dition, the midbilayer decane phase probably does not behave ideally ($\tilde{\gamma}_A{}^b \neq 1$) as assumed in the analysis. In such circumstances $\tilde{\gamma}_A{}^b$ must differ from 1.0 to maintain parity of the decane activity in the bilayer and annulus as the amount of decane mixing with the acyl chains increases.

The dependence of bilayer thickness on voltage decreases as the length of the alkane used in the formation of the bilayer increases. Thus, for GMO/hexadecane membranes, the change in thickness accompanying a 150-mV potential is not detectable (*4*). A simple explanation of this effect is that the longer alkanes mix more thoroughly with the acyl chains than do the shorter ones. In such cases, $\tilde{X}_A{}^b$ would tend to become equal to $X_A{}^b$ and, by virtue of Eq. 3, a significantly larger potential would be required to achieve a given fractional change in $X_A{}^b$. However, since $X_A{}^a$ remains on the order of 0.995 even for hexadecane, more thorough mixing would demand that $\tilde{\gamma}_A{}^b$ be significantly greater than 1 to keep the alkane activity in the bilayer equal to that in the annulus. There is evidence in support of this hypothesis. I demonstrated earlier (*15*) that the enthalpy of solution (ΔH) of *n*-hexadecane in GMO/hexadecane bilayers is large and positive (4 kcal/mole). Since, in general, $\ln \gamma \propto H/T$ (*18*), it follows that $\tilde{\gamma}_A{}^b > 1$.

These results and conclusions indicate the difficulties inherent in making assumptions about the molecular meaning of the activity coefficient. Often, for example, $RT\ln\gamma$ is interpreted as due to differences in solute-solute and solute-solvent interaction energies. It is now clear, however, that the interpretation of γ depends on knowledge of the structure of the environment surrounding the solute. Thus, in this report, the gross activity coefficient ($\gamma_A{}^b$) of the decane in the bilayer is ~ 1.7, while if the assumption of a microscopic phase separation is made, $\gamma_A{}^b \simeq 1$ in the separate phase is adequate to explain the results.

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$$\gamma_t = \int_{z_1}^{z_2} [P_l{}^N - P_l{}^T(Z)]dZ$$

where $P_l{}^N$ is the component of the interior pressure normal to the bilayer (Z direction), $P_l{}^T(Z)$ is the component tangential to the bilayer (normal to Z), and $Z_2 - Z_1$ is the bilayer thickness. Properly, the pressure tensor for the bilayer must be known before the electrostrictive pressure effect can be completely and accurately described. I believe the basic physical mechanism of compression can be understood by assuming an isotropic pressure. A more complete discussion of the anisotropy in black films is given by D. M. Andrews [thesis, Cambridge University (1970), p. 146].
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12. Equations 2 and 3 may appear to be incomplete, since the surfactant molecules, GMO in this case, are also subjected to the pressure P_V. O. Alvarez and R. Latorre [*Biophys. J.* **21**, 1 (1978)] measured the electrocompression of GMO membranes containing negligible amounts of alkane and found only a 0.04 percent change in thickness for a 100-mV potential. This means that the area per GMO (the interfacial concentration) can change by no more than this amount. Compared to the alkane shift effect, this change in concentration is negligible and I need not include equations for GMO [see also (*5*)].
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16. The suggested phase separation is not viewed as complete; that is, the acyl chains are not viewed as containing no decane. Unpublished observations in my laboratory suggest that some decane must be dissolved in the acyl chain region. Essentially, under the saturation equilibrium conditions used for the present experiments, decane is distributed between the central decane phase and the acyl chain phases. The final amount and the distribution of decane in the bilayer depend on the activity of the decane in the annulus.
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Lipid Barrier to Water Exchange in Reptile Epidermis

Abstract. *Extraction of lipids from the shed epidermis of the terrestrial snake* Elaphe obsoleta obsoleta *increases cutaneous water loss in vitro as much as 15-fold. Partial denaturation of epidermal keratin without lipid extraction increases cutaneous water loss only twofold. Histological observations and thin-layer and gas-liquid chromatography of the lipid extracts indicate a complex mixture of polar and neutral lipids predominantly in the mesos layer of the cornified epidermis. Comparative measurements of cutaneous water loss in other species of snakes and a lizard show that permeabilities differ naturally but are essentially identical after lipid extraction. These findings establish the importance of lipids in the permeability barrier of reptilian skin and suggest that keratin or scale morphology are of nominal importance in limiting water exchange.*

One of the important roles of the epidermis is to limit the exchange of water and electrolytes between the organism and its environment. In this context, reptiles have attracted interest because they possess scales and may have very low rates of cutaneous water loss (CWL) (*1*). It had been assumed that reptilian CWL is negligible (*2*), but recent research demonstrated that there is considerable interspecific variability of CWL and that species exposed to more desiccative conditions have lower rates (*1*). The skin properties underlying these differences are not known; speculations frequently concern quantitative aspects of keratinization and the size and pattern of scales (*3*). Although keratinized tissues were shown to be water permeable (*4*), current data do not elucidate the mechanisms determining skin permeability of reptiles. We examined the functional role of epidermal lipids and found that they are critical to a water barrier function.

Squamate reptiles (lizards and snakes) produce unusual stratified epidermal generations periodically during a shedding cycle (*5*). We investigated the properties of squamate epidermis by using intact sheets of naturally shed skin (outer epidermal generation). We determined gravimetrically the rates of water movement across small patches of skin shed by snakes and a lizard. Skin specimens were laid across a fine nylon net stretched across the open top of a small plastic vial containing a water-saturated wick (*6*). Evaporative CWL rates were determined by periodically weighing (to the nearest 10 μg) the vials, which were

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Table 1. Percutaneous evaporative water flux (milligrams per square centimeter per hour) from shed epidermis untreated or with lipids extracted in a mixture of chloroform and methanol. All skin samples were taken from the middorsal region. Data are means ± standard errors, with number of replicates shown in parentheses.

Species	Habitat	Untreated	Extracted
Sauria			
Iguana iguana	Mesic terrestrial; semiaquatic	1.164 ± 0.094 (5)	1.984 ± 0.124 (4)
Serpentes			
Nerodia rhombifera	Semiaquatic	0.401 ± 0.062 (6)	2.475 ± 0.212 (6)
Nerodia sipedon			
Normal*	Semiaquatic	0.409 ± 0.039 (6)	2.677 ± 0.056 (8)
Scaleless*	Semiaquatic	0.368 ± 0.038 (9)	1.752 ± 0.284 (7)
Elaphe obsoleta	Terrestrial	0.142 ± 0.017 (6)	2.271 ± 0.045 (6)
		0.157 ± 0.038 (5)	2.254 ± 0.040 (6)
		0.180 ± 0.031 (6)	2.769 ± 0.141 (5)
		0.202 ± 0.012 (4)	2.385 ± 0.070 (5)
		0.219 ± 0.038 (6)	2.250 ± 0.071 (6)
Cerastes cerastes	Xeric terrestrial	0.160 ± 0.022 (5)	2.170 ± 0.092 (6)

*These snakes were littermates.

kept over Drierite in a desiccator (water vapor pressure, 0.25 kPa) maintained at 33°C. We compared CWL from normal epidermis and from epidermis that was treated to remove lipids or subjected to partial keratin denaturation. To remove lipids, sheets of epidermis were extracted for 24 hours in (i) chloroform and methanol (2:1), (ii) ethyl ether and ethanol (8:92), (iii) acetone followed by 24 hours in hexane, or (iv) all three in sequence. In other specimens, keratin bonds were partially denatured by (i) 0.1*N* NaOH for 24 hours, (ii) 6*M* urea for 24 hours, or (iii) both in sequence (*7*). In some specimens, both keratin denaturation and lipid extraction were performed sequentially. All of the extraction or denaturation procedures and water loss measurements were carried out at physiological temperatures (*8*).

Lipid extraction increased CWL from the epidermis of black rat snakes (*Elaphe obsoleta obsoleta*) by more than an order of magnitude (Table 1). Chloroform and methanol was the most effective extraction mixture, producing a roughly 15-fold increase in CWL (five replicates). The CWL was increased tenfold by the ethyl ether and ethanol extraction mixture and eight- to ninefold by acetone and hexane (five replicates each). Partial denaturation of protein (*7*) by NaOH (five replicates), urea (four replicates), or both increased CWL only twofold. We infer that the lipid barrier to water flux is bidirectional, since inverting the epidermis of *E. o. obsoleta* did not change rates of water loss (*t*-test, $P < .05$).

Comparisons of CWL in shed skin from four species of snake and one lizard demonstrated that epidermal permeability was increased by lipid extraction in all five species. As expected, rates of water loss from untreated skin varied interspecifically and were related to habitat (Table 1). After lipid extraction CWL rates were similar in all species and ranged between 50 and 70 percent of evaporative rates from a free water surface.

Our measurements corroborate findings (*9*) that CWL from mutant scaleless snakes is the same as that from normal scaly conspecifics and show that lipids are important in determining permeability regardless of the presence or absence of scales (Table 1). Compared to lipids, structural features (either keratin or scale morphology per se) are of minimal importance in limiting water exchange through reptilian skin.

Using conventional thin-layer and gas-liquid chromatography, we identified a rather complex mixture of neutral and polar lipids in epidermal extracts from *E. o. obsoleta* (Fig. 1). The compositional features of the lipid complex resemble those of mammalian epidermis and are similar to the epicuticular lipids of arthropods, except that hydrocarbons are not predominant (*10*). Frozen sections (10 μm) of hydrated epidermis from *E. o. obsoleta* were stained with Oil Red O to reveal the distribution of lipids within the keratinized layers (*11*). Staining was very pronounced but was mostly limited to the mesos layer (*5*) (Fig. 1). After lipid extraction, skin sections were also stained to some extent by Oil Red O, indicating that the lipids in the epidermis are tightly complexed. Since the lipid extractions were not complete, the lipid permeability barrier is probably even more significant than the present data indicate. Routine light microscopy of epidermis after lipid extraction revealed no evidence of structural changes.

Measurements of CWL from reptiles after cellophane stripping of the epidermis led Maderson *et al.* (*12*) to

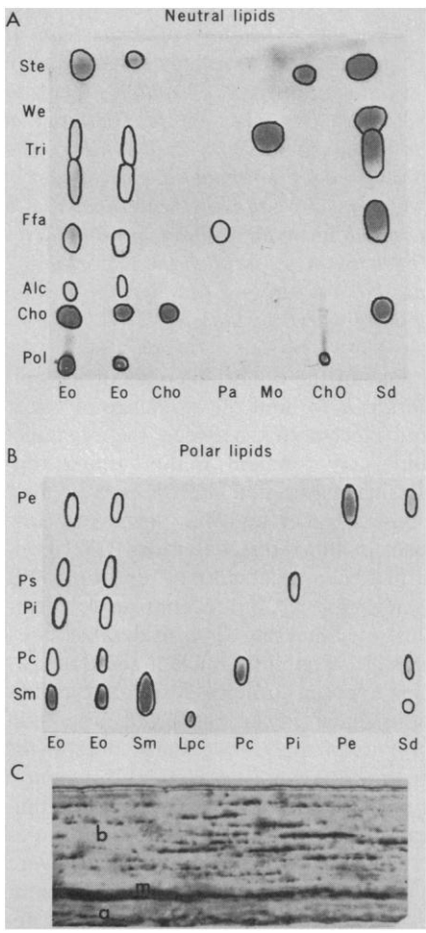

Fig. 1. (A and B) Thin-layer chromatograms of lipids from middorsal epidermis of *E. o. obsoleta* (*Eo*). Neutral lipids: sterol ester (*Ste*); wax ester (*We*); triglyceride (*Tri*); free fatty acid (*Ffa*); alcohols (*Alc*); cholesterol (*Cho*); polar lipids (*Pol*); palmitic acid (*Pa*); methyl oleate (*Mo*); cholesterol oleate (*ChO*); and standard mixture (*Sd*) of cholesterol, oleic acid, triolein, methyl oleate, and cholesterol oleate. Polar lipids: phosphatidylethanoline (*Pe*); phosphatidylserine (*Ps*); phosphatidylinositol (*Pi*); phosphatidylcholine (*Pc*); sphingomyelin (*Sm*); lysophosphatidylcholine (*Lpc*); and standard mixture (*Sd*) of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine. After extraction and purification, the distribution of neutral and polar lipids was demonstrated chromatographically. The solvent for neutral lipids consisted of hexane, diethyl ether, and formic acid (80:20:2) (*18*); for polar lipids, the solvent consisted of chloroform, methanol, acetic acid, and water (25:15:4:2) (*19*). Lipids were made visible by charring after spraying with sulfuric acid–dichromate and were outlined with ink to enhance contrast. Wax esters were verified in a separate procedure with oleoyl palmitate used as a reference standard. (C) Frozen section of shed middorsal epidermis from *E. o. obsoleta* stained with Oil Red O (*11*) and hematoxylin (×100). There is a prominent band of red stain in the mesos layer (*m*); less stain is evident in the alpha (*a*) and beta (*b*) layers.

conclude that the "physiological" permeability barrier resides within the alpha (probably including the mesos) layer, and while it was thought that the beta layer could play some role in reducing integument permeability, its function was interpreted as primarily mechanical. Their conclusions are compatible with our findings, since the mesos layer was disturbed during the cellophane stripping (*12*).

Since lipids are known to determine epidermal permeability in numerous terrestrial organisms, including certain amphibians (*13*), it is surprising that lipids were not investigated previously in relation to limiting water exchange in reptiles. It has been known since the 1950's that extracting lipids with organic solvents increased the permeability of mammalian skin (*14*), but this information has been overlooked by comparative physiologists (*15*). In mammals, the function of the epidermal permeability barrier apparently depends on intercellular lipids derived from epidermal lamellar bodies (Odland bodies, membrane-coating granules) (*16*). Similar bodies were found in the reptilian mesos layer, but their function remains to be clarified (*17*).

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Rat Model for Carcinogenesis in Ureterosigmoidostomy

Abstract. *A rat model is used to study the carcinogenesis that occurs when urine is surgically diverted into the fecal stream, as in ureterosigmoidostomy. Adenocarcinoma of the colon occurs adjacent to the urine inlet. It is completely prevented by proximal diversion of the feces, implying that fecal carcinogens are activated locally by the urine or the urothelium.*

Adenocarcinoma of the colon mucosa is a recognized complication of ureterosigmoidostomy, an operation used for many years to make up for the loss of the bladder in cancer surgery, extrophy of the bladder, bladder incontinence, and other conditions. The tumor, which develops adjacent to the junction of the ureter with the bowel, occurs 500 times as often as in the population at large and, in children so operated, 7000 times as often as in all persons under age 25. The latency period is 5 to 50 years (*1*).

In the past two decades, many thousands of children have had an isolated segment of sigmoid colon (a colon "conduit") interposed between their ureters and a cutaneous stoma, thus dispensing with the need for their abnormal urinary bladder. It is not known whether adenocarcinoma will appear in this large group, because the etiological mechanisms behind carcinogenesis in ureterosigmoidostomized patients have not been investigated. We used a rat model to test our own hypothesis of the etiological factors involved in this continuing problem.

Adenocarcinoma of the bladder is the most common kind of tumor developed in patients with extrophy of the bladder, a rare congenital malformation in which microscopic islands of colonic epithelium are present on the urothelial surface of the urine-bathed bladder (*2*). We hypothesized that colonic epithelium is especially susceptible to urine-borne carcinogens, and therefore planned long-term experiments in which rats were treated with carcinogens active in either the urinary tract or the gastrointestinal tract. The variables were considered to be urine, feces, and the two different epithelia. We joined the urinary stream to the colon as in a ureterosigmoidostomy or excluded the feces from the urine-bathed distal colon segment by a proximal colostomy (Fig. 1). Our results clearly indicate that a rat model is adequate for the study of carcinogenesis in ureterosigmoidostomy (Table 1). Adenocarcinoma of the colon appeared adjacent to the junction of the epithelia where the urine joined the fecal stream. And, contrary to our hypothesis, the colonic mucosa did not prove to be particularly susceptible to urine-borne carcinogens. No adenocarcinoma appeared at the bladder-colon junction unless feces were flowing by.

To simulate ureterosigmoidostomy in rats, we divided the urethra and both vasa to free the bladder base with its attached ureters and lateral vascular pedicles. We resected most of the dome of the bladder and used a running 7-0 suture of polyglycolic acid to join the remaining patch to an opening cut in the anterior surface of the rectal wall. To exclude the feces and simulate a colon conduit, the

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