9 Prokopy, R. J., in: Management of Insect Pests with Semiochemicals, p. 512. Ed. E. R. Mitchell. Plenum Press, New York 1981.
10 Boller, E. F., in: Management of Insect Pests with Semiochemicals, p. 457. Ed. E. R. Mitchell. Plenum Press, New York 1981.
11 Boller, E. F., and Hurter, J., Ent. exp. appl. *39* (1985) 163.
12 Boller, E. F., Schöni, R., and Bush, G. L., Ent. exp. appl., submitted.
13 Staedler, E., and Katsoyannos, B. I., Mitt. schweiz. ent. Ges. *52* (1979) 444.
14 Staedler, E., in: Chemical Ecology of Insects, p. 3. Eds W. J. Bell and R. T. Carde. Chapman and Hall, London 1984.
15 Boller, E. F., in: Handbook of Insect Rearing, vol. 2, p. 135. Eds P. Singh and R. F. Moore. Elsevier, Amsterdam 1984.
16 Chen, P. S., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 10, p. 195. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, 1985.
17 Hayes, K. C., Nutr. Rev. *43* (1985) 65.
18 Marnela, K. M., Morris, H. R., Panico, M., Timonen, M., and Laehdesmaeki, P., J. Neurochem. *44* (1985) 752.

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Short Communications

Permeability of arthrodial membrane to water: A first measurement using in vivo techniques

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Summary. Despite differences in surface morphology and fine structure, the permeabilities of untreated scorpion sternite and pleural cuticle to water are similar (0.69 versus 0.79 $\mu g \cdot cm^{-2} \cdot h^{-1} \cdot mm\ Hg^{-1}$). Hexane applied to pleural membrane increased its permeability 9-fold, but neither hexane nor chloroform : methanol had much effect on sternite permeability. When sternite cuticle was treated with 1.0 N KOH followed by chloroform : methanol, permeability increased about three times over control values. In contrast, cockroach pronotum, which is 17 times more permeable than scorpion sternite, exhibited a marked increase in permeability when treated with just hexane. In both the scorpion pleuron and cockroach pronotum, disruption of the lipid barrier caused by rubbing is partially responsible for the higher permeabilities observed following solvent treatment.

Key words. Arthrodial membrane; cockroach; cuticle; cuticular lipids; permeability; scorpion; water loss.

Arthrodial or 'intersegmental' membrane of arthropods occurs between true metameric segments, at the joints of appendages, and at the bases of hairs and bristles. Because the proteins in this cuticle do not tan, arthrodial membrane remains soft and flexible, permitting adjacent sclerotized segments to move relative to one another[1]. In most insects and arachnids, arthrodial membrane represents a relatively small, albeit critical, percentage of an individual's total surface area. However, during certain stages of reproduction or often following a large meal, arthrodial membrane unfolds and/or stretches to accomodate increased abdominal volume. At this time the surface area of arthrodial membrane can equal or exceed that of sclerotized ('hard') cuticle and, thus, becomes a potentially important site for transcuticular water loss.

Despite its functional importance, arthrodial membrane has received far less study than its counterpart, sclerotized cuticle. Filshie and Hadley's[2,3] ultrastructural examination of the dorsal sclerites and adjacent intersegmental membrane of the scorpion *Hadrurus arizonensis* is one of the few studies that have compared the two cuticle types. They found that the well-defined exocuticle of sclerite is absent in the intersegmental membrane. They also found differences in the fine structure of the epicuticle and in the pore/wax canal complex in intersegmental membrane. These differences might significantly alter the permeability of scorpion arthrodial membrane since the epicuticle is the site of the principal barrier to water efflux in most terrestrial arthropods. Until recently, however, it was not possible to test this experimentally.

Materials and methods. To address this question, we used miniature ventilated capsules that could be attached directly to the lateral pleuron of gravid female *H. arizonensis*. A capsule of similar design was used recently in conjunction with our transpiration monitor to measure electronically the permeability of cricket thorax in vivo[4]. With this system, dry air (0.5 $ml \cdot min^{-1}$) enters the capsule inlet tube, acquires moisture that is diffusing across the cuticle, and then exits via an outlet tube into an aluminum oxide sensor. The moisture content of the humidified air alters the impedance of the sensor. The signal is amplified and plotted on a millivolt recorder as a continuous tracing. Details of the system's design, operational characteristics, and calibration methods are given in Hadley et al.[5].

Test scorpions were cooled before being secured to a thin metal plate with strips of tape. The plate was then attached to a ring stand with the scorpion positioned on its side. The capsule was then lowered carefully until its base firmly contacted the lateral membrane. The capsule base was attached to the membrane using epoxy applied to the outer edge of the capsule. After drying, the entire unit was transferred to an insulated Plexiglas chamber into which air of desired temperature (± 0.5 °C) was circulated. The temperature of the capsule was measured with a copper-constantan thermocouple connected to a BAT-12 thermometer. The inlet and outlet ends of the capsule were connected to the transpiration monitor and the system flushed of residual moisture. Readings were taken only after the tracing had stabilized (3–6 h). Similar in vivo determinations were made with the capsule attached to the ventral sclerite (= sternite) of *H. arizonensis* and to the pronotum of the cockroach *Periplaneta americana*.

Our comparison of sclerotized cuticle and arthrodial membrane included untreated cuticle (controls) and cuticle that had been lightly swabbed (Q-tips) with either a lipid solvent, KOH, or distilled water (shams). Scorpion surface lipids, extracted from regional washings (small hexane-soaked cotton balls held by forceps) or from dissected soft cuticle, were analyzed by conventional thin layer and gas chromatographic techniques[6]. The surface detail (SEM) and fine structure (TEM) of the cuticle were also determined for the pleural (arthrodial) membrane and sternite, as the previous ultrastructural study had investigated dorsal sclerites and adjacent intersegmental membrane.

Comparisons between control and treatment permeability mean

Permeability of sclerotized (sternite, pronotum) cuticle and arthrodial membrane (pleuron) in the scorpion *Hadrurus arizonensis* and the cockroach *Periplaneta americana*. Test conditions were 35°C and 0% RH for the scorpion and 30°C and 0% RH for the cockraoch

Treatment	*Hadrurus* Sternite	Pleuron	*Periplaneta* Pronotum
Control	0.69 ± 0.13* (8)	0.79 ± 0.23 (6)	11.67 ± 0.37 (6)
Hexane	0.42 ± 0.11 (5)	7.16 ± 1.53 (7)	84.65 ± 22.87 (6)
KOH/Cl: methane	2.26 ± 0.98	—	—
Sham	—	4.59 ± 2.41 (3)	56.16 ± 11.50 (3)

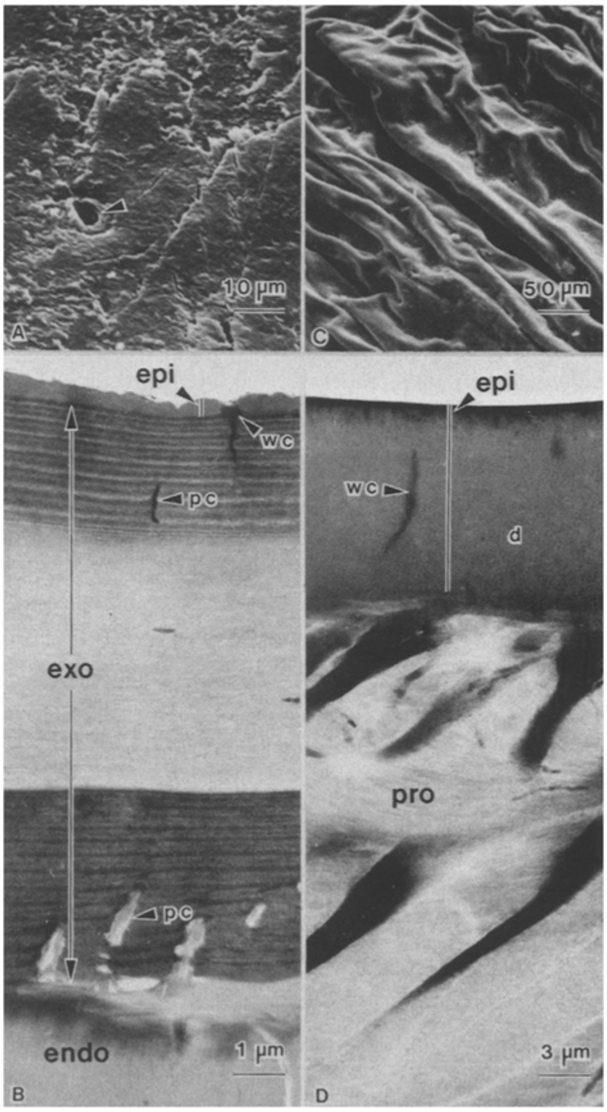
* $\mu g\ H_2O\ cm^{-2} \cdot h^{-1} \cdot mm\ Hg^{-1}$ ($\bar{x} \pm$ SE, sample size)

values within a given cuticle type and between the sternite and pleuron of *Hadrurus* were made using the Student's t-test. Probability values of < 0.05 were taken to indicate statistical significance.

Results. There was no significant difference in the permeability of untreated sternite cuticle and arthrodial membrane (table). The two cuticle types, however, responded quite differently to lipid solvents. Prewashing the sternite surface with hexane produced a slight decrease in permeability, whereas there was no change when chloroform:methanol or KOH was applied separately (data not shown). Sternite permeability did increase 3.3 times when KOH treatment was followed by chloroform:methanol (table). In contrast, pleural membrane that had been lightly swabbed with hexane exhibited a 9-fold increase in permeability over that exhibited by untreated controls. The cockroach cuticle responded still differently. The sclerotized pronotum (untreated) was approximately 17 times more permeable than the sclerotized scorpion sternite, yet following hexane treatment, the permeability of the pronotum still rose sharply. The mean permeabilities of hexane-treated and sham-treated pleuron (scorpion) and pronotum (cockroach) were both significantly higher than their respective controls. Our results using shams indicate that in both cuticle types, barrier disruption caused by rubbing accounted for over 60% of the increased permeability exhibited by hexane-treated cuticle. Shams were not run on sternite cuticle as it was apparent from the solvent treatments that rubbing did not affect the permeability of the scorpion sternite.

Morphologically, the sternite is a relatively smooth, platelike piece of sclerotized cuticle with a thickness of about 100–150 µm. Small fissures and raised encrustations give it a bas-relief when viewed with the SEM (fig. A). Scratches, probably caused by soil abrasion, and the openings to dermal gland ducts are clearly evident at low magnification. In thin section, the sternite features a well developed epicuticle (circa 0.3 µm thick) and a sharply demarcated three-layered exocuticle that overlies the endocuticle. Numerous pore canals pass through the endo- and exocuticle to join with wax canals just beneath the epicuticle (fig. B). The fine structure of the scorpion lateral pleuron differs in several respects. Unlike most arthrodial membrane, the pleuron here is quite tough and is actually thicker (300–350 µm) than the sternite. It is also compacted into many deep folds (fig. C) that greatly expand in gravid females to produce a more 'rolling' surface. The exocuticle, which is well-defined in the sternite, is absent in the pleuron. In addition, the dense layer of the epicuticle is over four times thicker (1.3 µm) than its counterpart in the sternite, and the wax canals are simple, unbranched channels that appear to traverse the entire width of the epicuticle (fig. D). Both types of cuticle have lipids associated with their surface. Small to trace amounts of hydrocarbons, wax esters, free fatty acids, alcohols, and sterols (principally cholesterol) were detected in hexane extracts using TLC. The hydrocarbon fraction was further analyzed by GLC. All hydrocarbon molecules were saturated and ranged from 18 to over 43 carbon atoms in sclerotized cuticle and from 18 to 39 carbon atoms in the pleural membrane. Qualitatively, the molecular composition is similar; however, shorter chain *n*-alkanes (C_{20}–C_{26}), which represent only 8% of the total hydrocarbon fraction in sclerotized cuticle, account for nearly 25% of the total in soft cuticle, while long chain branched components ($> C_{35}$) are more abundant in sclerotized cuticle.

Discussion. The permeability, ultrastructural, and chemical composition data in combination provide considerable insight into the nature and effectiveness of the waterproofing features of both arthrodial and sclerotized cuticle. The intuitive notion that penetration of water occurs more rapidly through arthrodial membrane[7] is obviously not the case, at least in this scorpion species. As in sclerotized cuticle, lipids associated with the epicuticle of arthrodial membrane are a principal barrier to diffusion.



A Surface of sternite (= ventral sclerite) of *Hadrurus arizonensis* containing opening to single dermal gland duct (arrow). Scale bar, 10 µm. *B* Transverse section of outer part of sternite showing the thin epicuticle (epi), exocuticle (exo), and upper border of endocuticle (endo). Pore canals (pc) pass through endo- and exocuticle to join with wax canals (wc) at junction of exo- and epicuticle. Scale bar, 1 µm. *C* Greatly folded surface of pleural membrane that connects dorsal sclerites with ventral sclerites. Scale bar, 50 µm. *D* Transverse section of the pleural membrane showing epicuticle (epi) with its thickened dense homogeneous layer (d) and upper portion of the hydrated procuticle (pro). Electron dense filaments connect to the inner face of the dense layer. Wax canal filaments (wc) appear to penetrate width of epicuticle. Scale bar, 3 µm.

Because of the extraction procedures used and the small quantity of lipid removed, we are unable to determine accurately the surface density of the lipid in the respective cuticle. Hydrocarbons, whose nonpolar properties make them well suited for barrier function[8], are a major constituent of the surface lipids of both cuticle types. Differences in the molecular composition of the hydrocarbon fractions are probably not sufficient to significantly affect permeability, although the greater percentage of long chain hydrocarbon molecules in sclerotized cuticle should theoretically enhance its diffusion resistance[8]. Any advantage conferred by this compositional difference may very well be countered by the increased thickness of the lipid-rich inner epicuticle in arthrodial membrane. A thicker inner epicuticle in arthrodial membrane may also help compensate for the absent exocuticle; however, the latter's role in contributing to barrier function in sclerotized cuticle remains speculative.

The response of the different cuticle types to chemical treatments and mechanical abrasion (shams) sheds additional light on their barrier properties. The lipid barrier on the surface of arthrodial membrane appears to be quite labile judging by the marked increase in permeability following hexane application or simply mild rubbing. In contrast, the barrier function provided by surface lipids associated with sclerotized cuticle was unaffected by the same treatments. A strong base in combination with chloroform:methanol was required to disrupt this barrier and even then the increase in permeability was less than observed for arthrodial membrane treated with solvent alone. These data suggest that in sclerotized cuticle the epicuticular lipids are more tightly bound to proteins and/or are covered by a substance or layer that is essentially lipid-insoluble. There is histochemical evidence for an appropriately located layer consisting of acid and neutral mucopolysaccharides in the cuticle of the scorpion *Heterometrus liurus*[9]. Such a coating would dissolve when the cuticle is treated with KOH, leading to the increased permeability observed. Functionally, the layer would also protect that portion of the scorpion integument that is most likely to experience damage due to soil abrasion.

Despite the much greater permeability of cockroach cuticle, epicuticular lipids still represent a major barrier component, as their removal produces much higher transcuticular water loss rates. The reduced barrier effectiveness of cockroach lipids can be explained in part by their unique composition. Cockroach surface waxes are composed mainly of hydrocarbons (85–95%), with the unsaturated molecule *cis, cis*-6,9-heptacosadiene accounting for 71% of the total fraction[10]. Together, these hydrocarbons produce a mobile, grease-like coating rather than the hard wax coating found in scorpion cuticle and in most other xeric-adapted arthropods. Not only is this grease less effective in retarding water loss, but its thermal stability is also lower. In fact, phase changes that probably lead to the restructuring of these superficially-deposited hydrocarbons and, hence, to increased permeability begin at temperatures as low as 30°C. Furthermore, the coating over the surface lipids that protects and contributes to the waterproofing barrier in sclerotized scorpion cuticle is either absent or poorly developed in the cockroach.

Without an effective barrier to water efflux, transpiration across scorpion arthrodial membrane would lead to rapid dehydration, especially in gravid females during hot, dry summer conditions. Nonetheless, these findings cannot be applied indiscriminately to all arthropods or, for that matter, even to other scorpion species. During our study we also measured the permeability of the pleural membrane of the scorpion *Pandinus imperator*, a large, tropical species that occurs in lowland rainforests. The mean value for pleural membrane in two gravid females was $11.95\ \mu g \cdot cm^{-2} \cdot h^{-1} \cdot mm\ Hg^{-1}$. This value, which is comparable to rates observed for the cockroach pronotum, is also about 2.5 times greater than the permeability of the sternite in the same two scorpions. Obviously, species differences and habitat conditions will have a major bearing on the respective cuticular permeabilities and the mechanisms that have evolved to minimize water loss.

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1 Neville, A.C., Biology of the Arthropod Cuticle. Springer-Verlag, Berlin 1975.
2 Filshie, B.K., and Hadley, N.F., Tiss. Cell *11* (1979) 249.
3 Hadley, N.F., and Filshie, B.K., Tiss. Cell *11* (1979) 263.
4 Hadley, N.F., Machin, J., and Quinlan, M.C., Physiol. Zool. *59* (1986) 64.
5 Hadley, N.F., Stuart, J.L., and Quinlan, M.C., Physiol. Zool. *55* (1982) 393.
6 Hadley, N.F., and Jackson, L.L., Insect Biochem. *7* (1977) 85.
7 Noble-Nesbitt, J., Pestic. Sci. *1* (1970) 204.
8 Hadley, N.F., The Adaptive Role of Lipids in Biological Systems. Wiley-Inter Science, New York 1985.
9 Tandan, B.K., Somadder, K., Kumar, P., Churchman, L.C., Rastogi, V.L., and Gupta, P.D., Biol. Mem. *2* (1977) 222.
10 Jackson, L.L., Comp. Biochem. Physiol. *41B* (1972) 331.

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Effect of glucose administration on bilirubin excretion in the rabbit

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Summary. The effect of i.v. infusion of glucose on the hepatic handling of bilirubin was examined in rabbits. A significant increase in the excretion of conjugated bilirubin into the bile was observed, accompanied by a decrease in bilirubinemia. Hepatic bilirubin concentrations were lowered and the UDP-glucose concentrations and liver UDP-glucuronosyl and UDP-glucosyl transferase activities increased.

Key words. Bilirubin; glucose; glucuronosyl transferase; rabbit; bilirubinemia; conjugates, bilirubin.

The hepatic handling of bilirubin depends on a series of different processes: plasma transport and translocation across the sinusoidal membrane of the liver cell, storage in the hepatocyte, conjugation and transfer into the bile. Previous studies in different species have demonstrated that glucose administration can lead to increased bilirubin excretion in fasted animals, with or without decreases in bilirubinemia[1,2]. The possibilities of increased bilirubin conjugation or of alterations in bilirubin bind-