and among organs within the same species. In leaves, thickness and mass were reported to be in the range of 0.5 to 14 micrometer and < 20 to 600 micrograms per square centimeter, respectively (*1*). In special cases such as interstomatal cavity, a very thin cutin layer (0.15 to 1.0 μm) might be found, whereas in organs such as fruits with well-developed cuticle, the cutin content may reach 1.5 milligrams per square centimeter (*4, 10, 11*). Development of cuticle may have been a crucial factor in the colonization of lands by plants (*12*). However, the occurrence of cutin does not appear to be limited to land plants since the polymeric material from a sea grass, *Zoestra marina*, which grows submerged on coastal shorelines contains covalently attached hydroxy fatty acids identical to those found in the cutin of land plants (*7*). Cutin in land plants is embedded in wax, and in some instances the wax occurs in layers so that the cuticle shows a lamellar structure, although more often the cuticle presents an amorphous appearance under the electron microscope (*1, 9, 13*) (Fig. 1).

The author is director of the Institute of Biological Chemistry and professor of biochemistry (Biochemistry/Biophysics Program), Washington State University, Pullman 99164.

0036-8075/80/0530-0990$02.00/0

Underground parts of plants such as roots and tubers (*14*, *15*) as well as wound periderm formed on any organ, irrespective of the location of the organ, are protected by a different type of polymer, called suberin, which contains polyester domains (*16*, *17*). Like cutin, suberin is also deposited at an extracellular location, on the plasma membrane side of the cell wall (*18*). Electron microscopic examination of the suberized regions show a lamellar structure comprised of light and dark bands (Fig. 1) most probably representing waxes and the polymer, respectively (*19*, *20*). Ultrastructural and cytochemical evidence indicates that suberin-type polymers are present in other plant organs such as the endodermis (Casparian bands), epidermis, hypodermis, and exodermis of roots (*21*), the bundle sheaths of grasses (*22*), the boundary between the secretory organs (such as glands and trichomes) and the rest of the plant (*23*), the sheaths around idioblasts such as those containing ammonium oxalate crystals or mucilage (*24*), and pigment strands of grains such as wheat (*25*).

Summary. Cutin, a biopolyester composed of hydroxy and epoxy fatty acids, is the barrier between the aerial parts of higher plants and their environment. Suberin, a polymer containing aromatics and polyesters, functions as a barrier in underground parts, wound surfaces, and a variety of internal organs. The composition and probable structure of these polymers are discussed. The biosynthesis of the hydroxy, epoxy, and dicarboxylic acids of the polyesters from the common cellular fatty acids is elucidated. An extracellular enzyme transfers the hydroxy and epoxyacyl moieties from their coenzyme A derivatives to the growing polyester. The enzymes acting in the biodegradation of the polyesters have been isolated from fungi, pollen, and mammals and characterized. The function and possible practical implications of these polyester barriers are briefly discussed.

Composition of Phytopolyesters

Since a pectinaceous layer is used to attach the cuticle to the epidermal cell wall, disruption of this layer by chemicals such as ammonium oxalate and oxalic acid, $ZnCl_2$ and HCl, or cuprammonium ion and hydrolytic enzymes such as pectinase can be used to isolate the cuticle (*1*, *2*). Enzymatic removal of the residual carbohydrates and removal of soluble waxes by solvent extraction leave behind the insoluble polyester cutin (Fig. 1). But suberin, being attached tightly to cell walls, can be obtained only as an "enriched" preparation by techniques similar to those employed for the isolation of cutin. The insoluble polymeric material can be depolymerized by (i) alkaline hydrolysis, (ii) transesterification with sodium methoxide or boron trifluoride in absolute methanol, and (iii) hydrogenolysis with $LiAlH_4$ in tetrahydrofuran (Fig. 2). Depolymerization with $LiAlD_4$ introduces deuterium at specific positions, indicating the nature of the reducible groups originally present in the polymer, and therefore is very useful in structural studies. The

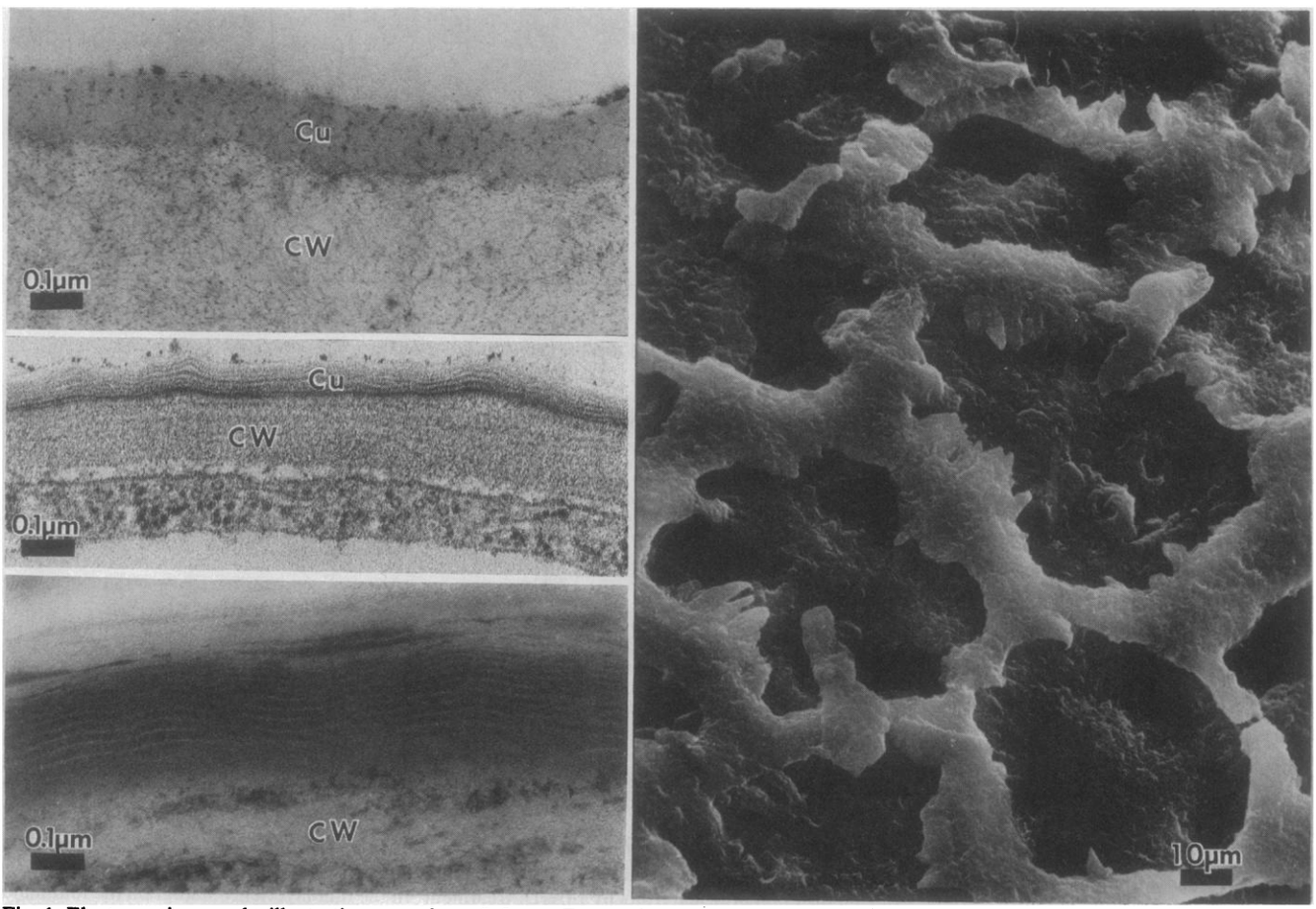

Fig. 1. Electron micrographs illustrating amorphous (left top, *Tropaeolum majus*) and lamellar (left middle, *Atriplex semibaccata*) cuticle, lamellar structure of potato suberin (left bottom), and scanning electron micrograph (right) of the tomato fruit cutin showing the protrusions that help to anchor the polymer to the fruit by fitting into the intercellular grooves. *Cu*, cuticle; *CW*, cell wall.

mixture of monomers obtained from the depolymerization techniques can be subjected to thin-layer chromatography (TLC) and combined gas-liquid chromatography–mass spectrometry (GLC-MS) (Fig. 2). The aliphatic carbon chains containing functional groups such as carbonyls and hydroxyls undergo diagnostic cleavages so that, in most cases, the mass spectra provide sufficient information for the identification of the monomers.

Cutin is composed of two families of hydroxy and epoxy fatty acids—a C_{16} family and a C_{18} family (Fig. 3). The major component of the former is dihydroxypalmitic acid, in which one hydroxyl group is at the ω-carbon and the other at C-10, C-9, C-8, or C-7; usually a mixture of positional isomers is present with one positional isomer dominating. The dihydroxypalmitate from papaya and tomato cutin has the *L* configuration, and it is probable that this configuration is a general property of the dihydroxypalmitate of cutin from all sources (*26*). The major components of the C_{18} family are oleic acid, ω-hydroxyoleic acid, ω-hydroxy-9,10-epoxystearic acid, and 9,10,18-trihydroxystearic acid, together with analogous compounds containing an additional double bond at C-12. In plant organs, which rapidly expand, the C_{16} family of monomers predominates, whereas in slower growing organs with thicker cuticle a mixture of the two families of monomers is found (*2*, *8*). Small amounts of phenolic acids such as *p*-coumaric acid and ferulic acid are also esterified to cutin, and other types of compounds might also be covalently attached (*27*). Cutin preparations from most plants leave 10 to 30 percent of the mass as insoluble residue after the depolymerization procedures. Whether this residue represents linkages, such as ether bonds, that are resistant to the cleavage techniques used, or the presence of polymers other than cutin itself is not known. However, it is clear that cutin is largely a polyester comprising mainly hydroxy and epoxy fatty acids.

The results of chemical analyses conducted on the polymeric materials from a variety of plant tissues (*1*, *2*, *4*, *7–9*, *15*, *16*, *28*, *29*) suggest that a high content of phenolics and the presence of a high pro-

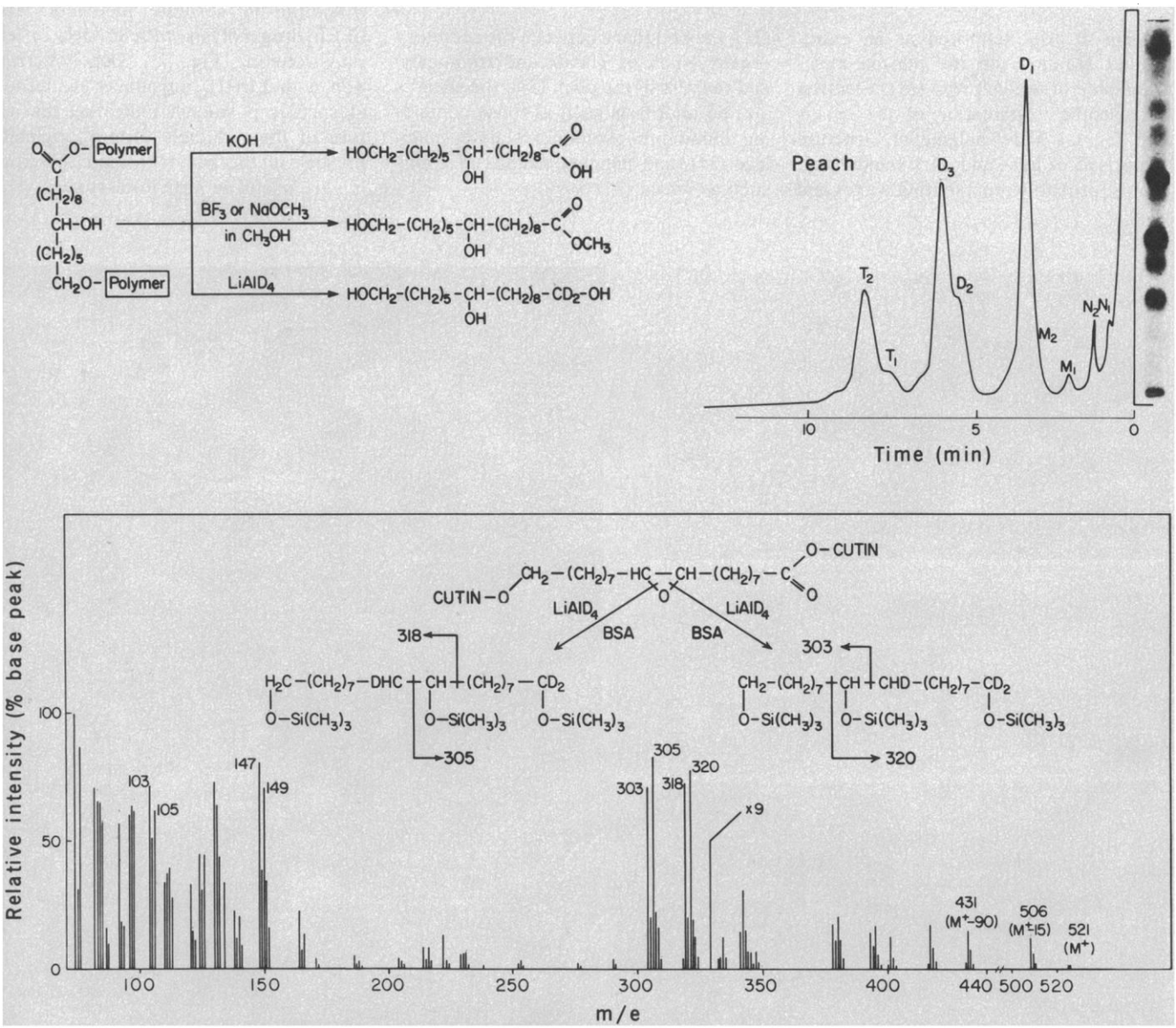

Fig. 2. (Top left) Chemical methods used to depolymerize the polyesters; (top right), thin-layer and gas-liquid chromatograms (as trimethylsilyl derivatives) of the monomer mixture obtained from the cutin of peach fruits by $LiAlD_4$ treatment. In the thin-layer chromatogram the four major spots are, from the bottom, C_{18} tetraol, C_{18} triol, C_{18} triol, and diols. N_1, C_{16} alcohol; N_2, C_{18} alcohol; M_1, C_{16} diol; M_2, C_{18} diol; D_1, C_{16} triol; D_2 and D_3, unsaturated and saturated C_{18} tetraol, respectively. (Bottom) Mass spectrum of component D_3 in the gas chromatogram; *BSA*, bis-*N*, *O*-trimethylsilyl acetamide.

portion of ω-hydroxy acids (mainly ω-hydroxypalmitic acid and ω-hydroxyoleic acid), the corresponding dicarboxylic acids and fatty acids as well as alcohols containing more than 18 carbon atoms distinguish suberin from cutin (*2, 15*). The more highly oxygenated epoxy and polyhydroxy acids that are major components of cutin are usually only minor components of suberin although there could be exceptions to this generalization (*2, 28*). Alkan-2-ols with an odd number of carbon atoms (C_{15} to C_{21}) were recently found in the polymeric materials obtained from several species of plants, and this component appears to be more common in suberin than in cutin (*7*). In general, monomer composition of suberin is much more complex than that of cutin.

Proposed Model for Cutin and Suberin

In cutin, the monomers are held together largely by intermonomer ester linkages. X-ray diffraction studies showed that cutin is an amorphous polymer with no indication of crystallinity (*30*). The composition of the monomers shows that hydroxyl groups are in excess of the number of carboxyl groups, and depolymerization of cutin with $LiBH_4$ (which reduces only esterified but not free carboxyl groups) showed that only a few percent of the monomers have free carboxyl groups (*7*). Chemical modification of the free hydroxyl groups in the polymer by two different techniques, followed by depolymerization and product analyses, reveals that most of the free hydroxyl groups in the polymer are secondary hydroxyl groups (*3, 11*). Thus, most of the ester linkages in the polymer involve primary hydroxyl groups. In agreement with this conclusion, pancreatic lipase, which catalyzes hydrolysis of primary alcohol esters, catalyzed extensive depolymerization of cutin (*31*). Such studies also indicated the presence of cross-links involving secondary hydroxyl groups, although the extent of cross-linking could not be measured accurately (*3, 11*). On the basis of the available information the model shown in Fig. 3 was proposed for cutin composed of only the C_{16} family of monomers (*3*).

The tentative model shown in Fig. 3 was proposed for the structure of suberin (*3*). This polymer contains a phenolic matrix that might be structurally somewhat similar to lignin; to this matrix are attached aliphatic domains that might have some resemblance to cutin. This complex is probably covalently attached to the cell wall via phenolic residues in a manner analogous to that found in lignin (*32*). This conclusion is based on the following observations:

1) Suberized cell walls respond positively to staining tests for phenolics (*33*); and drastic depolymerization techniques, such as nitrobenzene oxidation, give rise to the aromatic aldehydes, such as vanillin and *p*-hydroxybenzaldehyde, which are also obtained from lignin under similar conditions (*7*).

2) The aromatic components of suberin are probably less substituted and contain fewer methoxy groups than those of lignin (*3, 7, 34*).

3) Depolymerization by alkaline hydrolysis or hydrogenolysis ($LiAlH_4$) liberates aliphatic components that are somewhat similar to those obtained from cutin, with the exceptions noted earlier.

4) Studies with suberizing tissues indicate that both phenolic acids (*35*) and fatty acids (*36*) are involved in the biosynthesis of suberin and that phenolic acids are not synthesized in tissue slices, which do not suberize their cell walls (*35*).

5) The composition of the aliphatic monomers suggests that hydroxyl groups are not in excess of the number of carboxyl groups present and that the aliphatic components themselves can hardly form an extensive polymer. Consistent with the idea that the aliphatic polymer participates in cross-linking of the phenolic matrix, the ω-hydroxyl groups of the aliphatic monomers in potato suberin are not free (*3*).

Even though there is little doubt that suberin contains both phenolics and aliphatics, the possibility that the two polymers are not covalently attached to each other cannot be ruled out. Isolation of fragments representing the proposed linkages between the aromatic and aliphatic components would be a major advance in our understanding of this polymer.

Biosynthesis of Cutin

Application of modern biochemical methods to the biosynthesis of the polyesters began with the finding that rapidly expanding leaves of *Vicia faba* incorporated labeled precursors into cutin (*37*). Subsequent experiments with labeled precursors and labeled synthetic intermediates demonstrated the steps shown in Fig. 4 in tissue slices. More recently, the enzymes involved in every step in this pathway have been demonstrated in cell-free preparations (*2*).

The most common members of the C_{16} family of monomers of this polyester are generated by ω-hydroxylation of palmitic acid, with subsequent hydroxylation at C-10 or C-9. This sequence was suggested (i) by the demonstration that exogenous [1-^{14}C]palmitic acid was incorporated into both ω-hydroxypalmitic acid and dihydroxypalmitic acid and (ii) by the direct conversion of exogenous labeled ω-hydroxypalmitic acid to dihydroxypalmitic acid in slices of fruit skin and leaves. The present ω-hydroxylation reaction is the only instance of ω-oxidation of a fatty acid with a known function. It is catalyzed by particulate preparations from *V. faba* with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and O_2 as the required cofactors (*38*). This hydroxylating enzyme, located in the endoplasmic reticulum, was inhibited by agents that inhibit mixed function oxidases such as metal ion chelators, NaN_3, and thiol-directed reagents. Involvement of cytochrome P-450 in this reaction remains in doubt because light did not reverse the inhibition caused by CO.

The introduction of the mid-chain hydroxyl group involves a direct hydroxylation, rather than hydration of an olefin (*39*). This mid-chain hydroxylation, catalyzed by enzymes located in the endoplasmic reticulum, required NADPH and O_2 as cofactors, and photoreversible inhibition by CO demonstrated the involvement of cytochrome P-450 (*38*). This reaction was also inhibited by thiol-directed reagents as well as by inhibitors of mixed function oxidases such as NaN_3 and metal ion chelators. Even though both ω-hydroxylation and C-10 hydroxylation occur in the endoplasmic reticulum, the difference in the sensitivity of the two reactions to CO suggests that two different hydroxylases are involved. It appears that the two hydroxylated monomers generated by the endoplasmic reticulum are subsequently transported to the extracellular location, possibly as the activated thioesters (*40*), and are incorporated into the growing polymer. However, little is known about how the monomers are transported to the site of the polymer synthesis.

The biosynthetic pathway for the C_{18} family of monomers (Fig. 4) was suggested by the specific incorporation of exogenous, labeled oleic acid, linoleic acid, and linolenic acid, but not of stearic acid, into the appropriate 18-hydroxy acids, 18-hydroxy-9,10-epoxy acids, and 9,10,18-trihydroxy acids in slices of fruit skin and leaves (*41*). Conversion of exogenous, labeled ω-hydroxyoleic acid into the epoxy and trihydroxy acids and the direct conversion of synthetic labeled 18-hydroxy-9,10-epoxyoleic acid into

9,10,18-trihydroxy stearic acid were also demonstrated with tissue slices (*42*). Pentahydroxystearic acid, which is a component of cutin in some plants (*43*), was shown to be derived from linoleic acid by stepwise epoxidation and hydration of the double bond at C-9 and subsequently at C-12 in the leaves of *Rosemarinus officinalis* (*44*).

The two reactions characteristic of the synthesis of the C_{18} family of acids, namely epoxidation and epoxide hydration, were demonstrated with cell-free preparations from leaves and fruit skin. Epoxidation of 18-hydroxyoleic acid was catalyzed by a particulate (the sediment from centrifugation at 3000*g*) preparation from young spinach leaves with NADPH, O_2, adenosine triphosphate (ATP), and coenzyme A (CoA) as required cofactors. This reaction, which apparently required activation of the carboxyl group seven methylenes away from the epoxidation site, involved cytochrome P-450 (*45*). A free hydroxyl group at the ω-carbon and the *cis* double bond were essential structural features for the substrate. Epoxide hydration of 18-hydroxy-9,10-epoxystearic acid was catalyzed by a similar particulate preparation obtained from the skin of rapidly expanding apple fruits (*46*). Consistent with the natural abundance of the *threo*-diastereoisomer in apple cutin, the hydrase generated *threo*-9,10,18-trihydroxystearic acid from 18-hydroxy-*cis*-9,10-epoxystearic acid. This enzyme, unlike the catabolic epoxide hydrases from animals, was specific for the 18-hydroxyepoxy acid substrate. It appears that epoxidation and epoxide hydration are catalyzed by enzymes that are closely associated with the cuticular matrix, and ω-hydroxy acids generated by the endoplasmic reticulum are presumably transported to these enzymes.

The synthesis of the polyester from the monomers involves transfer of monomers to the growing polymer from an activated intermediate. Thus, incorporation of labeled hydroxy acids into cutin, catalyzed by a particulate preparation containing cuticular membranes (3000*g* pellet) obtained from the excised epidermis of *V. faba* leaves, required ATP and CoA as cofactors (*47*). That the insoluble, labeled material generated in such preparations represented cutin synthesis was shown by the observation that only cutinase, but no other polymer-hydrolyzing enzymes, released the label from the insoluble residue. The polymer-synthesizing enzyme could be dissociated from the endogenous primers contained in such particulate preparations by ultrasonic treatment, resulting in a soluble enzyme preparation that required exogenous primers. With this primer-dependent enzyme preparation it

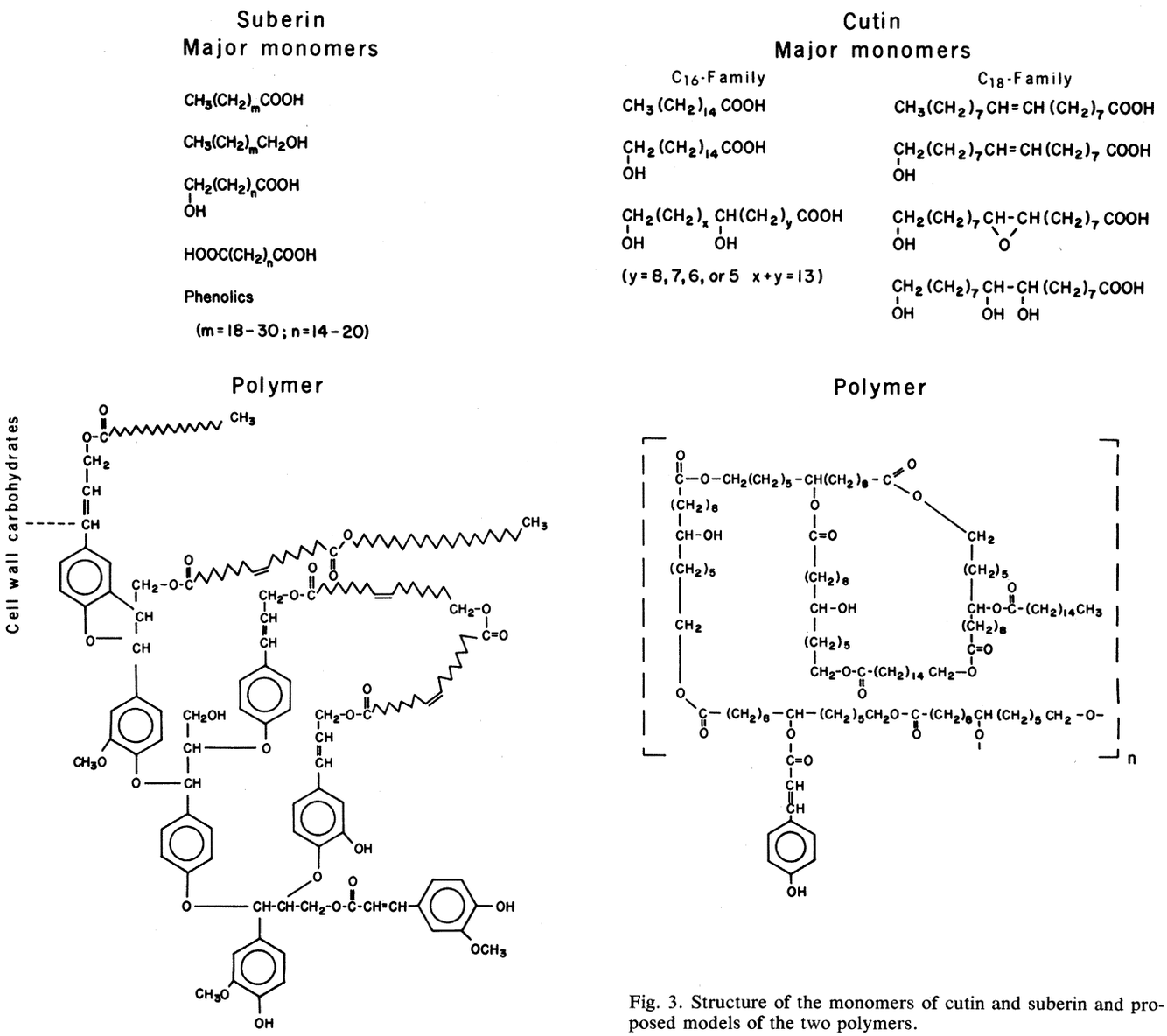

Fig. 3. Structure of the monomers of cutin and suberin and proposed models of the two polymers.

was shown that hydroxyacyl-CoA:cutin transacylase transferred the hydroxyacyl moiety to the free hydroxyl groups present in the primer (Fig. 4).

Biosynthesis of Suberin

The finding that wound-healing potato tuber disks synthesize suberin with a composition similar to that of the natural suberin of intact tubers (*16*) permitted the use of the wound-healing potato tissue for biosynthetic studies on suberin. From such studies, a probable pathway (Fig. 5) for the biosynthesis of the major aliphatic components of suberin was proposed (*2, 3*). Incorporation of exogenous labeled acetate and oleate into the aliphatic components of suberin suggested that the synthesis of the major suberin components, ω-hydroxyoleic acid and the corresponding dicarboxylic acid, occurred only after a 3-day lag period after wounding (*36*) although chain elongation (synthesis of aliphatic chains > C_{18}) was induced much earlier (*37, 48*). It is likely that a membranous elongating system catalyzes chain elongation with malonyl-CoA as the substrate and NADPH as the reductant. The CoA esters of the very long fatty acids thus generated are probably reduced by acyl-CoA reductase as described in other plant systems (*2*). In addition to the reactions mentioned above, ω-hydroxylation and other hydroxylation reactions (*38*), including the hydroxylation of the aromatic monomers of suberin, are also catalyzed by endoplasmic reticulum (*49*). Thus, the appearance of rough endoplasmic reticulum along the plasma membrane during the early phases of suberization and of smooth endoplasmic reticulum during the rapid deposition of suberin (*17, 18*) are consistent with the idea that, during the early phase, the membrane-bound ribosomes synthesize the microsomal enzymes that subsequently catalyze the synthesis of suberin.

The conversion of ω-hydroxy fatty acids to dicarboxylic acids is the unique reaction involved in the biosynthesis of the major aliphatic components of suberin. This activity appears in wound-healing potato disks after a 3-day lag period (*50*). Effects of actinomycin D and cycloheximide on suberization, and on the appearance of ω-hydroxy acid dehydrogenase activity, suggested that the transcriptional and translational processes directly responsible for the synthesis of these aliphatic components of suberin occurred between the third and fourth day after wounding. The conversion of ω-hydroxy acids to the corresponding dicarboxylic acids occurred in a two-step process involving a ω-oxo acid as the intermediate. ω-Hydroxy fatty acid dehydrogenase, the enzyme that catalyzes the first step, was induced during wound healing, but the ω-oxo acid dehydrogenase activity, necessary for the second step, was present in the fresh as well as in the wound-healing tissue.

ω-Hydroxy fatty acid dehydrogenase was purified to homogeneity from wound-healing potato tissue (*50*). This NADP specific enzyme is a dimer of 30,000-dalton protomers. Apparent K_m (Michaelis constant) values for NADP, 16-hydroxypalmitate, NADPH, and 16-oxo-palmitate were 100 μ*M*, 20 μ*M*, 5 μ*M*, and 7 μ*M*, respectively. The equilibrium constant at *p*H 9.5 and 30°C was $1.4 \times 10^{-9} M$. Unexpectedly, hydride from the A side of the nicotinamide of NADPH was transferred to the 16-oxo acid by the purified enzyme at nearly the same rate as that from the B side. Chemical modification studies and the effects of *p*H on K_m values gave information about the active site of ω-hydroxy fatty acid dehydrogenase (Fig. 5). An arginine residue participates in binding of the pyridine nucleotide while the ε-amino group of lysine interacts with the ω-carboxyl group of the substrate, and a histidine residue probably functions as the proton donor or acceptor. Studies of

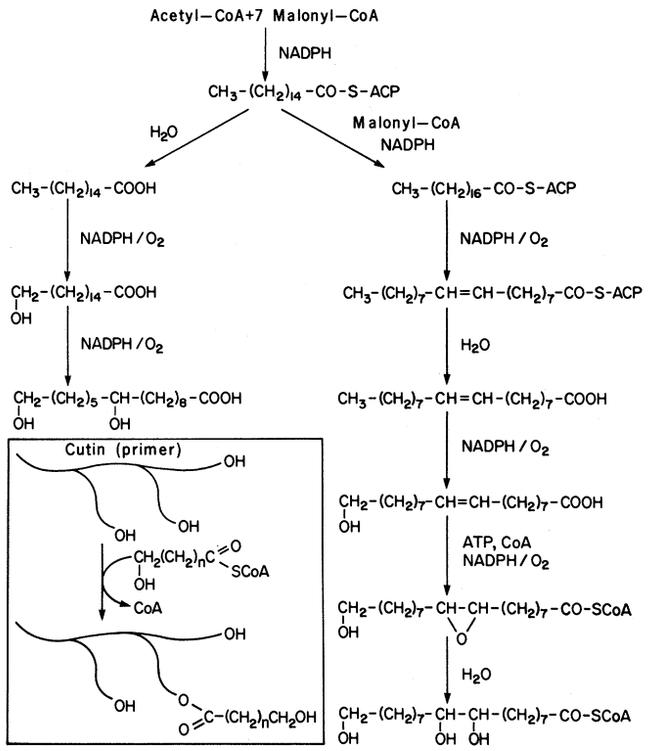

Fig. 4. Biosynthesis of cutin monomers and the polymer from the monomers (bottom left). *ACP*, acyl carrier protein.

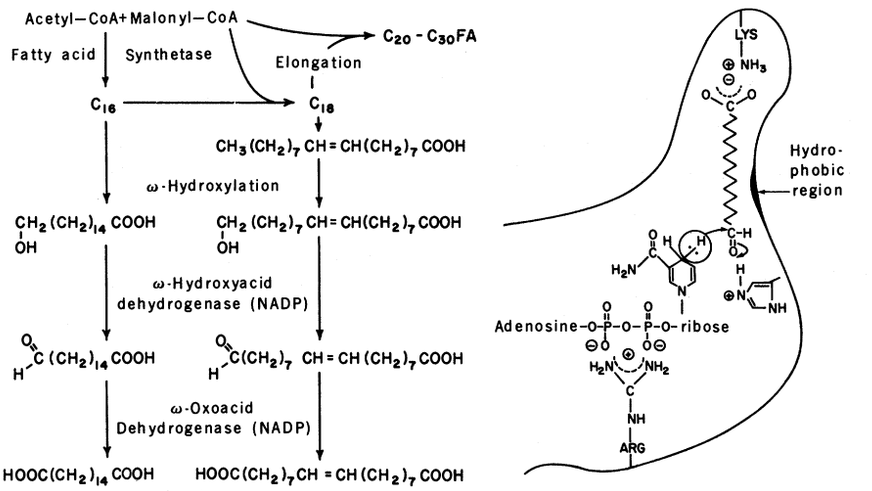

Fig. 5. Biosynthesis of the aliphatic monomers of suberin (left) and the proposed mechanism of action of ω-hydroxy fatty acid dehydrogenase (right).

substrate specificity strongly suggested that a hydrophobic region in the protein interacts with the aliphatic chain. It appears that the enzyme which catalyzes oxidation of ω-hydroxy fatty acid utilizes a mechanism similar to that used by other dehydrogenases, but has evolved a binding site which interacts not only with the aliphatic chain but also with the distal carboxyl group.

Regulation of Biosynthesis of the Polyesters

Cutin synthesis is a specialized function of the epidermal layer of cells. Even though this layer of cells is capable of incorporating labeled acetate into the hydroxy fatty acids of cutin in vitro (*3*), the internal photosynthetic tissue might contribute some fatty acids for cutin synthesis in intact leaves. Generally, the rate of synthesis of cutin, as measured by incorporation of exogenous labeled precursors (*37*), and the activity of the enzymes involved in cutin synthesis appeared to reflect the rate of expansion of the organ (*45, 47*).

Developmental and environmental factors can also bring about changes in the composition of cutin. For example, in the cutin of very young tissues and in dark-grown stems of *V. faba*, 16-oxo-9-hydroxypalmitic acid is a major component, whereas this acid becomes a minor component on full growth of the tissue and on further growth of the dark-grown stem in the light (*8, 37*). Neither the biochemical explanation for such changes nor the possible functional significance of cutin composition is known.

Suberin synthesis is a general response to wounding, whether injury occurs as a result of external factors or by crushing brought about by growth such as that in tree barks. However, such an injury-related trigger is not apparent in the suberization associated with many of the barrier layers, such as the epidermis, hypodermis, and endodermis of roots, as well as the internal barriers mentioned earlier. In any case, since wounding gives a signal to the one or two layers of cells beneath the wound surface to synthesize suberin, it would appear that such a system would be useful in elucidating the molecular events that trigger suberization. Thorough washing of potato tuber tissue slices removed abscisic acid from the tissue and prevented suberization, and exogenous abscisic acid at least partly restored suberization in the washed tissue (*51*). Also in potato tissue culture, exogenous abscisic acid induced suberization in a dose-dependent manner. However, the effect of abscisic acid is indirect because suberization could be severely inhibited by washing potato tissue slices even 3 days after wounding, although abscisic acid was not removed by this washing. Presumably abscisic acid triggers a series of biochemical events, resulting in the formation of a suberization inducing factor, which in turn induces the synthesis of the enzymes involved in suberization.

Biodegradation of Polyesters

There is little evidence for turnover of extracellular polyesters during any stage of growth of plant organs. However, in specialized situations, localized enzymatic breakdown of polyesters probably occurs during the life of a plant. During expansion of plant organs, the cuticular polymer might undergo some bond cleavages affording additional sites for further biosynthetic elongation. In this case, the basic mechanism might resemble that used in the synthesis of cell walls in expanding tissues (*52*) in that a polymer-hydrolyzing enzyme might be involved in the "break and extend" mechanism of polymer synthesis that occurs during expansion of the organ. Cutinase was suggested to be responsible for creating the opening in the polyester above the newly differentiated sunken stomata (*53*).

The presence of phytopolyesters in ancient sediments (*54*) and in sewage sludge (*55*) suggests that these polymers are rather refractory to biodegradation. However, enzymes responsible for the hydrolysis of cutin have been isolated from fungi, pollen, and mammals.

Fungal Degradation of Polyesters

Several plant pathogens and saprophytes can grow on cutin as the sole source of carbon (*56*), suggesting that such organisms excrete a cutin-hydrolyzing enzyme (or enzymes). Cutinase has been purified from the extracellular fluid of cutin-grown phytopathogenic fungi, including *Fusarium solani pisi*, *Fusarium roseum culmorum*, *Fusarium roseum sambucinum*, *Ulocladium consortiale*, *Helminthosporum sativum*, and *Streptomyces scabies* (*57, 58*). Enzymes from all of these sources are similar in size (~ 25,000 daltons), amino acid composition, *p*H optimum (~ 10.0), and substrate specificity. These fungal enzymes contain an active serine residue that participates in catalysis, and, therefore, they are severely inhibited by organic phosphates. They have no free SH groups and therefore are not inhibited by SH-directed reagents. Cutinase from *F. solani pisi* and probably those from other sources contain one disulfide linkage that is essential for enzymatic activity (*8*). Fungal cutinases also catalyze hydrolysis of small substrates such as *p*-nitrophenyl esters of short-chain fatty acids (butyrate) but not of long-chain acids (palmitate). They generate oligomeric esters from cutin and catalyze hydrolysis of these oligomers to monomers.

Induction of cutinase by the insoluble polymer raised the interesting question as to how such a polymer, which cannot be absorbed into the cell, induces the synthesis of the enzyme. Under conditions of starvation, *F. solani pisi* presumably excretes extremely low levels of hydrolytic enzymes, including cutinase. The small amounts of hydrolysate thus generated might enter the cell and induce cutinase. This hypothesis finds support in the finding that low concentrations of cutin hydrolyzate (~ 80 μg/ml) induced cutinase in glucose-grown cells after glucose in the medium was depleted (*59*). Under such conditions, cutinase constituted the major protein (< 80 percent) of the extracellular fluid. Individual cutin monomers and their analogs induced cutinase production, and the essential structural feature of the inducer was found to be an aliphatic alcohol containing at least 16 carbon atoms.

Fungal Cutinases: Novel Glycoproteins

The fungal cutinases thus far purified are glycoproteins containing 3.5 to 6 percent carbohydrate (*58*). Five out of the seven enzymes examined contained alkali-labile *O*-glycosidic linkages with carbohydrates, whereas the other two (from *S. scabies* and *H. sativum*) contained alkali-resistant linkages, presumably *N*-glycosidic linkages involving asparagine.

Analysis of the products of treatment of cutinases with tritiated sodium borohydride (NaB^3H_4) showed several unexpected products revealing the presence of novel structural features (Fig. 6). Cutinase I from *F. solani pisi* contained mannose, arabinose, glucosamine (*N*-acetyl-), and glucuronic acid attached by *O*-glycosidic linkages to four amino acid residues. Two of them, serine and threonine, occur commonly in other glycoproteins, whereas the other two, β-hydroxphenylalanine and β-hydroxy-

tyrosine have apparently not been heretofore found in any other glycoprotein. However, all of the fungal cutinases did not contain all four of the hydroxyamino acids in *O*-glycosidic linkage: serine was present in all five proteins, β-hydroxyphenylalanine was present in four, threonine in three, and β-hydroxytyrosine in two of the cutinases thus far examined.

Direct evidence for the presence of these novel amino acids in cutinase has been obtained (*60*). Induction of cutinase in glucose-grown cells by small amounts of cutin hydrolyzate permitted the use of labeled phenylalanine as a precursor of these hydroxyamino acids. The labeled cutinase derived from this precursor was treated with anhydrous HF to remove the carbohydrates. Enzymatic (Pronase) hydrolysis of the resulting protein produced labeled phenylalanine, tyrosine, β-hydroxyphenylalanine, and β-hydroxytyrosine. Since β-hydroxytyrosine and β-hydroxyphenylalanine are destroyed during routine chemical hydrolysis of proteins, it is not known whether such amino acids occur widely in nature. These hydroxyamino acids are perhaps generated by post-translational hydroxylation of phenylalanyl and tyrosyl residues in the protein.

The amino terminal glycine of cutinase from *F. solani pisi* is attached to glucuronic acid by amide linkage; this type of amino terminal block has not been found in any other glycoprotein (*58*). Cutinase II, another isozyme from this organism, also contained the same structural features as those found in cutinase I. The five fungal cutinases, which contained *O*-glycosidic linkages, also contained the glucuronamide blocking group at the amino terminus.

Induction of cutinase was accompanied by the excretion of very small quantities (< 4 percent of cutinase) of a nonspecific esterase that catalyzed hydrolysis of a variety of small esters but not of cutin (*57*, *58*). Evidence that this protein might be a precursor form of cutinase, representing incomplete post-translational processing of the enzyme has been summarized (*3*). In this precursor, the polysaccharide chains presumably prevent access of the macromolecular substrate (cutin), but not small substrates, to the active site of the precursor form of the enzyme. Removal of the bulk of the carbohydrates would leave monosaccharide residues on the polypeptide, and the resulting enzyme would be cutinase. If this hypothesis holds good, this would be the first instance of processing of a proenzyme into enzyme via cleavage of carbohydrates. On the basis of the experimental evidence thus far obtained, the tentative models shown in Fig. 6 were proposed for the structure of cutinase and its probable precursor.

Pollen Cutinase

Another instance where cutin degradation might play a biologically important role is in sexual reproduction of higher plants. Since incompatibility reactions in some plants involve lack of penetration of the pollen tube through the cuticle of the stigma, cutinase was suggested to be involved in incompatibility (*61*). Suggestive evidence for the presence of cutinase was provided when it was shown that the addition of a cutin preparation to germinating pollen caused a slight increase in titratable acidity (*62*). More recently, it was shown that germinating nasturtium (*Trapaeolum majus*) pollen excreted a cutinase that catalyzed the hydrolytic release of all types of monomers from biosynthetically labeled cutin. Experiments with actinomycin D and cycloheximide indicated that cutinase, excreted by germinating pollen, was already present in the mature pollen and was not synthesized during germination (*9*). Cutinase from nasturtium pollen, recently purified to homogeneity (*63*), is a single polypeptide of 40,000 daltons with an isoelectric point of 5.5; it contains carbohydrates (about 7 percent), which are attached to the peptide by linkages stable to alkali, presumably by asparaginyl residues. This enzyme has a low *p*H optimum (around 6.5), and is extremely sensitive to SH-directed reagents but not to "active" serine-directed reagents such as diisopropyl fluorophosphate. Thus, the plant cutinase appears to be an SH hydrolase, whereas the fungal cutinase is a serine hydrolase. The amino acid composition and other properties of the fungal cutinases are also quite different from those of the pollen enzyme.

Polyester Degradation by Mammals

Polyesters contained in fruits and vegetables are consumed by animals, and herbivores obviously consume fairly large quantities of such polymers. Although microscopic studies failed to detect degradation of cutin in ruminants (*64*), studies with radioactive cutin have provided conclusive evidence that cutin consumed by the rat is degraded, most probably by pancreatic lipase, and that the monomers are absorbed and catabo-

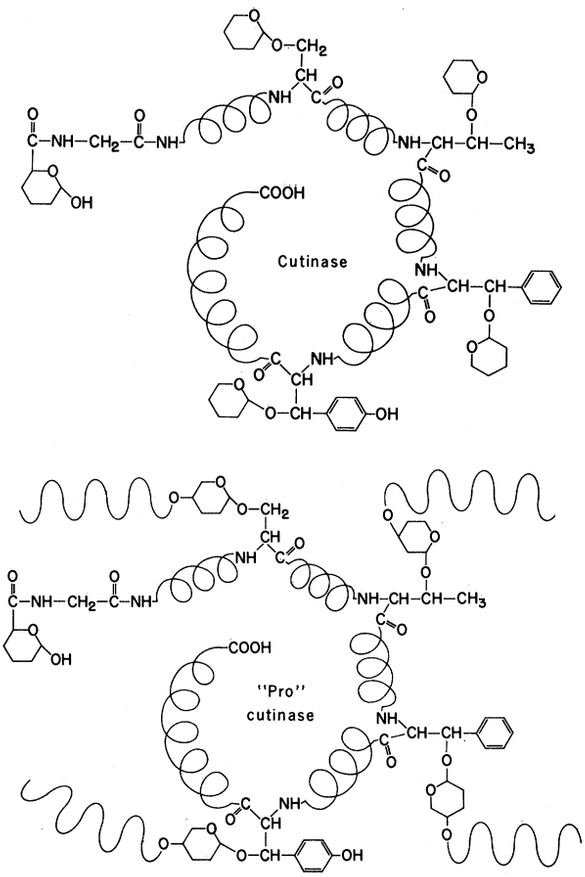

Fig. 6. Proposed model for cutinase and its possible precursor from the fungus *F. solani pisi*, illustrating the novel structural features found in these proteins. The pyranose ring represents *O*-glycosidically attached monosaccharide and the wavy lines represent oligosaccharide chains.

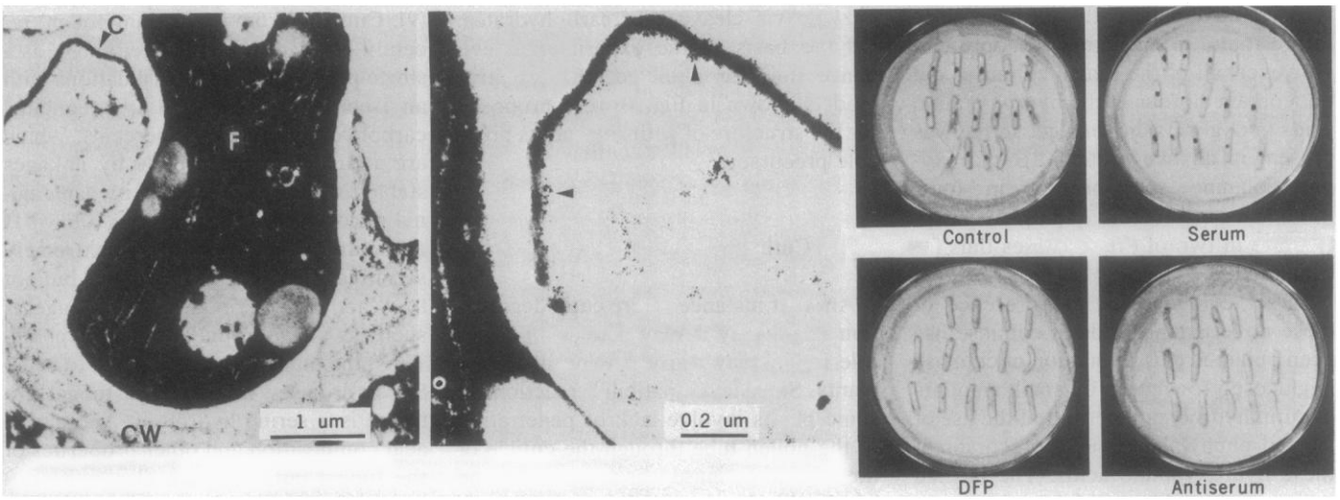

Fig. 7. (Left) Electron micrograph of the fungus *F. solani pisi* penetrating into the host plant (*P. sativum*) and (center) a magnified region of the cuticle. The dark spots (arrows) represent ferritin-conjugated antibody prepared against cutinase and thus show that this fungus excreted cutinase during penetration of the cuticle of its host. *C*, cuticle; *CW*, cell wall of the epidermis of the host tissue. (Right) Photograph of pea epicotyl segments 72 hours after inoculation with 5 μl of conidial suspension of *F. solani pisi* in water (*Control*), water containing 16 mg of protein per milliliter of immunoglobulin fraction from rabbit serum (*Serum*), water containing 1.75 mg of protein per milliliter of rabbit immunoglobulin from antiserum prepared against cutinase (*Antiserum*), and water containing 10 μM diisopropyl fluorophosphate (*DFP*). The dark spots represent infection.

lized (*31*). The enzyme that catalyzes hydrolysis of cutin in the hog was purified and identified as pancreatic lipase. Since pancreatic lipase from animals shows similar substrate specificity, it is most probable that the biopolyesters consumed by animals are at least partly degraded and utilized. Hydrolysis of dietary cutin would generate monomers, including potentially toxic epoxy acids. A microsomal epoxide hydrase, which catalyzes the hydration of the epoxy acid of cutin, was found in the intestinal walls of rabbits and rats, and this activity was especially high in the upper portions of the intestine (*7*). Such an enzyme probably plays an important role in detoxifying the epoxy acids, which are major components of cutin of fruits and of common vegetables such as spinach.

Function of Biopolyesters and Practical Implications

The major function of these polymers is that they constitute the structural component of extracellular biological barriers which, because of their metabolic inertness, do not require constant rebuilding. These barrier layers prevent diffusion of moisture or other molecules (or both) mainly because of the waxes deposited with the polymers (*20, 65*). The polyester barriers are, in general, weak cation exchangers, and hence are thought to play a role in ion uptake by plants as well as in leaching of ions from plants (*1*). Agricultural chemicals interact with the polyester barrier and associated waxes (*66*). This interaction is a crucial factor in the formulation of agricultural chemicals and in the practices used in the application and management of such chemicals.

The structural integrity of the plant cuticle is broken by pathogenic fungi causing extensive damage to plants and resulting in economic losses. The mode of penetration of cuticle by fungi has been controversial for the better part of a century (*1, 56, 67*). Arguments have been advanced favoring the hypothesis that the mere physical force of growth of the infection peg allows penetration, whereas, contrary to this hypothesis indirect evidence for the involvement of a cutin-hydrolyzing enzyme (cutinase) in this process has also been offered. With the availability of pure cutinase, it became possible to use ferritin-labeled antibodies and electron microscopy to test whether *F. solani pisi* excreted cutinase during penetration of its host *Pisum sativum* (*68*). The results were clearly positive (Fig. 7). Furthermore, specific inhibition of cutinase by antibodies and chemical inhibitors of this enzyme prevented infection of the host (*69*). This proof that enzymatic penetration of the cuticle is involved in pathogenesis not only helps to settle the long-standing controversy but also opens up the possibility of a totally new approach, targeted at cutinase, in combating fungi. In cases where fungal invasion involves enzymatic penetration of the cuticle, it might be possible to use less toxic "antipenetrants" in place of the highly toxic fungicides now in use. Even those fungi, which prefer entry through the so-called natural opening, namely stomata, must penetrate a cutin barrier in the substomatal cavity (*56*), and therefore they might also be prevented from infecting plants by stopping the action or the synthesis of cutinase.

Chemicals associated with cutin and suberin influence plant-pathogen interaction. Fatty alcohols, a common component of surface wax, stimulate fungal growth (*1*) and induce cutinase production by *F. solani pisi* (*59*). The phenolic acids, and possibly other toxic compounds, esterified to the polyesters might be released by the hydrolytic enzymes excreted by fungi and thus provide protection when needed. It might also be possible to covalently attach pesticidal chemicals to the cuticular polyester of growing plants so that they would not be leached out, but would be released only on attack by pests and thus afford timely protection with a minimal amount of pesticide.

Suberin has various functions in the normal development of a plant. For example, in the Casparian band of endodermis, the suberin layer helps to minimize apoplastic transport of water and solutes, as well as to protect the vascular tissue from microbial attack (*21, 70*). The suberin layer around the bundle sheaths of grasses is thought to have a major effect on CO_2 concentration, and hence on photosynthesis (*22*). The suberin in the pigment strands of grains is thought to control transport of materials into the grain during development (*25*). Suberin-containing barriers, such as those at the

base of secretory organs and the lining of juice sacs of citrus, function as a diffusion barrier, and thus protect the plant against the influx of secreted chemicals or moisture loss (*23*).

Suberin is also the defensive barrier which plant tissues synthesize to survive environmental threats involving wounding. For example, fungal infection triggers deposition of a polymeric material containing phenolic substances on the cell wall, and this barrier helps to prevent the spread of the invading pathogens in the host (*35, 71*). Also, mechanical wounding caused by any agent results in the deposition of a polymer containing phenolics and aliphatic components. Although the polymeric materials deposited on the cell wall under such conditions have been variously designated as, for example, lignin, induced lignin, lignin-like, and suberin, it appears likely that in most such cases the polymer represented wound-induced suberization, which is a general response to wounding in all organs (*16*). Whereas suberin is most probably a combination of aliphatic and aromatic components, the aforementioned polymers were examined for only the aromatics; therefore the terms suberin, lignin, and cutin were used too often without any reliable information on the total composition of the material. Induction of suberization would minimize water loss and decay, two of the major practical problems in dealing with roots and tubers in modern agriculture.

Conclusion

The extracellular polyesters of plants perform various functions both in the normal development of plants and in responding to environmental factors. We have just begun to understand the chemistry and biochemistry of these polymers. Very little is known about the intermolecular structure of the polymer. Whereas the biosynthetic pathways have been elucidated, none of the enzymes involved in the synthesis of this polymer has been purified, and little is known about the regulation of biosynthesis. The chemistry and biochemistry of suberin is even less well understood than those of cutin. One of the major problems in consolidating our knowledge about suberin is that this polymer has been examined, in many cases without recognizing it as suberin, for only the phenolic content or for only the aliphatic components but not for total composition and structure. Since suberin presents the complexity of lignin and contains polyester domains similar to that of cutin, a combination of techniques developed for lignin and cutin would be required to study this polymer. In view of the many important functions attributed to cutin and suberin in the life of plants, it is astonishing that so little is known about them. The novel chemical and biochemical problems that may be encountered in the study of these polymers should provide new intellectual challenges and their solutions hold much promise in devising new methods for the protection and utilization of plants.

References and Notes

1. J. T. Martin and B. E. Juniper, *The Cuticles of Plants* (St. Martin's, New York, 1970).
2. P. E. Kolattukudy and T. J. Walton, *Progr. Chem. Fats Other Lipids* **13**, 119 (1972); P. E. Kolattukudy, R. Croteau, J. S. Buckner, in *Chemistry and Biochemistry of Natural Waxes*, P. E. Kolattukudy, Ed. (Elsevier/North-Holland, New York, 1976), p. 290; P. E. Kolattukudy, in *Biochemistry of Wounded Plant Tissues*, G. Kahl, Ed. (de Gruyter, New York, 1978), p. 43; in *The Comprehensive Biochemistry of Plants*, P. K. Stumpf, Ed. (Academic Press, New York, 1980), vol. 4, p. 571.
3. P. E. Kolattukudy, *Recent Adv. Phytochem.* **11**, 185 (1977).
4. P. J. Holloway, E. A. Baker, J. T. Martin, *An. Quim.* **68**, 905 (1972).
5. D. H. Hunneman and G. Eglinton, *Phytochemistry* **11**, 1989 (1972).
6. A. B. Caldicott and G. Eglinton, *ibid.* **15**, 1139 (1976).
7. P. E. Kolattukudy, K. E. Espelie, T. S. Lin, R. E. Purdy, C. L. Soliday, K. Kronman, unpublished results.
8. P. E. Kolattukudy, R. Croteau, L. Brown, *Plant Physiol.* **54**, 670 (1974); K. E. Espelie, B. B. Dean, P. E. Kolattukudy, *ibid.* **64**, 1089 (1979).
9. M. Shaykh, P. E. Kolattukudy, R. Davis, *ibid.* **60**, 907 (1977).
10. P. J. Holloway, *Phytochemistry* **12**, 2913 (1973).
11. A. H. B. Deas and P. J. Holloway, in *Lipids and Lipid Polymers in Higher Plants*, M. Tevini and H. K. Lichtenthaler, Eds. (Springer-Verlag, New York, 1977), p. 293.
12. A. S. Crafts, *The Chemistry and Mode of Action of Herbicides* (Interscience, New York, 1961), p. 28.
13. N. D. Hallam and B. E. Juniper, in *The Ecology of Leaf Surface Microorganisms*, T. Preece and C. Dickinson, Eds. (Academic Press, New York, 1971), p. 3; H. S. Heide-Jorgensen, *Bot. Tidsskr.* **72**, 227 (1978).
14. C. H. Brieskorn and P. H. Binneman, *Z. Lebensm. Unters. Forsch.* **154**, 213 (1974); *Phytochemistry* **14**, 1363 (1975); B. Rodriguez-Miguens and I. Ribas-Marques, *An. R. Soc. Esp. Fis. Quim. Ser. B* **68**, 303 (1972).
15. P. E. Kolattukudy and V. P. Agrawal, *Lipids* **9**, 682 (1974); P. E. Kolattukudy, K. Kronman, A. J. Poulose, *Plant Physiol.* **55**, 567 (1975).
16. P. E. Kolattukudy and B. B. Dean, *Plant Physiol.* **54**, 116 (1974); B. B. Dean and P. E. Kolattukudy, *ibid.* **58**, 411 (1976).
17. N. Parameswaran, J. Kruse, W. Liese, *Z. Pflanzenphysiol.* **77**, 212 (1975); J. Wattendorff, *ibid.* **72**, 119 (1974).
18. P. Sitte, in *Lipids and Lipid Polymers in Higher Plants*, M. Tevini and H. K. Lichtenthaler, Eds. (Springer-Verlag, New York, 1977); p. 1; R. Barckhausen, in *Biochemistry of Wounded Plant Tissues*, G. Kahl, Ed. (de Gruyter, New York, 1978), p. 1.
19. P. Sitte, *Biochem. Physiol. Pflanz.* **168S**, 287 (1975); J. Wattendorff, *Z. Pflanzenphysiol.* **73**, 214 (1974); B. B. Dean, P. E. Kolattukudy, R. Davis, *Plant Physiol.* **59**, 1008 (1977).
20. C. L. Soliday, P. E. Kolattukudy, R. Davis, *Planta* **146**, 607 (1979).
21. D. S. van Fleet, *Bot. Rev.* **27**, 165 (1961); H. T. Bonnett, *J. Cell Biol.* **37**, 199 (1968); J. T. Tippet and T. P. O'Brien, *Aust. J. Bot.* **24**, 619 (1976); C. A. Peterson, R. L. Peterson, A. W. Robards, *Protoplasma* **96**, 1 (1978); A. J. Wilson and A. W. Robards, *ibid.* **95**, 255 (1978); P. Olesen, *ibid.* **94**, 325 (1978); M. G. Scott and R. L. Peterson, *Can. J. Bot.* **57**, 1040 (1979).
22. T. P. O'Brien and D. J. Carr, *Aust. J. Biol. Sci.* **23**, 275 (1970); W. M. Laetsch, *Annu. Rev. Plant Physiol.* **25**, 27 (1974); T. P. O'Brien and J. Kuo, *Aust. J. Bot.* **23**, 783 (1975); M. D. Hatch and C. B. Osmond, in *Encyclopedia of Plant Physiology (New Series)*, U. Huber and C. R. Stocking, Eds. (Springer Verlag, Berlin, 1976), vol. 3, p. 144; R. F. Evert, W. Eschrich, W. Heyser, *Planta* **138**, 279 (1978).
23. U. Lüttge, *Annu. Rev. Plant Physiol.* **22**, 23 (1971); E. Schnepf, in *Dynamic Aspects of Plant Ultrastructure*, A. W. Robards, Ed. (McGraw-Hill, New York, 1974), p. 331; P. Olesen, *Protoplasma* **99**, 1 (1979).
24. J. Wattendorff, Z. *Pflanzenphysiol.* **60**, 307 (1969); F. M. Scott and B. G. Bystrom, in *New Research in Plant Anatomy*, N. K. B. Robson, D. F. Cutler, M. Gregory, Eds. (Academic Press, New York, 1970), p. 15; J. Wattendorff, *Planta* **130**, 303 (1976); *Protoplasma* **95**, 193 (1978).
25. S. Y. Zee and T. P. O'Brien, *Aust. J. Biol. Sci.* **23**, 1153 (1970).
26. K. E. Espelie and P. E. Kolattukudy, *Lipids* **13**, 832 (1978).
27. R. Riley and P. E. Kolattukudy, *Plant Physiol.* **56**, 650 (1975).
28. P. J. Holloway, *Chem. Phys. Lipids* **9**, 158 (1972); *ibid.* **9**, 171 (1972); W. Jensen, *An. R. Soc. Esp. Fis. Quim. Ser. B* **68**, 871 (1972).
29. K. E. Espelie and P. E. Kolattukudy, *Plant Physiol.* **63**, 433 (1979); *Plant Sci. Lett.* **15**, 225 (1979).
30. V. P. Agrawal and P. E. Kolattukudy, unpublished results; L. A. Wilson and C. Sterling, *Z. Pflanzenphysiol.* **77**, 359 (1976).
31. A. J. Brown and P. E. Kolattukudy, *J. Agric. Food Chem.* **26**, 1263 (1978); *Arch. Biochem. Biophys.* **190**, 17 (1978).
32. K. Freudenberg, in *Constitution and Biosynthesis of Lignin*, K. Freudenberg and A. C. Neish, Eds. (Springer-Verlag, New York, 1968), p. 47; Y. Z. Lai and K. V. Sarkanen, in *Lignin: Occurence, Formation, Structure and Reactions*, K. V. Sarkanen and C. H. Ludwig, Eds. (Wiley, New York, 1971), p. 165.
33. M. G. Scott and R. L. Peterson, *Can. J. Bot.* **57**, 1063 (1979).
34. E. P. Swan, *Pulp Pap. Mag. Can.* **67**, T456 (1966); Y. Asada, T. Ohguchi, I. Matsumoto, in *Biochemistry and Cytology of Plant-Parasite Interaction*, K. Tomiyama, J. M. Daly, I. Uritani, H. Oku, S. Ouchi, Eds. (Elsevier, New York, 1976), p. 200.
35. G. Kahl, *Bot. Rev.* **40**, 263 (1974).
36. B. B. Dean and P. E. Kolattukudy, *Plant Physiol.* **59**, 48 (1977).
37. P. E. Kolattukudy, *Biochem. Biophys. Res. Commun.* **41**, 299 (1970); *Plant Physiol.* **46**, 759 (1970); *Biochemistry* **13**, 1354 (1974).
38. C. L. Soliday and P. E. Kolattukudy, *Plant Physiol.* **59**, 116 (1977); *Arch. Biochem. Biophys.* **188**, 338 (1978).
39. P. E. Kolattukudy and T. J. Walton, *Biochemistry* **11**, 1897 (1972).
40. T. J. Walton and P. E. Kolattukudy, *Biochem. Biophys. Res. Commun.* **46**, 16 (1972).
41. P. E. Kolattukudy, T. J. Walton, R. P. S. Kushwaha, *Biochemistry* **12**, 4488 (1973).
42. R. Croteau and P. E. Kolattukudy, *Arch. Biochem. Biophys.* **162**, 471 (1974).
43. C. H. Brieskorn and L. Kabelitz, *Phytochemistry* **10**, 3195 (1971).
44. R. Croteau and P. E. Kolattukudy, *Arch. Biochem. Biophys.* **162**, 458 (1974).
45. ———, *ibid.* **170**, 61 (1975).
46. ———, *ibid.* p. 73.
47. ———, *Biochem. Biophys. Res. Commun.* **52**, 863 (1973); *Biochemistry* **13**, 3193 (1974).
48. P. Bolton and J. L. Harwood, *Phytochemistry* **15**, 1501 (1976); C. Willemot and P. K. Stumpf, *Can. J. Bot.* **45**, 570 (1967).
49. J. M. Rhodes and L. S. C. Wooltorton, in *Biochemistry of Wounded Plant Tissues*, G. Kahl, Ed. (de Gruyter, New York, 1978), p. 243.
50. V. P. Agrawal and P. E. Kolattukudy, *Plant Physiol.* **59**, 667 (1977); *Arch. Biochem. Biophys.* **191**, 452 (1978); *ibid.* **191**, 466 (1978).
51. C. L. Soliday, B. B. Dean, P. E. Kolattukudy, *Plant Physiol.* **61**, 170 (1978).
52. Y.-S. Wong, G. B. Fincher, G. A. Maclachlan, *Science* **195**, 679 (1977); *J. Biol. Chem.* **252**, 1402 (1977).
53. D. J. Carr and S. G. M. Carr, *Protoplasma* **96**, 127 (1978).
54. G. Eglinton, D. H. Hunneman, K. Douraghi-Zadeh, *Tetrahedron* **24**, 5929 (1968); D. H. Hunneman and G. Eglinton, in *Advances in Organic Geochemistry, 1968*, P. A. Schneck and I. Havenaar, Eds. (Pergamon, Oxford, 1969), p. 157.
55. P. E. Kolattukudy and R. E. Purdy, *Environ. Sci. Technol.* **7**, 619 (1973).

56. W. Heinen and H. de Vries, *Arch. Mikrobiol.* **54**, 331 (1966); J. Shishyama, F. Arakai, S. Akai, *Plant Cell Physiol.* **11**, 937 (1970); L. Hankin and P. E. Kolattukudy, *Plant Soil* **34**, 525 (1971); R. E. Purdy and P. E. Kolattukudy, *Arch. Biochem. Biophys.* **159**, 61 (1973); G. van den Ende and H. F. Linskens, *Annu. Rev. Phytopathol.* **12**, 247 (1974).
57. R. E. Purdy and P. E. Kolattukudy, *Biochemistry* **14**, 2824 (1975); *ibid.*, p. 2832; C. L. Soliday and P. E. Kolattukudy, *Arch. Biochem. Biophys.* **176**, 334 (1976).
58. T. S. Lin and P. E. Kolattukudy, *Biochem. Biophys. Res. Commun.* **72**, 243 (1976); *ibid.* **75**, 87 (1977); *Physiol. Plant Pathol.*, in press; *Eur. J. Biochem.* in press.
59. T. S. Lin and P. E. Kolattukudy, *J. Bacteriol.* **133**, 942 (1978).
60. ———, *Arch. Biochem. Biophys.* **196**, 255 (1979).
61. M. Kroh, in *Pollen Physiology and Fertilization*, H. F. Linskens, Ed. (North-Holland, Amsterdam, 1964), p. 221; J. Heslop-Harrison, *Annu. Rev. Plant Physiol.* **26**, 403 (1975).
62. W. Heinen and H. F. Linskens, *Nature (London)* **191**, 1416 (1961); H. F. Linskens and W. Heinen, *Z. Bot.* **50**, 338 (1962).
63. I. B. Maiti, P. E. Kolattukudy, M. Shaykh, *Arch. Biochem. Biophys.* **196**, 412 (1979).
64. N. J. Chatterton and J. B. Powell, *Agron. J.* **66**, 812 (1974).
65. J. Schönherr, *Planta* **131**, 159 (1976).
66. W. van Valkenburg, Ed., *Pesticide Formulations* (Dekker, New York, 1973), p. 481; H. M. Hull, H. L. Morton, J. R. Wharrie, *Bot. Rev.* **41**, 421 (1975); J. Schönherr, in *Advances in Pesticide Science*, H. Geissbühler, Ed. (Pergamon, Oxford, 1979), part 3, p. 392.
67. J. T. Martin, *Annu. Rev. Phytopathol.* **2**, 81 (1964); D. J. Royle, in *The Biochemical Aspects of Plant-Parasite Relationships*, J. Friend and D. R. Threlfall, Eds. (Academic Press, New York, 1975), p. 161; J. R. Aist, in *Encyclopedia of Plant Physiology, New Series*, R. Heitefuss and P. H. Williams, Eds. (Springer-Verlag, Berlin, 1976), vol. 4, p. 197.
68. M. Shaykh, C. L. Soliday, P. E. Kolattukudy, *Plant Physiol.* **60**, 170 (1977).
69. I. B. Maiti and P. E. Kolattukudy, *Science* **205**, 507 (1979).
70. D. T. Clarkson and A. W. Robards, in *The Development and Function of Roots*, J. G. Torrey and D. T. Clarkson, Eds. (Academic Press, London, 1975), p. 415; I. B. Ferguson and D. T. Clarkson, *New Phytol.* **77**, 11 (1976).
71. J. Friend, in *Biochemical Aspects of Plant-Parasite Relationships*, J. Friend and D. R. Threlfall, Eds. (Academic Press, New York, 1976), p. 195; I. Uritani and K. Oba, in *Biochemistry of Wounded Plant Tissues*, G. Kahl, Ed. (de Gruyter, Berlin, 1978), p. 287.
72. I thank Dr. W. W. Thompson and R. W. Davis for providing the electron micrographs, and Drs. C. A. Ryan and R. Croteau for reading the manuscript. Supported by NSF grant PCM 7700927 and a grant from Washington State Tree Fruit Commission. Scientific Paper No. 5415, College of Agriculture Research Center, Washington State University, Pullman 99164.

Contribution of the Ocean Sector to the United States Economy

Giulio Pontecorvo, Maurice Wilkinson
Ronald Anderson, Michael Holdowsky

It is usually possible to discern the progress achieved and also the difficulties not yet overcome at different periods in the development of fields of inquiry by examining the technical tools which are used contemporaneously (1).

We report here on a project that has led to the creation of a new subdivision within the national income accounting system (NIAS). This subdivision divides gross national product (GNP) into two parts: an ocean sector and an all-other component (*2*). In 1972, with the GNP of the United States some $1171.1 billion, the aggregate value of the ocean sector was $30.6 billion. Although this number may at first glance seem small, it is roughly comparable to such industries as agriculture, mining, transportation, and communications.

In this article we describe the methodology and the calculation of the value of the ocean sector and its several subsectors (fisheries and coastal zone, for example) and address a number of specific interrelated problems:

1) The need to develop the statistical underpinning for the analysis of our ocean activity. This need stems primarily from the several pieces of legislation passed in the 1970's that involve substantive intervention by government in economic markets involving ocean economic activity (*3*).

2) Evaluation of the manner in which the value of government activity is measured within the NIAS. This is particularly important in the ocean sector where government accounts for the size of the sector (roughly one-third), and where government regulation has a major impact on the allocation of resources in numerous subsectors.

3) To suggest a methodology that may extend the utility of the NIAS in certain regional and natural resource problem areas, for example, ocean and agricultural policy.

4) The need to review the importance and the implications of the microeconomic theory underlying the NIAS for the measurement of economic activity, especially in the areas where market prices do not exist.

The new account measures the contribution of the ocean sector to the GNP, and is the first within the NIAS to be defined essentially on a spatial rather than a production sector basis. The ocean sector account measures both the aggregate contribution of the ocean to the GNP and the relative value of the several subsectors of the account, such as fisheries, transportation, and mining. Creation of the account may lead to model building that will analyze the linkages between the ocean sector and the rest of the economy. Such efforts would contribute to the U.S. ocean policy.

Why do we need an ocean policy, and what role, if any, would the ocean sector account in the NIAS play in the development of such policy? Although there is no simple answer we have shown previously that there is an economic need for such policies (*4*, *5*). The bases for the political and legal need are to be found in reports of the Stratton Commission (*6*), the Senate Commerce Committee (*7*), and in reports of the annual meetings of the Law of the Sea Institute, and the De-

Summary. The national income accounts have been reorganized to estimate the contribution to the gross national product of the ocean sector and its various subsectors for the year 1972. The new account is the first within the national income accounts to be organized along geographic, rather than productive, sectors. If properly updated and disseminated, this new account will give government and business interests a solid and consistent data base to measure, and choose among, the alternative uses of the oceans.

Drs. Pontecorvo and Wilkinson are professors and Dr. Anderson is an assistant professor at the Graduate School of Business and Mr. Holdowsky is a doctoral candidate in economics, Columbia University, New York 10027.

0036-8075/80/0530-1000$01.75/0