## Repellency of Skin-Surface Lipids of Humans to Mosquitoes

Abstract. *Skin lipids obtained from the washings, in acetone or diethyl ether, of the foreheads or arms of humans are very repellent to female* Aedes aegypti *when evaluated in a dual-port olfactometer. By means of thin-layer chromatography, the repellent substances can be separated from nonrepellent material also present in skin lipids.*

In the course of investigating the attractiveness of human skin to mosquitoes we have examined the role of skin lipids in the host-seeking behavior of female *Aedes aegypti* var. *queenslandensis* (Theo.).

Skin lipids were evaluated in a small (12 cm by 10 cm; 6 cm high) dual-port olfactometer modified from the design of Willis (*1*). A humidified airstream filtered through charcoal was split into two equal portions and passed through glass coils submerged in a constant-temperature water bath which heated the airstreams to 27°C. The air flowed through the apparatus at a rate of 2 liters per minute, as measured by two glass float flowmeters. The lipid samples, because of their insolubility in water, were tested dry in round-bottom flasks. One airstream passed through a flask containing the sample to be tested, and the other through a dry empty flask. Each stream then flowed directly through a Buchner funnel (5 cm in diameter) into the test cage, the two funnels being mounted side by side. The two open ends of the cage were covered with 40-mesh nylon net.

Repellency was measured by counting the numbers of mosquitoes that distributed themselves in the test airstream or the control stream. Twenty mosquitoes were used for each test, counts being made once every minute for 10 minutes. In addition, the control and sample ports were reversed and the samples retested to eliminate effects of position.

Extracts of lipids were prepared by washing the elbows, hands, or forehead in diethyl ether for 1 to 2 minutes. Ether was removed from the solution under reduced pressure, and a mixture of skin lipids remained.

Benzene was used as a developing solvent for separation by thin-layer chromatography of the nonpolar lipid components on plates containing silica-gel G. Spots were detected by immersion of the plate after development in a tank containing iodine crystals. The following $R_F$ values were obtained: origin, 0.11, 0.54, 0.92. Larger quantities of skin lipids extracted in either diethyl ether or acetone were chromatographed on plates containing thick layers of silica-gel G, and were developed with benzene. Skin-lipid components were detected by spraying one side of the plate with iodine in methanol, and the regions containing material sensitive to iodine vapor, as well as the intermediate regions between the iodine-sensitive materials, were cut out and extracted with chloroform. After removal of the chloroform under reduced pressure, these substances were evaluated in the olfactometer. In order to keep the surface area of the sample constant, in subsequent runs (Nos. 76.0 to 76.6, Table 1) equal weights of samples were dissolved in equal volumes of diethyl ether and concentrated under reduced pressure in round-bottom flasks of the same size.

By means of the thin-layer chromatography we found that the pattern of spots developed by iodine vapor was remarkably consistent both for extracts from different individuals and for extracts from the three body regions.

The repellency values for the crude extracts (all of which were repellent) are shown in Table 1 together with values for various lipid fractions extracted from the silica-gel G and a value for a standard tropical insect repellent, *m*-diethyltoluamide (DEET), which was tested for comparison. Repellency corresponded to the less polar spots detected by iodine. It is interesting that the spot at the origin, which undoubtedly was composed of mixtures of more polar materials, was not repellent to female *Aedes aegypti*. In some instances, it appeared to be attractive.

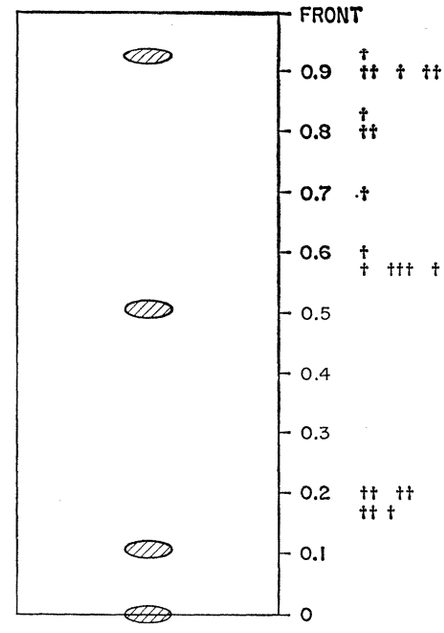


Fig. 1. Thin-layer chromatography of skin lipids. Developing solvent, benzene; adsorbent, silica-gel G (†, ††, †††); increasing degree of repellency, as determined in the olfactometer, of material extracted from the plate at the $R_F$ indicated. These measurements are from a number of separate chromatograms and the $R_F$ values vary somewhat.

Figure 1 shows the correspondence

Table 1. Repellency of human skin lipids to mosquitoes, as determined in an olfactometer. Each value represents the mean of ten 1-minute observations. Skin lipids were obtained from both males and females. Samples 76.0 to 76.6 were and samples 37 to 40 were not evaluated on a weight-constant surface area basis. Student's *t*-test was used for determining the significance ($p < .01$) of olfactometer data.

| Sample | | Identification | Olfactometer data | | |
|---|---|---|---|---|---|
| | | | No. of mosquitoes | | |
| No. | Weight (g) | | Control port | Sample port | IR* |
| 37.0 | 0.73 | Hands and elbows washed in 1500 ml of ether (8 people) | 12.7<br>18.6 | 7.3<br>1.4 | 27<br>86 |
| 38.0 | 0.47 | Acetone extract of skin lipids | 15.4<br>12.1 | 4.6<br>7.9 | 54<br>21 |
| 39.0 | 0.30 | Ether extract of arm (1 person) | 17.5 | 2.5 | 75 |
| 40.0 | 0.28 | Ether extract of hands (7 people) | 14.6 | 5.4 | 46 |
| | | Water versus water | 10.4 | 9.6 | 4 |
| | | DEET versus water | 14.5 | 5.5 | 45 |
| | | DEET versus lipids | DEET/8.8 | Lipid/11.2 | |
| 76.0 | 0.005 | Ether extract of elbows (11 people) | 13.4 | 6.6 | 34 |
| | | *Fractions obtained by thin-layer chromatography* | | | |
| 76.6 | 0.005 | $R_F$, 0.8 –front | 13.3 | 6.7 | 33 |
| 76.5 | 0.005 | $R_F$, 0.65–0.8 | 10.0 | 9.9 | |
| 76.4 | 0.005 | $R_F$, 0.40–0.65 | 11.7 | 8.3 | 17 |
| 76.3 | 0.005 | $R_F$, 0.25–0.40 | 10.3 | 9.7 | 3 |
| 76.2 | 0.005 | $R_F$, 0.10–0.25 | 11.8 | 8.2 | 18 |
| 76.1 | 0.004 | $R_F$, 0.0 –0.10 | 10.6 | 9.4 | |

* Index of repellency $= \frac{\text{No. of mosquitoes on control side} - \text{No. on sample side}}{\text{Total No.}} \times 100$

between the location of the spots detected by iodine and the repellency as determined in the olfactometer. We have not yet identified the repellent substances. The significance of skin lipids in terms of their effect on the behavior of mosquitoes can only be conjectured. Perhaps the attraction of the host to the mosquito depends on a balance between naturally occurring repellents and attractants.

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**References and Notes**

1. E. R. Willis, *J. Econ. Entomol.* **40**, 770 (1947).
2. Supported by the U.S. Army Medical Research and Development Command, Department of the Army, on contracts DA-40-193-MD-2466 (W.A.S.) and DA-49-193-2465 (H.M.).

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## Clostridium botulinum Type F from Marine Sediments

Abstract. Clostridium botulinum *Type F has been demonstrated in two samples of marine sediments. One sample was taken 83 kilometers off the coast of California; the other, 100 kilometers off the coast of Oregon. Cultures of this type have not been reported previously in the United States, and only once before in the whole world.*

In a survey on the incidence of *Clostridium botulinum* in the coastal areas of Alaska, Washington, Oregon, and California, two samples of marine sediment have yielded cultures of *Cl. botulinum* Type F. The first and only previous culture of *Cl. botulinum* Type F was isolated from a homemade liver paste connected with an outbreak of human botulism on the Danish island Langeland (*1*). One of the five persons who ate the liver paste suffered no harm, but three had severe attacks of botulism, and the fifth person died three days later (*2*). This culture has since been described as a prototype strain designated as *Cl. botulinum* Type F (*3*).

The marine sediments that yielded cultures of *Cl. botulinum* Type F in this laboratory were collected 83 kilometers from the coast of California (sample A) and 100 kilometers from the coast of Oregon (sample B). Sample A came from a depth of 1646 meters at 42°N latitude and sample B from a depth of 1326 meters at 43°N latitude.

Portions (approximately 5 g) of these mud samples were inoculated into 25 ml of broth containing glucose, peptone, trypticase, beef infusion, and ground meat, a modification of Dolman's medium (*4*). The inoculated tubes were incubated anaerobically (95 percent nitrogen and 5 percent carbon dioxide) at 25°C for 5 days. A portion of the broth was then centrifuged at 10,000 rev/min (12,000*g*). The supernatant was tested for toxicity by injecting two mice (Swiss Webster strain) intraperitoneally with 0.4 ml of a 1:2 dilution of the supernatant and gelatin-phosphate buffer. Characteristic symptoms of botulism, if present, occurred within 20 hours after injection.

The toxin was identified by injecting pairs of mice with 0.5 ml of mixtures composed of 0.4 ml of a 1:2 dilution of the supernatant and 0.1 ml of one of the following antitoxins: a polyvalent antitoxin (Types A, B, C, E, and F in equal concentrations); or an individual antitoxin (Types A, B, C, D, E, or F). Heat lability of the toxin was determined by injecting a pair of mice with 0.4 ml of a 1 : 2 dilution of the sample supernatant that had been heated for 10 minutes at 100°C and then cooled. All mice were observed for at least 6 days.

The data supporting the identification of the culture as *Cl. botulinum* Type F are summarized in Table 1. Neutralization of the toxin was achieved in both samples only by the Type F antitoxin and by a polyvalent antitoxin containing Type F. Heating the supernatant for 10 minutes at 100°C inactivated the toxin. The toxin of sample A was not neutralized by Types A, B, C, D, or E antitoxin, since the mice injected with this toxin died within 19 to 23 hours and had characteristic symptoms of botulism. The same results were obtained for sample B with the exception (not shown in Table 1) that Type E antitoxin showed some neutralization of the toxin, but only when the Type E antitoxin was in large excess. Cross neutralization between Type F and Type E was also reported from the Denmark isolate (*1, 3*). The titer of the toxin from both cultures was 6 to 20 minimum lethal doses (mouse) per milliliter of medium.

Table 1. Neutralization reaction pattern of cultures identified as *Clostridium botulinum* Type F. Results are given as number of mice dead out of number tested. S is supernatant; HS, heated supernatant.

| Source of toxin | Anti-toxin | Result | |
|---|---|---|---|
| | | Sediment sample A | Sediment sample B |
| S | None | 6/6 | 6/6 |
| S | ABCEF | 0/2 | 0/2 |
| S | A | 4/4 | 4/4 |
| S | B | 4/4 | 4/4 |
| S | C | 4/4 | 4/4 |
| S | D | 2/2 | 2/2 |
| S | E | 4/4 | 4/4 |
| S | F | 0/6 | 0/6 |
| HS | None | 0/2 | 0/2 |

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**References and Notes**

1. V. Moller and I. Scheibel, *Acta Path. Microbiol. Scand.* **48**, 80 (1960).
2. B. B. Jensen and F. Hahnemann, *Ugeskrift Laeger* **121**, 1363 (1959).
3. C. E. Dolman and L. Murakami, *J. Infect. Diseases* **109**, 107 (1961).
4. C. E. Dolman, *Can. J. Public Health* **48**, 187 (1957).
5. Supported by funds made available by AEC and administered by means of a contract between the U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries, and the U.S. Atomic Energy Commission. Antitoxin obtained from Department of Health, Education, and Welfare, Communicable Diseases Center, Atlanta, Georgia.

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## Deformity of Forelimb in Rats: Association with High Doses of Acetazolamide

Abstract. *Deformities of the right forelimb occurred in a number of offspring of rats given high doses of the carbonic anhydrase inhibitor, acetazolamide, during pregnancy. In most cases this was the only deformity found. More than 20 times the usual therapeutic dose rate used for humans was required to produce this effect in rats.*

We have found a remarkably specific and reproducible malformation of the right front extremity in a number of offspring of rats given a diet containing large concentrations of acetazolamide during pregnancy. Acetazolamide (2-acetylamino-1,3,4,-thiadiazole-5-sulfonamide) is a potent carbonic anhydrase inhibitor which has been used as a diuretic in human patients since 1953.