Other uses of liposomes include encapsulation of hormones, antineoplastic agents, poly I:poly C for interferon induction, anti-viral antibodies and anti-viral drugs.

In our laboratory, poliovirus, an adenovirus SA7, and the anti-neoplastic agent bleomycin (BLM) have been encapsulated. The BLM was used to treat human hepatocellular carcinoma and carcinoma of the oesophagus cells *in vitro*, with very good results. Lower doses of drug could be used, and action on the cellular DNA was more rapid when compared with the free drug. Experiments are now in progress to increase the specificity of liposomes for the two malignant cell types so that different drugs can be targeted to them.

It can therefore be seen that one of the greatest potentials of these vesicles is in the improvement of pharmacological specificity. Drugs can be directed to the actual target site, thereby reducing harmful side effects. In addition, they may be useful in bypassing restricting membranes that normally render the cell resistant to drug action or viral replication. □

Cuticular Hydrocarbons and Evaporative Water Loss in Two Tenebrionid Beetles from the Namib Desert

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Water loss and cuticular lipid composition were examined in two species of tenebrionid beetles from the Namib Desert. Onymacris plana, *a large diurnal species, exhibited significantly lower water loss rates (mg g^{-1} h^{-1}) 30°C, 35°C and 40°C than the smaller, nocturnal* Lepidochora discoidalis. *Rates for both species were lower than those of most desert insects. Hydrocarbons were the most abundant cuticular lipids* (O. plana, *81%;* L. discoidalis, *76%), and were saturated in both species. Over 33 hydrocarbon components were detected in* O. plana, *ranging in length from 23 to over 40 carbon atoms; n-alkanes accounted for 66% of the hydrocarbon fraction.* Lepidochora discoidalis *hydrocarbons contained only 23 components (20-35 carbon atoms), with n-alkanes accounting for 94% of the total. The chemical properties of the hydrocarbons are discussed in relation to cuticular permeability and compared with the properties of the cuticular hydrocarbons of North American desert tenebrionid beetles.*

Waterverlies en die lipiedsamestelling van die kutikulum van twee Namibwoestyn-Tenebrionidae is ondersoek. Onymacris plana, *'n groot daglewende spesie, het 'n beduidend kleiner waterverlies (mg $g^{-1}h^{-1}$) vertoon by 30°C, 35°C en 45°C as die kleiner naglewende* Lepidochora discoidalis. *Die waterverliestempo vir albei spesies is egter laer as dié van die meeste woestyninsekte. Koolwaterstowwe maak die belangrikste deel van die lipiede van die kultikulum uit (*O. plana, *81 persent;* L. discoidalis, *76 persent) en is by albei spesies versadig. Meer as 33 koolwaterstofkomponente is in* O. plana *gevind; hulle het in lengte van 23 tot meer as 40 koolstofatome gewissel;* n-*alkane het 66 persent van die koolwaterstoffraksie uitgemaak. Die koolwaterstowwe van* L. discoidalis *het slegs 23 komponente besit (20 tot 35 koolstofatome) en* n-*alkane het 94 persent van die totaal uitgemaak. Die koolwaterstowwe se chemiese eienskappe word bespreek met betrekking tot hul rol in die bepaling van membraandeurlatendheid en word met die kutikulumkoolwaterstowwe van Noord-Amerikaanse woestyn-Tenebrionidae vergelyk.*

Introduction

Recent studies on desert tenebrionid beetles have attempted to relate epicuticular lipid composition to cuticular permeability. Hadley[1] found that the quantity of surface hydrocarbons and the percentage of long-chain hydrocarbon molecules increased in the beetle *Eleodes armata* during summer when the potential for water loss was greatest. Similar results were obtained on winter active beetles acclimated to 35°C. These compositional changes should result in a decreased cuticular permeability, in the light of findings with plasma membrane and artificial bilayer lipids.[2] In a subsequent study, Hadley[3] correlated cuticular transpiration and transition temperature of *E. armata* and three other sympatric desert tenebrionids (*Cryptoglossa verrucosa, Centrioptera muricata,* and *C. variolosa*) with the quantity and chemical nature of their epicuticular lipids. Although these beetles as a group exhibited cuticular features that corresponded with their xeric existence, slight interspecific differences in water loss confounded any relationship between cuticular lipid and permeability.

We have now investigated two tenebrionid beetle species, from the Namib Desert, with contrasting activity times, temperature preferences and water relations. *Onymacris plana* is a large black beetle, active by day in summer in exposed desert habitats; its rate of water loss is among the very lowest for Namib tenebrionids.[4] *Lepidochora discoidalis,* in contrast, is a small light-coloured beetle active largely at night. Water loss rates for this genus are significantly higher.[4] We report on the quantity, molecular size and composition of hydrocarbon components of the cuticle of these two species together, and compare their water loss rates.

Materials and methods

Beetles were collected fresh from the dune sea of the Namib Desert and shipped by air to the laboratory in Arizona. Upon arrival they were placed in large plastic trays containing a sand substrate and provided with food. No tests were conducted until there had been ample time for recovery and rehydration.

Water loss rates were determined using a flow-through chamber placed in a walk-in environmental room. A recir-

Table 1. Water loss rates of *Onymacris plana* (n = 7) and *Lepidochora discoidalis* (n = 10) in dry air at various temperatures.

Air temperature	25°C	30°C	35°C	40°C
O. plana				
mg g^{-1} h^{-1}	1.10 ± 0.12	1.34 ± 0.15	1.45 ± 0.16	2.90 ± 0.22
µg mm^{-2} h^{-1}	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	2.2 ± 0.1
L. discoidalis				
mg g^{-1} h^{-1}	1.13 ± 0.12	2.84 ± 0.31	5.56 ± 0.50	8.12 ± 0.63
µg mm^{-2} h^{-1}	0.5 ± 0.1	1.0 ± 0.1	1.9 ± 0.1	2.7 ± 0.2

Beetles were exposed to each temperature for four hours. Values represent mean ± one standard error.

Table 2. Lipid and hydrocarbon quantities extracted from the cuticle of *Onymacris plana* and *Lepidochora discoidalis*.

Beetles	*n*	Pooled mass (g)	Mean mass (g)	Total lipid (mg)	Hydrocarbon (mg)	Hydrocarbon: lipid ratio	Hydrocarbon (µg/mm²)
O. plana	18	12.61	0.70	6.62	5.36	0.81	0.32
L. discoidalis	29	2.33	0.08	3.21	2.44	0.76	0.36

culating air pump forced air through a tube of Drierite and then through the chamber at a rate of 1.0 litre/min. Chamber humidity did not exceed 5% during the tests. Weight losses, assumed to result only from water loss, were determined over four-hour intervals. Tests were conducted at 5°C increments between 25°C and 40°C. Surface areas were estimated using the equation:

Surface area = k (mass)$^{0.67}$

Values for the constants in the equation were based on Edney's measurements.[4]

Epicuticular lipids were removed by immersing the beetles in redistilled hexane for 15 min. The lipid extract was filtered, evaporated under nitrogen, and weighed to 0.01 mg. The hydrocarbons were separated from other lipids by eluting the extract with hexane through silicic acid columns,[5] then dried and weighed. Aliquants of the hydrocarbon fraction were spotted on thin layer chromatography plates impregnated with silver nitrate, to check for unsaturation. Gas chromatographic analysis of hydrocarbons was performed on 3.2 x 1830 mm glass columns

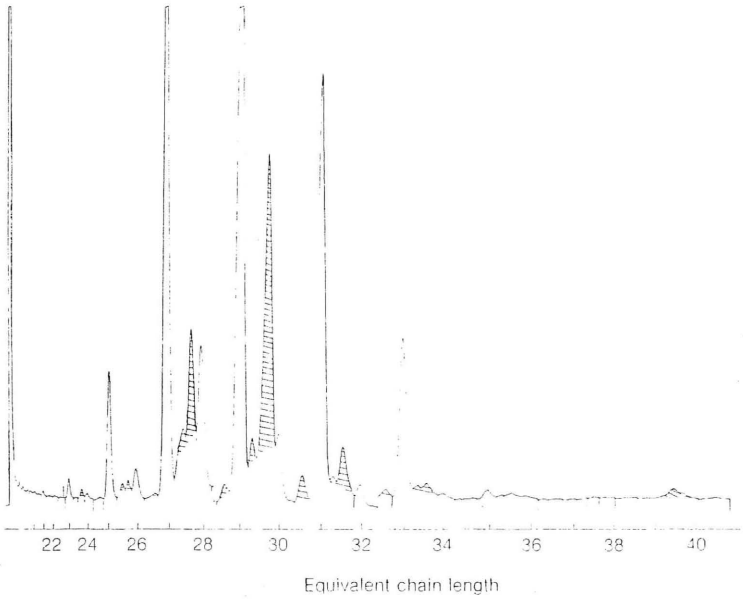

Fig. 1. Gas liquid chromatogram of *n*-alkanes (clear peaks) and branched alkanes (cross-hatched peaks) from a surface extract of adult *Onymacris plana*. Separations were performed on 3.2 x 1 830 mm glass columns of Gas Chrom Q coated with 3% OV-101, temperature programmed from 220°C to 300°C at 2°C/min.

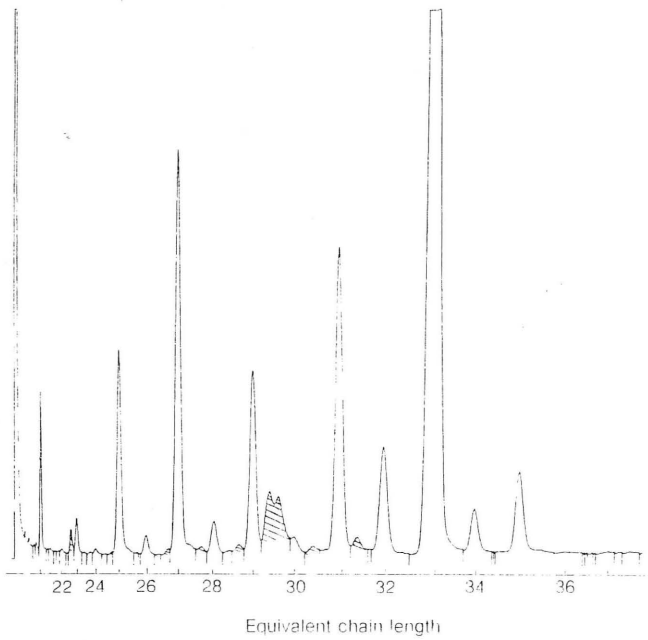

Fig. 2. Gas liquid chromatogram of *n*-alkanes (clear peaks) and branched alkanes (cross-hatched peaks) from a surface extract of adult *Lepidochora discoidalis*. Separations performed as stated in Fig. 1.

packed with 3% OV-101 on Gas Chrom Q and programmed from 220° to 300°C at 2°C/min. Procedures for identifying peaks, quantifying and separating *n*-alkanes from branched alkanes have been described previously.[6]

Non-hydrocarbons were eluted from the silicic acid columns with chloroform, dried and separated into classes by thin layer chromatography on Silica Gel G plates developed in hexane: diethyl ether: formic acid (80:20:2, volume/volume/volume). Lipid bands were detected by charring and identified against known standards.

Results

Water loss rates for *O. plana* and *L. discoidalis* are presented in Table 1. Between 25°C and 35°C, rates of water loss per unit of body mass (mg g^{-1} h^{-1}) for the much larger *O. plana* were relatively independent of temperature (Q_{10} = 1.32), but doubled between 35°C and 40°C (Q_{10} = 4.00). The rate of increase in water loss between 25°C and 35°C for *O. plana* was slightly lower when expressed per unit surface area ($\mu g\ mm^{-2}\ h^{-1}$). Water loss rates per unit body mass for *L. discoidalis,* in contrast, increased exponentially between 25°C and 35°C (Q_{10} = 4.92), but showed a reduced rate of rise between 35°C and 40°C (Q_{10} = 2.13). Per unit body mass, water loss rates for *O. plana* were significantly lower than those for *L. discoidalis* at 30°, 35° and 40°C, but only at 35°C and 40°C when expressed per unit surface area ($P < 0.01$; Student's *t*-test). Both species survived the four-hour exposure to 40°C.

Chromatographic separation of extracted cuticular lipids revealed the presence of hydrocarbons, free fatty acids, and triglycerides in the *O. plana* material, and hydrocarbons, alcohols and cholesterol in the *L. discoidalis* material. Considerable unidentified pigment was also present in the lipid extracts of both species. Lipid/hydrocarbon ratios excluding the pigment, are given in Table 2. Hydrocarbons accounted for most of the identified lipid in both species. Hydrocarbons were 3.75 times more abundant in *O. plana* when expressed per beetle, but when expressed per unit surface area, calculated from the mean mass of the sample, hydrocarbon surface densities for *L. discoidalis* (0.36 $\mu g/mm^2$) were essentially equal to those for *O. plana* (0.32 $\mu g/mm^2$).

Silver nitrate chromatography indicated that the hydrocarbons of both species were saturated. Gas chromatographic analysis revealed the presence of 33 hydrocarbon components in *O. plana,* the components ranging in chain length from 23 to over 40 carbon atoms. There were only 23 components in *L. discoidalis,* ranging in chain length from 20 to 35 carbon atoms (Figs 1 and 2; Table 3). Straight-chain *n*-alkanes predominated over branched alkanes in both species, especially in *L. discoidalis*. In *O. plana* C_{29} and C_{31} were the two most abundant *n*-alkanes and C_{29b} the major branched alkane, whereas in *L. discoidalis*, C_{33} accounted for over 50% of the total hydrocarbon fraction. Branched components were not identified by mass spectrometry, but patterns of fractional equivalent chain lengths (Table 3) suggest the presence of at least 2- or 3-methyl branched and internally branched molecules.[6]

Table 3. Hydrocarbon composition of *Onymacris plana* and *Lepidochora discoidalis* cuticle. Values (%) represent means of three replicate runs; values less than 0.1% indicated as trace.

GLC peak no.	Equivalent chain length		*O. plana*	*L. discoidalis*
20	20.0		—	1.06
22b	22.7		—	0.29
23	23.0		0.14	0.47
24	24.0		trace	trace
25	25.0		1.42	4.08
25b	25.5		0.18	—
25b	25.7		0.24	—
26	26.0		0.46	0.47
26b	26.6		0.11	—
27	27.0		9.11	9.31
27b	27.5 (.3)†		1.49	0.21
27b	27.7 (.6)		3.02	0.25
28	28.0		2.98	0.93
28b	28.3		0.19	—
28b	28.5 (.6)		1.08	0.37
29	29.0		26.19	5.14
29b	29.3		2.46	2.23
29b	29.7 (.6)		17.02	2.35
30	30.0		2.66	0.55
30b	30.5 (.4)		1.49	0.30
31	31.0		18.21	9.69
31b	31.3 (.4)		0.89	0.30
31b	31.7		3.38	—
32	32.0		1.09	3.83
32b	32.7		0.20	—
33	33.0		3.38	52.71
33b	33.4		0.20	—
33b	33.7		0.49	—
34	34.0		0.12	1.45
35	35.0		0.20	2.92
35b	35.7		0.16	—
36	36.0		trace	trace
38b	38.5		0.10	—
39b	39.7		0.74	—
40b	40.7		0.10	—
		n-alkane	66.0%	93.6%
		branched	34.0%	6.4%

† The decimal in parentheses is the fractional part of the equivalent chain length value for the corresponding branched component in *L. discoidalis*.

Discussion

The differences in rates of water loss per unit body mass between *O. plana* and *L. discoidalis* (Table 1) probably reflect differences in both body size and permeability. Edney[4] found that large beetles lost a smaller proportion of their original mass per day than did small beetles when all were fasted in dry air at 27°C. His tests included *O. plana* and two species belonging to the genus *Lepidochora*. In our study, the much larger *O. plana* lost a significantly lower proportion of its body mass than did *L. discoidalis* at 30°C and above. When expressed per unit surface area, the differences between the species were diminished, but the data still indicate a lower permeability for *O. plana* at 35°C and 40°C. Whatever the basis for the observed differences in water loss, the values are consonant with the activity patterns of the two species; *O. plana,* which is subjected to daytime desert extremes, exhibited the lowest rates at the high temperatures.

A more useful measure of permeability can be obtained by dividing the rate of water loss per unit surface area by the vapour pressure difference (mmHg) between body core and environment to account for the force tending to move water across the cuticle.[7] Permeabilities calculated in this manner were identical for the two species at 30°C (0.031 $\mu g\ mm^{-2}\ h^{-1}\ mmHg^{-1}$), but were significantly lower for *O. plana* at 35°C and 40°C (0.026 and 0.040) compared to *L. discoidalis* (0.045 and 0.049). Edney[7] has prepared a table which permits comparison of permeability and cuticular resistance for a variety of arthropods from various habitats. The values were obtained for *O. plana* and *L. discoidalis at* 30°C (and even higher temperatures) are somewhat lower than the permeabilities exhibited by other xeric insects, including tenebrionid beetles from the Sonoran Desert (*Centrioptera muricata,* 0.063; *Cryptoglossa verrucosa,* 0.084). In fact, only

desert scorpions typically exhibit lower permeabilities (*Androctonus australis,* 0.008; *Hadrurus arizonensis,* 0.012). More important to our study is the observation that, although the permeability of *L. discoidalis* increases more rapidly than that of *O. plana* at higher temperatures, its cuticular transpiration is still well below that of most desert species. The problem for *L. discoidalis* is really one of body size, for even transpiration at these low rates would soon deplete critical water supplies. By restricting surface activity to cooler, more humid night hours, however, transpiration is greatly reduced and body water is conserved.

The amounts of extractable surface lipids and hydrocarbons in *O. plana* (0.05 and 0.04% of body mass, respectively) and in *L. discoidalis* (0.14 and 0.11% of body mass, respectively) are comparable to those found for Sonoran Desert tenebrionid beetles.[3] The predominance of the hydrocarbon fraction in *O. plana* (81%) and *L. discoidalis* (76%) is another feature shared with Sonoran Desert tenebrionids, although percentage values for the latter (more than 90%) are even higher. Hydrocarbon predominance, however, is apparently not characteristic of the family Tenebrionidae, because hydrocarbons account for only 10% of the cuticular lipids of adult *Tenebrio molitor* and *T. obscurus,* and only 7% of the total extracted lipid of adult *Tribolium castaneum* and *T. confusum.*[8,9] Differences in extraction solvents and extraction times account for some of the apparent percentage differences between species.

The composition of surface hydrocarbons in tenebrionid beetles can vary greatly between species.[3] *Onymacris plana* and *L. discoidalis* are no exceptions, as gas chromatography indicated differences in the molecular size range of the hydrocarbon components, in the ratio of *n*-alkanes to branched alkanes, in the relative amounts of individual components, and possibly in the types of branched components present (Figs 1 and 2, Table 3). Straight-chain hydrocarbons having an odd number of carbon atoms predominated in both species, a pattern which is typical of insects in general.[5] Relative amounts of these specific *n*-alkanes, however, showed little similarity in the two species (Table 3). In *O. plana,* the percentage contribution of the odd-numbered *n*-alkanes decreased on either side of the principal component (C_{29}, 26.2% of total). In *L. discoidalis*, a single odd-numbered *n*-alkane (C_{33}) accounted for over 50% of the total hydrocarbon fraction; relative amounts of the shorter odd-chain *n*-alkanes were variable. The dominance of a single component and the overall high percentage of *n*-alkanes (93.6%) makes *L. discoidalis* unique among tenebrionid beetles and perhaps among insects.

The higher permeability of *L. discoidalis* at 35°C and 40°C is difficult to explain solely on the basis of differences in the quantity and composition of the surface hydrocarbons of the two species. The thickness of the hydrocarbon film on the cuticle surface does not appear to be a factor, as the surface densities of hydrocarbon in *O. plana* (0.32 $\mu g/mm^2$) and *L. discoidalis* (0.36 $\mu g/mm^2$) were nearly equal. An inverse correlation between cuticular permeability and surface density was found when the xeric scorpion, *Hadrurus arizonensis,* was compared to the mesic scorpion, *Uroctonus apacheanus,*[10] but in *Eleodes armata,* a Sonoran Desert tenebrionid with significantly higher water loss rates per unit surface area than either Namib beetle, the surface density is approximately five times higher (1.68 $\mu g/mm^2$).[1] The hydrocarbons of both *O. plana* and *L. discoidalis* are saturated and contribute to low permeability.[11,12] Long-chain hydrocarbon molecules, a third feature characteristic of impermeable membranes, are present qualitatively in *O. plana* but absent in *L. discoidalis*. They constitute only one per cent of the total hydrocarbon fraction of *O. plana*, however, an amount not likely to influence transpiration across the cuticle significantly. The role of non-hydrocarbon constituents and their interactions with hydrocarbons in the cuticular waterproofing process of the two species is unknown. The possible contribution of respiratory transpiration to water loss rates observed at higher temperatures in *L. discoidalis* is also unknown.

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