water could facilitate such plastic flow is that hydrolysis takes place along pre-existing dislocation lines where Si — O — Si bridges are already broken. As the temperature is raised this model would suggest that the silanol groups become sufficiently mobile to move with the dislocations, breaking and re-forming Si — O — Si bridges as they go. If the number of dislocations were 10^9 cm^{-2}, as found in natural quartz (*5*), then only one one-thousandth of the observed water would take part in this dislocation motion. Whatever the precise mechanism, the process is thermally activated, presumably as the mobility of the silanol groups is increased sufficiently.

Since all silicates have Si — O — Si or Si — O — M bridges (where M is a metal ion) which are susceptible to this type of hydrolysis, this temperature-induced water weakness may apply to silicates in general. We have observed such weakness in olivine and feldspar rocks, deformed in a hydrous environment.

These observations raise the possibility of great weakness in the earth's deeper crust and outer mantle at temperatures far below the melting point.

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Permeability of Insect Cuticle to Water and Lipids

Abstract. *Insect cuticle presents a paradox: permeability to water may vary at different times, while lipids penetrate readily. Electron microscopy shows that the epicuticle is penetrated by filaments of wax 60 to 130 Å in diameter. These are believed to be lipid-water liquid crystals in the middle phase. The variable permeability to water, and other properties of the cuticle, may be due to phase changes.*

Some components of insect cuticle are among the most impermeable to water of all natural membranes. Typical rates of water loss by insects are lower than 0.1 mg cm^{-2} hr^{-1} (*1*). Lipids on the other hand penetrate the membrane fairly freely. In apparent contradiction of these observations, water passes through cuticle very rapidly under certain conditions: for example, when an insect is immersed in oil, drops of water soon appear all over its surface (*2*). In an atmosphere of high humidity water passes into mealworm larvae at a rate as high as 0.4 mg cm^{-2} hr^{-1}, compared with a maximum loss rate of 0.08 mg cm^{-2} hr^{-1} in dry air. Thus cuticle at different times can be permeable or impermeable to water and yet allow lipids to pass (*3*). An interpretation of some observations on the structure of the epicuticle may resolve this problem and throw light on other properties of this important membrane.

The epicuticle in various insects has been examined by electron microscopy. It consists of a clearly demarcated dense layer, the cuticulin, which is like a plasma membrane in that it can be resolved as a double layer; it overlays a thicker homogeneous region as thick as 1 μ. These layers are always penetrated by filaments of wax 60 to 130 Å in diameter which give rise to the surface wax layers (Fig. 1). An irregular porous layer of cement and a monolayer of lipid sometimes can be seen on the outside. The cement is frequently impregnated with wax which may form a white powdery bloom at the surface. The cuticulin and usually the dense layer completely cover the insect, except over some sensory areas. It is probable that the channels occupied by the wax filaments serve not only to bring wax to the surface, but also permit lipids to enter an insect.

Theories explaining the peculiar permeability properties of cuticle depend largely upon the nature of the wax filaments and the spaces they occupy. When first seen these structures were termed wax canal filaments and wax canals (*4*); lipid forms with this filamentous shape were then unknown. In material fixed with osmium and stained with lead, they appeared as dense lines of indefinite length in an orderly hexagonal array when densely packed. In some preparations the filaments have been resolved as tubes. They have, therefore, the structure expected for liquid crystals of lipid and water in the middle phase. These have been described on the basis of x-ray diffraction studies as "hexagonal arrays of cylinders of indefinite length, water is outside, and the hydrophilic groups of the lipid molecules sit on the surface" (*5*). The most probable arrangement of lipids in the epicuticle is shown in Fig. 2, in which the lipid-water liquid crystals are continuous with the monolayer and other lipids at the surface.

The properties of lipid-water systems of this sort are of particular interest. The arrangement gives the maximum surface area for reactions involving the lipids. Large amounts of lipid-soluble substances can be incorporated in solution in the regions occupied by the hydrocarbon ends of the chains. The main and subsidiary lipid components can diffuse freely within the liquid-crystalline structures, which can also flow in any direction towards regions where the lipid ceases to be in a liquid-crystalline phase.

These properties and the arrangement of molecules in the structure proposed in Fig. 2 can account for the following aspects of cuticle permeability.

The transport of wax to the surface of the cuticle is a general problem in insects. There has been no satisfactory explanation of the mechanism by which this insoluble, inert material is moved from the epidermal cells across a wide hydrophilic endocuticle and through the epicuticle. The structure proposed in Fig. 2 suggests that polar lipid molecules are synthesized by the cells and are continually added to the inner ends of the wax canal filaments which would flow through the epicuticle as the surface wax is absorbed in the

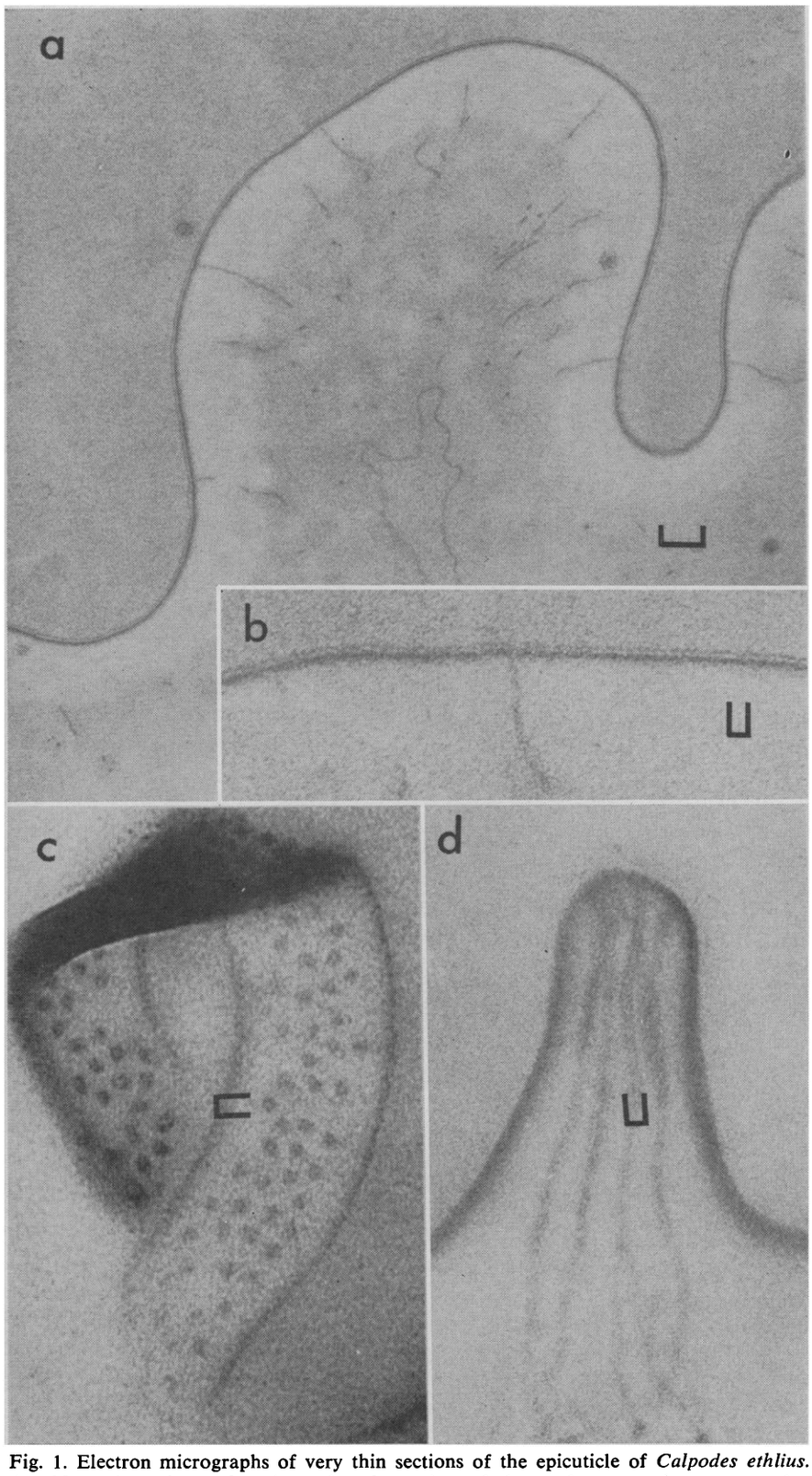


Fig. 1. Electron micrographs of very thin sections of the epicuticle of *Calpodes ethlius*. (*a*) Normal to the surface just after formation of the surface monolayer; one of a series through focus (× 57,000; scale, 1000Å). (*b*) The cuticulin and monolayer (× 226,000; scale 100Å). (*c*) Tangential to the surface through the wall of a wax-secreting tubercle. A group of middle-phase lipid-water liquid crystals are cut transversely in hexagonal array (× 218,000; scale, 100Å). (*d*) Normal to the surface through the wall of a wax-secreting tubercle, showing a middle-phase cylinder in longitudinal section (× 218,000; scale, 100Å).

meshwork of the cement or as it crystallizes to form the solid surface bloom. Nonpolar lipids could diffuse through the central hydrocarbon ends of the polar lipids composing the middle-phase cylinders. The wax canal filaments and the surface monolayer are probably polar lipids since they are fixed by osmium tetroxide; they differ from most of the surface wax, which is unfixed and mainly hydrocarbon.

The permeability of cuticle to water is altered on immersion in oil. One of the immediate effects of immersing an insect in oil would be solution and loss of the surface wax, and very rapid outward flow of all cuticular lipids. The water droplets which appear on the surface of an insect could have passed through the spaces vacated by the lipid-water liquid crystals.

Nonabrasive dusts desiccate insects as effectively as abrasive ones. If lipid solvents and crystallization to solid wax blooms can drag the middle-phase filaments through the wax canals and even leave them filled with water, then a similar effect would be expected for adsorbent dusts at the surface. With the wax in the canals replaced by water, the subsequent desiccation is readily explained.

The cuticle is permeable to oil-soluble molecules (pheromones, telergones, hormones, insecticides) which can disperse rapidly over the surface. When an autoradiographic technique was used to trace the spread of a radioactive preparation of di-iodo octadecane from the tarsi of adult *Phormia* over the body (*6*), activity spread over the whole of the integument within a few minutes. This is most easily explained if the surface monolayer of wax is liquid, allowing rapid diffusion.

Olfactory organs take up odorous compounds from the air. Filaments have been described in the olfactory organs of various insects; they have been likened to the neurofibrils found in nerves (*7*). Neurofibrils, however, are considerably larger, and the olfactory organ structures described look very much like a middle-phase lipid-water system. This would provide an ideal surface for trapping oil-soluble molecules from the air. Liquid-crystalline phases are known to be able to take up large amounts of other lipids dissolved within the hydrocarbon regions (*8*). The surface area for absorption is large, and the liquid state could ensure the rapid diffusion of odorous

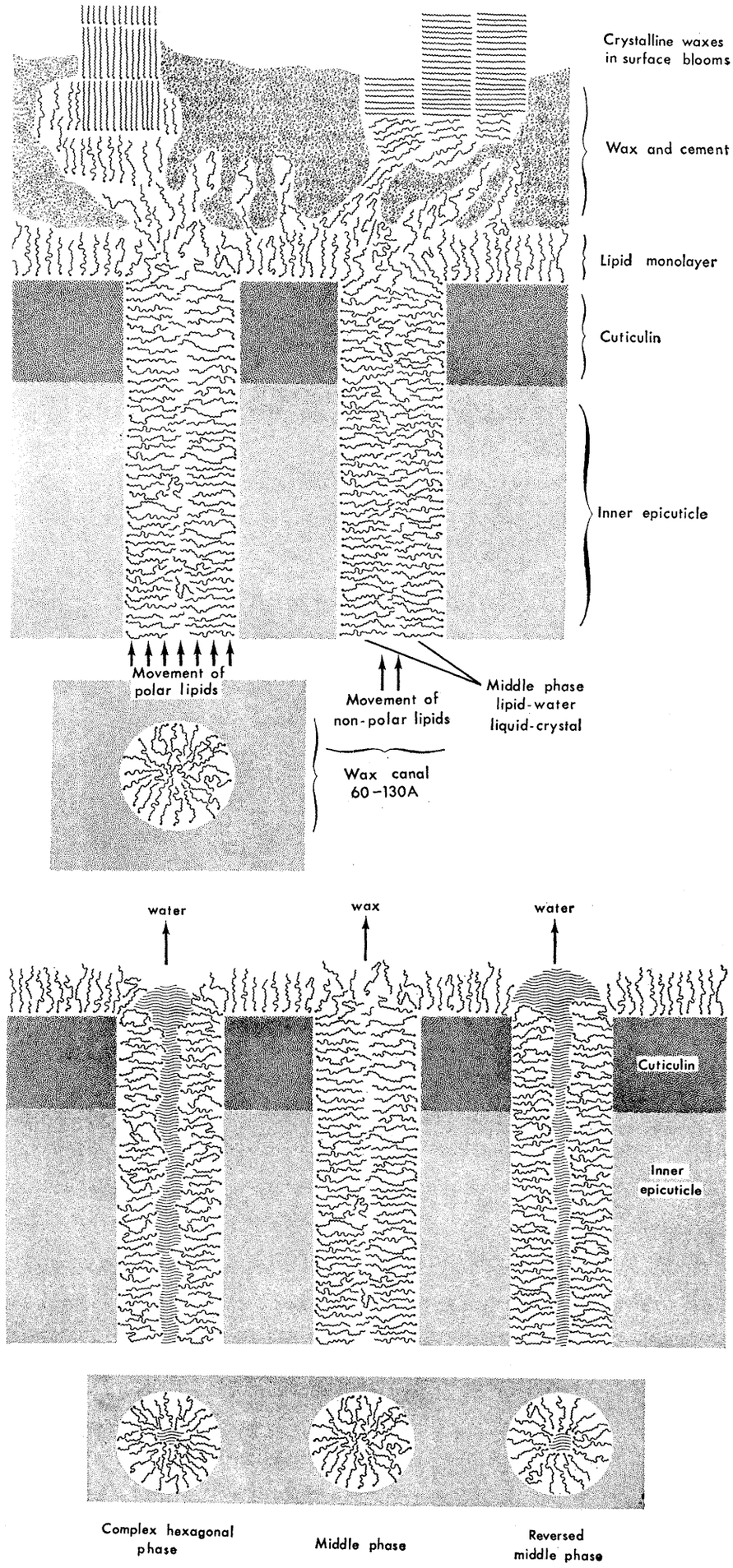
Fig 2 (top right). Diagram of the most probable arrangement of lipid molecules in the epicuticle. The inner epicuticle may extend for about 1 μ, that is, about 20 times the thickness shown.

Fig. 3 (bottom right). Three lipid-water liquid-crystalline phases known from in vitro studies (*5*, *10*). If the middle phase (center) could change to either the complex hexagonal or the reversed middle phase within the epicuticle, there would be an increase in permeability to water.

molecules from the surface to a site for stimulating the receptor. It will be interesting to see if all olfactory organs have lipids in this form.

The permeability to water of the same cuticle may vary at different times. Although a cockroach loses water very slowly, a drop of water on the cuticle rapidly enters the insect (*9*). The middle phase structure in Fig. 2 is only one of several described by Luzzati and Husson from x-ray diffraction studies. For example, Fig. 3 shows the structure of the complex hexagonal phase and the hexagonal phase typical for phospholipids, in which the orientation of the molecules is reversed (*10*). The phase depends on the temperature and on the proportions of lipid and water in the system. If the lipids in the cuticle change their phase (Fig. 3) under the influence of environmental humidity or of changes within the cuticle, the variable permeability to water can be explained. The role of such lipid-phase changes in controlling membrane permeability deserves further study. It may be significant that the structure of the desmosomes, which link insect epidermal plasma membranes together, may be likened to lipids in the reversed middle or hexagonal phase; and hexagonal arrays in synaptic disc membranes and elsewhere (*11*) parallel to the middle-phase cylindrical structure.

Molting fluid is resorbed through new cuticle which is at the same time protected from its action. When the old endocuticle is dissolved by the molting fluid it is simultaneously resorbed through the new epicuticle; this process poses a problem of protection for the new cuticle. The lipid-water filled channels, or wax canals, could form a molecular sieve allowing water and small molecules to pass through, but preventing the entry of hydrolytic enzymes.



The cuticle is impermeable to water despite the variety of chain length of the lipid molecules. The main barrier to water loss is believed to be a lipid monolayer in which the molecules are tightly packed (*9*). This crystalline degree of order would be difficult to achieve with the lipids of variable chain length which occur in insect cuticles. On the other hand, such mixtures might readily form liquid crystals and liquid monolayers. Critical temperatures for water loss could then be explained as phase changes in liquid-crystalline systems.

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Immune Reactivity in Mice Thymectomized Soon after Birth: Normal Response after Pregnancy

Abstract. *Female CBA mice that had been thymectomized soon after birth were mated with normal male T6 mice. After delivery of at least one litter, the parous females exhibited normal immune reactions to sheep erythrocytes and skin homografts. The transplacental passage of a humoral substance from the thymus glands of the developing fetuses is suggested as the mechanism responsible for the restoration of immunological responsiveness in neonatally thymectomized parous female mice.*

Mice thymectomized soon after birth characteristically exhibit diminished immune reactivity to certain particulate antigens and to skin homografts (*1, 2*). This diminished reactivity can be restored to normal in a variety of ways, such as (i) by intravenously injected isologous adult lymph node and spleen cells (*3*) and by relatively large numbers of isologous thymus cells (*4*), (ii) by subcutaneous grafts of intact isologus (*1*) or homologous (*5*) thymus tissue during the first week of life, and (iii) by intraperitoneal implants of intact isologous (*6, 7*) neonatal or embryonic thymus tissue in cell-tight Millipore diffusion chambers.

The foregoing restorative methods are also effective in preventing the wasting disease which is a common feature that follows neonatal thymectomy in some strains of mice. In the experiment reported here, pregnancy induced a restoration of immunological reactivity in neonatally thymectomized female mice.

Mice of the CBA strain were either thymectomized or given a sham operation within 16 hours of birth. At weaning, the females and males were separated, and only the females were used in subsequent procedures. When the females were 5 to 8 weeks old the absolute number of lymphocytes in the peripheral blood was determined for each mouse. At 8 weeks of age the neonatally thymectomized females were nonselectively divided into two groups. One group was mated with normal, healthy T6 males, whereas the other group was not allowed to mate and served as a control group. The female mice that received the sham operation comprised another control group and they also were not allowed to mate. After each female in the mated group had delivered one or two litters, another count of the absolute number of lymphocytes in the peripheral blood was performed on all the mice in the mated group, and the neonatally thymectomized control group. By this time all the mice were 13 to 17 weeks of age. Each mouse in all three groups was then challenged intraperitoneally with washed sheep erythrocytes (0.2 ml of a 20 percent suspension in saline). Ten days after challenge the titer of the serum antibody to sheep erythrocytes was determined, the blood having been obtained from the orbital sinus. Finally, each parous female was given a graft of full-thickness male T6 skin and Ak skin. The skin grafts were performed from 1 day to 1 month postpartum. Mice in the two control groups were grafted only with full-thickness Ak skin. After removal of the protective dressings on the eighth postoperative day, each graft was examined daily for visual evidence of rejection. From the time of weaning throughout the experimental period, each mouse was weighed twice weekly. Mice dying of wasting disease or those killed at the conclusion of the experiment were autopsied, and the retrosternal area was examined microscopically for thymus remnants. Only data from completely thymectomized female mice are included in the results.

Nine of the 17 mice which were mated with T6 males became pregnant, and each delivered at least one litter of mice prior to the 13th week of age. The other eight mice died of wasting disease before becoming pregnant or delivering any litters. Although the lymphocyte counts prior to mating of these eight mice are included in the results below, these mice were neither given skin grafts nor injected with sheep erythrocytes. Similarly, four of the 16 unmated control mice thymectomized at birth died of wasting disease before hemagglutination titers or skin homografts could be performed, and seven died before another count of the lymphocytes could be made.

The numbers of circulating lymphocytes were similar (Fig. 1) in the two groups of the thymectomized mice before they were nonselectively separated into a mated experimental group (17 mice) and an unmated control group (16 mice). In each group the lymphocyte ranged from 800 to 5100 per cubic millimeter, with a count of less than 4000 in most mice. The lymphocyte counts in ten 6- to 8-week-old sham-thymectomized mice ranged from 4400 to 10,400 per cubic millimeter, with all but one of the mice having a count of more than 6600. The number of lymphocytes after birth ranged from 2300 to 4800, whereas at a corresponding age in the neonatally thymectomized control group the count ranged from 1100 to