sorption rate constant of 2.85 hr^{-1}, and an elimination rate constant of 0.25 hr^{-1} (half-life ~3 hr). The theoretical C_{max}, reached at 1 hr, was 7.58 μg/ml. A similar set of parameters was obtained when the equation was applied to the means of all 27 treatments, which gave an excellent fit (r^2 = 0.998).

The results of an analysis of variance of the parameters are shown in Table VI. Of the three parameters, as well as C_{max} and t_{max}, none showed any significant formulation or time effect. However, analysis of variance revealed a statistically significant between-subject variation ($p < 0.05$) for all parameters studied, including k_e. Since all subjects were of the slow inactivator phenotype, the finding with this small sample lends support to the proposed existence of additional minor genes involved in the elimination of isoniazid (2), although other mechanisms, *e.g.*, multiphasic absorption, cannot be ruled out entirely.

In conclusion, no significant differences in the relative rate or extent of bioavailability could be demonstrated in three Canadian isoniazid formulations. In slow acetylator subjects, the drug was absorbed rapidly from the gut and was eliminated with a half-life of approximately 3 hr.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 14, 1976, from the *Drug Research Laboratories, Health Protection Branch, Ottawa, Ontario, K1A 0L2, Canada.*

Accepted for publication March 11, 1977.

The authors thank Dr. L. Eidus for valuable discussions, Dr. W. Tostowaryk for the statistical analyses of the results, and Dr. J. C. K. Loo and Mr. P. Fitzsimmons for setting up the computer program.

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NOTES

Influence of Human Skin Surface Lipids on Protection Time of Topical Mosquito Repellent

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Abstract □ Skin surface lipids were extracted from volunteers who had been ranked according to the duration of protection from mosquitoes by diethyltoluamide. These lipids were analyzed by GLC–mass spectrometry for their fatty acid contents. Correlations were found between total skin lipid content and protection time of diethyltoluamide and between certain fatty acid concentrations in the skin lipids and the protection time of diethyltoluamide.

Keyphrases □ Lipids, skin surface—effect on protection time of diethyltoluamide, humans □ Diethyltoluamide—protection time, effect of skin surface lipids, humans □ Repellents, insect—diethyltoluamide, protection time, effect of skin surface lipids, humans

Topical mosquito repellents protect some individuals longer than others. In studies of repellents applied to forearms, the repellency duration with diethyltoluamide (I) against female *Aedes aegypti* (yellow fever) mosquitoes varied in a reproducible way among the volunteers[1]. This report investigated the role of skin surface lipids in affecting the protection time of I toward mosquitoes.

Surface lipids contain certain components that are repellent to *A. aegypti* mosquitoes when evaluated in a dual-port olfactometer (1, 2). The primary repellency of these lipids is derived from the volatile fatty acids, whereas the hydrocarbon fraction exhibits a degree of repellency, primarily due to the unsaturated components present (3).

[1] W. Akers, unpublished data.

Table I—Ranking of Volunteers Based on Descending Order of Duration of Protection of I toward Biting of Female *A. aegypti* Mosquitoes

Volunteer	Average Protection Time, hr, for 0.32 mg of I/cm^2 ± *SD*[a]
P	10.0 (2) ± 2.12
S	9.7 (8) ± 2.24
B	9.2 (3) ± 0.58
A	8.5 (4) ± 2.16
D	8.3 (4) ± 0.50
R	6.5 (5) ± 0.71
LT	6.2 (3) ± 1.53
L	5.6 (8) ± 1.13
M	5.3 (5) ± 1.10
T	5.0 (6) ± 0.55
C	4.5 (5) ± 0.71

[a] Number in parentheses is the number of replicates.

Skin surface lipids have been studied for their chemical composition (4–9), and considerable information concerning their composition has been developed using GLC and mass spectrometric techniques.

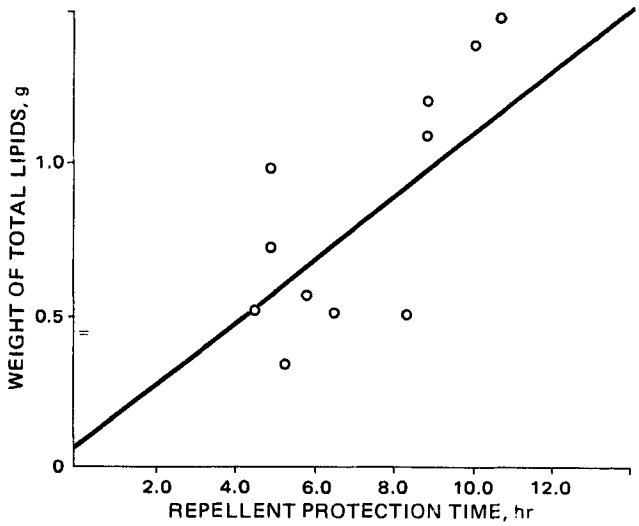

Figure 1—*Repellent protection time of I on individuals as a function of the total skin surface lipid weight.*

EXPERIMENTAL

Attractancy and Repellency—Relative individual attractiveness to mosquitoes was determined using a probing test. Each subject had a 9 × 12-cm foam pad with a 7 × 10-cm hole in the center placed on his or her ventral forearm. The participating volunteer placed this forearm under a 5 × 8-cm screen-covered hole in the center of the bottom of a standard test cage containing 250 avid female mosquitoes. The foam pad prevented cage contamination by the individual's forearm. The number of mosquitoes landing and probing in a 1-min period was recorded.

Each volunteer exposed his or her arm under each of six test cages in a random order with at least a 1-min rest between exposures. All subjects were tested over a 4-hr period on the same day. The average number of mosquitoes probing in 1 min was taken as a measure of the individual's attractiveness to mosquitoes.

Duration of repellent protection time against mosquitoes was determined by application of 0.32 mg of I/cm^2 to a 7 × 10-cm test site on the ventral forearm. Once each hour, the forearm was covered by a plastic sleeve with a 5 × 8-cm hole corresponding to the repellent-treated area. The forearm was inserted into a cage containing 250 avid female *A. aegypti* mosquitoes for a 3-min exposure. The test exposure was repeated hourly until two bites were received on the treated site; that time was recorded as the protection time. The protection times reported are averages over 18 months of testing for each individual (Table I).

Lipid Collection—For the lipid analysis, six subjects consistently exhibiting a longer duration of repellent protection than the average were selected from a volunteer population of 22 along with five additional individuals who consistently exhibited a shorter duration of protection than the average. Acetone extractions were made on each subject three times weekly for 4 weeks as follows. The subject held his or her elbow over a glass funnel assigned exclusively to that individual. Analytical grade acetone, 50 ml, was used to rinse the elbow in each extraction, and acetone extractions were stored in polyethylene bottles in a freezer during the 4 weeks of collection. Subjects used only plain soap[2] and no perfumes for 1 week prior to and during the 4 collection weeks.

GLC–Mass Spectral Analysis of Fatty Acids—Standard amounts (500 μg) of *cis*-13-docosenoic acid were added to the total lipid sample from each volunteer. After derivatization with bis(trimethylsilyl)trifluoroacetamide, the sample was submitted to analysis. An SE-30 column was used for GLC separations. The results are shown in Table II for each volunteer.

Multiple Regression Analysis—The relative effect of each fatty acid on mosquitoes is influenced by two factors in addition to the molar quantity: the intrinsic repellency of the fatty acid molecule to mosquitoes and the relative volatility of the fatty acid. Both factors were included in the relative intrinsic repellency determined (10) in olfactometer testing against mosquitoes. The molar concentration of each fatty acid (Table II) was multiplied by the relative intrinsic repellency (10) to obtain a factor suitable for correlation with the individual attractiveness to mosquitoes and the individual protection times afforded each subject by the repellent.

A multiple regression from the literature (11) was run on the fatty acid intrinsic repellency product as a function of first the protection time and then the attractiveness to mosquitoes. The hierarchial regressional analysis was performed stepwise so that the covariant factors with the highest correlations were introduced into the regression equation first. Saturated fatty acid fractions C_{13} and C_{15} and unsaturated fatty acid fractions C_{14}, C_{15}, C_{16}, and C_{17} were found to be multicollinear with the C_{17} saturated fatty acid fraction (Table III); the six fractions were omitted from the regressional analysis, using the approximation that the partial correlation coefficient for C_{17} would describe their effect on the dependent variable.

The multiple regression analysis equation of dry protection time *versus* the relative fatty acid repellency factor (*RF*) is:

$$\text{dry protection time} = 8.5\,(\text{m}M\ C_{17} \times RF_{C17}) + 50.1\,(\text{m}M\ C_{11} \times RF_{C11}) - 36.1\,(\text{m}M\ C_7 \times RF_{C7}) - 7.9\,(\text{m}M\ C_{16} \times RF_{C16}) + 3.3\,(\text{m}M\ C_{18} \times RF_{C18}) - 0.1\,(\text{m}M\ C_{12} \times RF_{C12}) + 3.5 \quad \text{(Eq. 1)}$$

The significance = 0.042. The repellency factors from Skinner *et al.* (10) are: RF_{C17}, 23; RF_{C11}, 52; RF_{C7}, 61; RF_{C16}, 4; RF_{C18}, 9; and RF_{C12}, 35. The coefficients for the attractiveness correlation are different from those for the protection time correlation. One exception is the coefficient C_{11}, which has the opposite sign in the attractiveness compared to the protection time correlation; that is, negative attractiveness is positive repellency or duration of protection.

The multiple regression analysis equation of attractiveness (Att) *versus* the relative fatty acid repellency factor is:

$$\text{Att} = -44.7\,(\text{m}M\ C_{14} \times RF_{C14}) - 85.9\,(\text{m}M\ C_{11} \times RF_{C11}) + 54.0\,(\text{m}M\ C_{15U} \times RF_{C15U}) + 32.71 \quad \text{(Eq. 2)}$$

The significance = 0.049. The repellency factors from Skinner *et al.* (10) are: RF_{C14}, −2; RF_{C11}, 52; and RF_{C15U}, 17.

DISCUSSION

The duration of repellent protection has a significantly positive correlation ($\alpha < 0.05$) with the amount of lipid recovered from the skin (Fig. 1). Disregarding for the moment the repellent nature of the fatty acids, the correlation can be explained in terms of the film-forming nature of lipids on the skin. One might surmise that the lipids and repellent form a mixture, suppressing the evaporation rate from the skin surface. Gabel *et al.* (12) demonstrated that a lower evaporation rate increases the duration of repellent protection; moreover, musks, vanillin, and vanillin derivatives mixed with I increase repellent persistence when applied to the skin by suppressing repellent evaporation (13). Nicolaides (9) noted the possibility of free fatty acids and mono- and diacylglycerols forming films to reduce evaporative losses of water from the skin. Therefore, the fatty acids may influence repellent protection time by a physical mech-

[2] Ivory.

Table II—Fatty Acid Composition of Skin Surface Lipids from Volunteers

Subject	Fatty Acid Saturated, mg												Fatty Acid Unsaturated, mg					Total Lipid Extract, g
	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	
P	0.1	0.3	0.7	1.7	0.3	6.9	3.2	68.7	43.5	133.4	12.2	38.3	5.8	4.3	47.6	10.7	64.6	1.39
S	0.0	0.2	0.4	1.0	0.1	28.0	2.1	71.0	34.4	139.5	7.3	52.4	3.3	3.1	27.7	6.9	85.1	1.21
B	0.2	0.1	0.2	0.5	0.2	8.4	2.0	50.6	29.9	12.9	7.0	35.1	2.4	3.4	27.2	7.1	58.7	1.10
A	0.2	0.4	0.6	1.9	0.4	15.1	0.6	42.4	11.7	116.3	4.9	42.4	1.4	1.5	25.4	3.2	84.2	0.77
D	0.0	0.1	0.5	0.5	0.2	3.3	0.4	16.4	9.1	64.0	2.4	18.4	0.6	1.0	13.0	1.8	33.0	0.51
R	0.0	0.1	0.3	0.5	0.1	5.1	0.3	15.1	4.9	37.4	2.1	18.1	0.5	0.4	5.9	1.6	31.1	0.52
LT	0.2	0.2	0.5	0.6	0.2	6.4	1.3	42.1	19.4	108.2	4.1	34.6	1.9	2.1	26.6	5.6	83.3	0.99
L	0.0	0.1	0.2	0.5	0.2	6.4	1.0	27.8	15.4	57.9	3.2	16.9	1.5	1.1	14.2	3.7	38.3	0.57
M	0.1	0.3	0.2	1.3	0.1	22.6	0.2	23.7	5.0	51.1	2.1	20.0	0.8	0.3	8.2	1.4	43.7	0.35
T	0.0	0.4	0.3	1.6	0.0	30.4	0.2	35.4	14.1	118.0	4.9	38.9	1.7	1.5	23.0	5.0	66.5	0.73
C	0.1	0.1	0.2	0.4	0.5	3.4	0.1	13.1	4.8	50.3	1.8	18.3	0.6	0.3	9.9	1.5	49.5	0.53

anism in addition to their repellent effects.

Since the fatty acids exhibit repellency to mosquitoes, the effect of fatty acids on individual attractiveness to mosquitoes was considered. Nicholaides (9) indicated that lipases from microbial sources on the skin hydrolyze triacylglycerols to yield fatty acids and that the amount of hydrolysis increases the longer the lipids remain on the skin. Regression analysis of the total weight of extract from each individual produced a significant negative regression coefficient ($\alpha < 0.05$). Therefore, fatty acids in total appear to exhibit a negative attractiveness or positive repellency.

When multiple regression analysis was employed to ascertain the relative effects of fatty acid fractions on individual attractiveness to mosquitoes, C_{14} and C_{11} saturated fatty acids exhibited the highest degree of correlation with negative attractiveness. These fatty acids have a higher volatility than the longer chain fatty acids constituting the bulk of the lipid extract. A higher volatility might indicate more repellent effect against mosquitoes in the air above the skin.

In the second multiple regression analysis, the relation between repellent protection time and the fatty acid fractions was considered. The long chain fatty acids, including those multicollinear with C_{17} (Table III), dominate the regressional coefficients, indicating a positive correlation between the fatty acid fractions and repellent protection time. It cannot be determined whether or not the dominance of the long chain fatty acids in this correlation is a result of film-forming action with the repellent or of an actual repellent effect of these low volatility fatty acids; however, the second coefficient (C_{11}) indicates that a relatively volatile fatty acid, from the mosquito repellency consideration, does exhibit a positive correlation with repellent duration of protection.

With the current data, the multiple regression analysis gives only inferential coefficients so that the precise identification of the fatty acid components affecting attractiveness or protection time is tenuous. The low volatility, long chain fatty acids probably affect repellent persistence by a film-forming mechanism, which slows the evaporative (and possible penetrative) loss of repellent from the skin surface.

Further studies of these factors are needed. Understanding how these factors influence the protection time of repellents should help in the development of more effective repellents.

Table III—Correlation Coefficients Exhibiting a High Degree of Collinearity

C_{13}	0.919					
C_{15}	0.947	0.947				
C_{14U}	0.961	0.941	0.948			
C_{15U}	0.936	0.955	0.985	0.915		
C_{16U}	0.953	0.900	0.937	0.949	0.941	
C_{17U}	0.937	0.930	0.967	0.955	0.970	0.970
	C_{17}	C_{13}	C_{15}	C_{14U}	C_{15U}	C_{16U}

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 3, 1977, from the *Life Sciences Research Division, Stanford Research Institute, Menlo Park, CA 94025.*

Accepted for publication March 9, 1977.

Supported by U.S. Army Medical Research and Development Command Contracts DADA-170-70-C-0112 and DADA-170-70-C-0109.

The authors thank Kathy L. Zeller, Steven F. Bayles, and Ronald K. Shimmin for assistance.

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