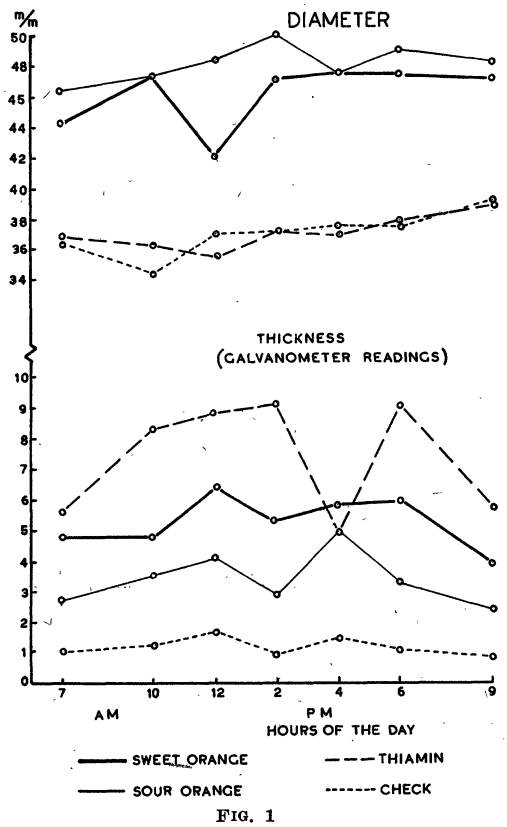

FIG. 1

on *Phytophthora*—in living plant material. Disks of the tissues, such as the bark of citrus trees, 5 mm in diameter, are detached aseptically and placed on the surface of a water-agar or Czapek-agar medium in Petri dishes. The disk is removed after 4 hr, and the place is inoculated with *Phytophthora*. Ordinary agar (*Gelidium*) contains factor *L*, but its effect is slight at the concentration of 2% used in these experiments. In 4%, and especially in 6%, water-agar, *Phytophthora* in 3 days produces thalli made up of extremely sparse hyphae, up to a diameter of 4 cm.

Using this method, the thalli obtained with the bark of sweet orange, a susceptible species, were slightly but significantly larger than those of sour orange (*C. aurantium* L.), a highly resistant species. The thalli obtained with Coronel, a highly susceptible variety of sweet orange, were larger than those of the Pera variety which is rather resistant (*4*).

In the following experiment the effect of both factor *L* (diam of thalli) and thiamin (thickness of mycelial mat as indicated by galvanometer readings) was measured. Disks of bark of 5 sweet orange trees and 5 sour orange trees were detached aseptically with a cork-borer at 7:00, 10:00, and 12:00 A.M. and at 2:00, 4:00, 6:00, and 9:00 P.M. Large, 15-cm Petri dishes, containing 2% Czapek agar, received one disk of sweet orange bark, one of sour orange bark, and one of filter paper containing 0.4 μg thiamin. After 4 hr the disks were removed, and the corresponding place was inoculated with *C. citrophthora* (Sm. & Sm.) Leonian. As a check a fourth inoculation was made at another point.

The results are shown in Fig. 1. Each point represents the mean of 5 readings, except in a few cases in which only 4 readings were made. It is seen that there is no significant difference in the diameter of cultures at the places where disks of sour orange and sweet orange bark had been placed, but they were significantly larger than the controls and the thalli of the thiamin disks. As in previous experiments, thiamin did not affect the diameter.

The thickness of the mycelial mat as shown by the galvanometer readings was greatest for the thiamin disks and was substantially greater for the sweet orange disks than for the sour orange ones. No indication of an hourly change in the above effects is detectable.

This experiment, as well as previous ones, gives support to the theory that the differences in susceptibility of citrus species to *Phytophthora* are due, at least in part, to the amount of growth substances in the bark of the host. Factor *L* would account for the differences between varieties of sweet orange, whereas thiamin, or substances producing the same effect as thiamin on *Phytophthora*, would account for the difference between the sweet orange and the sour orange.

Lesions of *Phytophthora* gummosis show a characteristic concentric zoning in the inner bark of citrus, each zone corresponding to a period of 24 hr. This zoning can be explained by a periodicity of growth of the fungus in the bark, and such a periodicity might be the result of changes in the amount of growth substances in the bark during the period of 24 hr. No indication of such a periodicity was found in this experiment.

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Studies on Arthropod Cuticle. VII, Patent and Masked Carbohydrate in the Epicuticle of Insects[1,2]

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The histochemical test for polysaccharides and glycoproteins involving the application of Schiff's re-

[1] Paper No. 2693, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

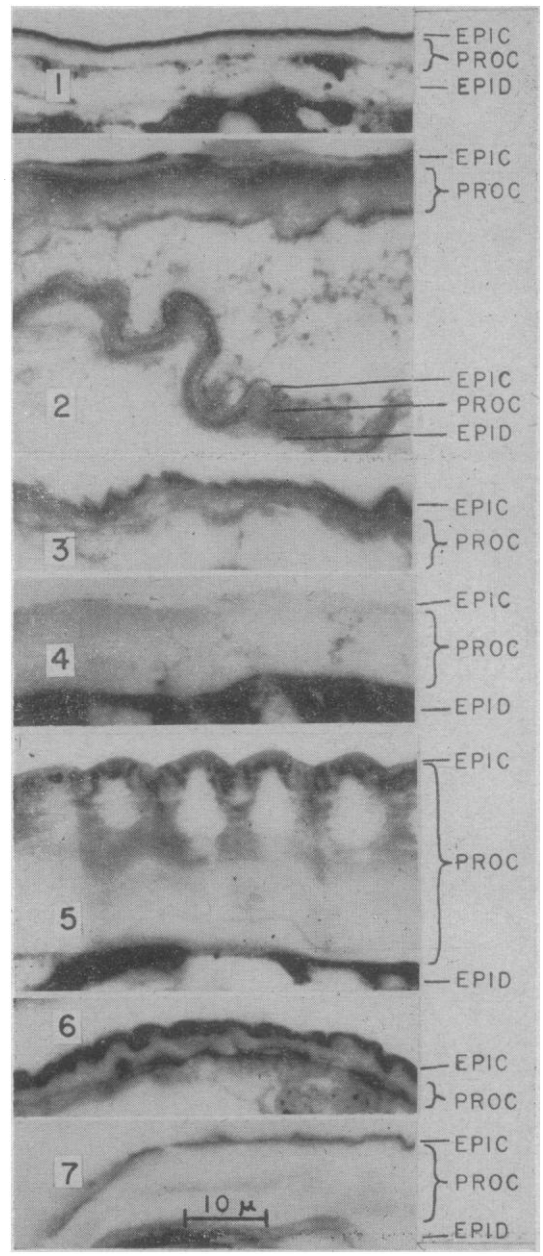
[2] The work described in this paper was done under terms of a contract between the Office of the Surgeon General, U. S. Army, and the University of Minnesota. Under the terms of this contract the Army neither restricts nor is responsible for the opinions or conclusions of the author.

agent after oxidation with periodic acid (*1*) is not always positive even though carbohydrate may demonstrably be present. This can readily be shown by examination of the staining reactions of cuticle sections from insects. The procuticle fraction of these cuticles contains the polysaccharide chitin, usually to the extent of 25–60% of the dry weight (*2*). Pure chitin gives an intense red color with this test (provided the sample has not been air-dried); soft procuticle, especially in early stages of cuticle development, gives a pink to light-red color; lightly sclerotized cuticle that has not yet developed any distinct coloration of its own stains only a very faint pink or, in some species, not detectably; more strongly sclerotized cuticles, ranging in natural coloration from light- to dark-amber, are not detectably altered in color by this technique. However, sclerotized cuticle that is negative to this reaction may be treated with hot 10% NaOH solution to purify the chitin; it then gives an intense reaction. With the fairly well-known chemical chitin (*2*), then, we have a polysaccharide which in its natural condition may be either patent or masked to the periodic acid–Schiff's reaction.

A similar situation is encountered with the insect epicuticle, the extremely thin outer subdivision of the cuticle, except that here the carbohydrate has not been identified. The cuticle of insects and other arthropods consists typically of (1) a relatively thick inner chitin-protein portion called the procuticle (which may become differentiated into a sclerotized exocuticle and an unsclerotized endocuticle), and (2) a very thin outer portion characterized by the absence of chitin or at least by being dispersed by hot alkaline solutions and hence negative to the van Wisselingh color test for chitin detection. Qualitative reactions have been used to indicate that the epicuticle contains protein, lipid (probably as lipoprotein), sterol, waxes, polyphenols, and perhaps enzymes involved in sclerotization (*2, 3*). There are no reports in the literature of a carbohydrate being present in the epicuticle, but an unidentified carbohydrate was extracted from *whole* cuticles by Trim (*4*).

In the course of a study in progress on the cuticle of bees, it was noticed that the epicuticle sometimes did not stain red after the periodic acid–Schiff's reagent treatment. Accordingly, a short survey was made covering various representative insect groups and stages. The technique of Hotchkiss (*1*) was followed, using his acid solution B. (A few tests with acid solution A, which aids in the retention of water-soluble compounds, gave the same results.) Control slides were run concurrently with the omission of oxidation by periodic acid; they were consistently negative. Test sections included the adult integument of the firebrat (*Thermobia domestica*), a cockroach (*Periplaneta americana*), a beetle (*Tribolium confusum*), and the honeybee (*Apis mellifica*); and larval or nymphal integuments of a bug (*Sinea diadema*), the wax moth (*Galleria mellonella*), a mosquito (*Aedes aegypti*), and a blowfly (*Sarçophaga bullata*).

The epicuticle was recognizable as a single red line in the larva of *Aedes* (Fig. 1) and in the spiracular chamber and tracheae of a teneral adult of *Tribolium*. The entire cuticle was stained pink without the epicuticle being distinguishable from the procuticle in larvae of *Galleria* (Fig. 5) and in prospective sclerites



FIGS. 1–7. Photomicrographs of 4-μ sections of specimens fixed in Carnoy's fluid and stained only with the periodic acid–Schiff's reagent. All at same magnification. *EPIC* = epicuticle, *EPID* = epidermis, *PROC* = procuticle. (1) Larva of *Aedes aegypti*, (2) larva of *Sarcophaga bullata* showing loosened second instar cuticle with the developing third instar cuticle beneath it, (3) intersegmental membrane of a teneral adult of *Periplaneta americana*, (4) prospective sclerite of a teneral adult of *P. americana*, (5) larva of *Galleria mellonella*, (6) developing intersegmental membrane of adult antenna from old pupa of *Apis mellifica*, (7) intersegmental membrane of antenna from a teneral adult of *A. mellifica*.

of a teneral adult of *Periplaneta* (Fig. 4). The epicuticle was recognizable as composed of two layers, only the outer of which stained red, in the intersegmental membranes of *Thermobia, Periplaneta* (Fig. 3), and adult *Apis* (Fig. 7), in prospective adult sclerites from old *Apis* pupae, and in both larval and puparial cuticles of *Sarcophaga* (Fig. 2). The outer epicuticle stained red, the inner epicuticle pink, in early developmental stages of the adult cuticle in the pupa of *Apis* (Fig. 6). The epicuticle was unstained, and the procuticle nearly or completely negative also, on the dorsum of *Thermobia,* partly or fully sclerotized sclerites of adult *Periplaneta* and *Apis,* and both sclerites and intersegmental membranes of *Sinea* and *Tribolium.*

Accepting this demonstration of a carbohydrate (polysaccharide?) in the epicuticle of numerous insect species, the question of how to interpret the case of those species that give negative results arises. An answer for the honeybee is available. When the adult cuticle is being formed late in pupal life, the outer epicuticle stains red and the inner epicuticle stains pink over all of the antenna (Fig. 6); later in pupal life the outer epicuticle of the sclerites stains only pink, the inner epicuticle seemingly not at all; and still later, at the time of emergence of the adult from the pupa, the outer epicuticle of the intersegmental membrane stains red (Fig. 7), that over the sensory pore plates stains pink, and that over the general sclerites and setae does not stain at all. Less complete data outlined in the preceding paragraph suggest that the same explanation will hold for *Periplaneta* and *Tribolium.* Also, it seems relevant to point out an observed correlation: when the epicuticle stains intensely, the procuticle stains moderately, whereas when the epicuticle is negative, the chitin-containing procuticle is also negative (*Apis, Sinea,* and *Tribolium*) or nearly so (*Thermobia*). Admitting that negative evidence is not proof, the suggestion is still obvious that the insect epicuticle consistently contains a carbohydrate component but that in some species or regions the carbohydrate becomes masked by sclerotization processes.

To the biologist, demonstration of a carbohydrate in the epicuticle is of minor importance in comparison to the concomitant demonstration that epicuticle may vary in composition not only from species to species but also from area to area on one species. Thus, commonly, two types of epicuticle can be shown on one species, and in the honeybee at least three types can be demonstrated on the antennae.[3] Since the work of Wigglesworth and Beament has shown that the epicuticle is the major portion of the effective barrier between the insect and its environment (at least for the passage of water) (*3*), the demonstration of local gross variations is important and, unfortunately, complicates precise analyses of penetration through the cuticle.

[3] Visible differentiation can also be made with Mallory's connective tissue stain, which at different developmental stages and on various areas gives red, blue, purplish, or no staining to the epicuticle of honeybee antennae.

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The Fluoride Content of Placental Tissue as Related to the Fluoride Content of Drinking Water[1]

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Current programs to fluoridate water supplies as a dental caries control measure have stimulated interest in the extent to which the fluoride might be retained by the various tissues of the body. Considerable evidence is available to show that much of the fluoride is excreted in the urine, but there is little information on the possible accumulation or storage of the fluoride not excreted.

A previous study of normal human blood fluoride concentrations of residents of Rochester and Newburgh, N. Y. (*1*), has disclosed blood fluoride as a function of the fluoride concentration in drinking water. Higher levels of blood fluoride were noted for residents of Newburgh, where the fluoridated water supply contains 1.0–1.2 ppm fluoride, than for residents of Rochester, where the supply contains approximately 0.06 ppm.

In this investigation samples of placentae were obtained from the afterbirth of normal patients residing in Rochester and Newburgh, and the fluoride contents determined by the method of Smith and Gardner (*2*) as described for blood. Table 1 shows the distribution of the fluoride content of the placental samples as found for the two cities.

Of the Rochester samples 58% contained less than 50 μg/100 g of tissue, whereas only 17% of the Newburgh samples were in this range. Only one Rochester sample contained more than 200 μg/100 g, but 6 of the Newburgh samples contained more than this concentration. The Rochester samples had a mean concentration of 0.74 ppm fluoride and the Newburgh samples 2.09 ppm, almost three times as much. In the study of blood fluoride concentrations (*1*) the Rochester samples had a mean value of 0.014 ppm fluoride and the Newburgh samples 0.040 ppm—also almost three times as much. Thus the increased level of

[1] This article is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.