L-Lactic Acid: A Mosquito Attractant Isolated from Humans

Abstract. *L-Lactic acid was the major component in material isolated from humans that was active as an attractant for female yellow fever mosquitoes,* Aedes aegypti *(L.). The L(+)-isomer was several times as attractive as the D-isomer. Good correlation was found between the attractiveness of an individual to mosquitoes and the quantity of lactic acid present in an acetone washing of his hand.*

The nature of the mosquito attractant in emanations from warm-blooded animals has been the subject of investigation and conjecture for many years. We have now identified L-lactic acid as the major component in material isolated from humans that attracts female yellow fever mosquitoes, *Aedes aegypti* (L.).

Attractive material was first isolated from condensate obtained from a flowing nitrogen stream passing over a human arm and through a cold trap (about −15°C); the quantity of attractant obtained was too small for identification. Larger amounts were then obtained by washing the forearms of subjects with acetone. When these solutions were treated with ammonia and evaporated to dryness under reduced pressure, they yielded a residue that contained the attractive material.

The attractive substances in the acidified residue from arm washings were separated by thin-layer chromatography on silica gel with a developing solvent containing butanol, acetic acid, and water (75 : 18 : 75) and were located by spraying with a solution of bromocresol green indicator adjusted to *p*H 9.8. The major attractive component was found in the zone having the same R_F value (0.60) as L-lactic acid. The acid was recovered by eluting this zone with acidified acetone, adding ammonia to alkalinity, and evaporating to dryness. A minor amount of an unidentified substance showing slight attractant activity was also found at R_F 0.1 to 0.2. Material eluted from other sections of the chromatogram showed no appreciable activity in the mosquito bioassay.

The identity of the attractant eluted from the zone at R_F 0.60 was confirmed as lactic acid by thin-layer chromatography on silica gel developed with an ethanol, ammonia, water (80 : 5 : 15) system and with an acetone, methanol, water (4 : 4 : 2) system, and by chromatography on paper developed with an ethanol, ammonia, water (80 : 5 : 15) system and with an ethyl acetate, acetic acid, water (3 : 1 : 1) system; spots were eluted from the paper with ethanol. In all tests, the attractant gave a single spot with the same R_F value as authentic L-lactic acid. Further confirmation was obtained from the infrared spectrum of the ammonium salt (KBr disc), which agreed with that of ammonium L-lactate. The gas chromatographic retention time (*1*) of the methyl ester obtained by treating the acidified extractive from the zone at R_F 0.60 with diazomethane agreed with that of L-lactic acid similarly methylated. The presence of lactic acid in the attractant material trapped from a gas stream that had passed over a human arm was then tested for and found by paper chromatography.

An enzymatic assay (*2*) specific for L-lactic acid demonstrated the presence of 14 mg of L-lactic acid in the acidified residue from acetone washings of 800 arms. A portion of this material equivalent to 10 μg of L-lactic acid had essentially the same attraction for mosquitoes as 10 μg of authentic L-lactic acid. In three tests, L-lactic acid was five times as attractive to mosquitoes as an equal quantity of D-lactic acid.

The olfactometer used in the bioassays was a Plexiglas (*1*) enclosure (173 by 65 by 100 cm), designed to bring purified outside air (free of human emanations) into a chamber containing 200 to 300 female mosquitoes via two traps (*3*). A subject put his arm through one of two rubber sleeves located just ahead of the traps, and the outside air was allowed to sweep over the forearm and hand and attract mosquitoes into the traps. Degree of attraction was gauged by the percentage of mosquitoes attracted by the arm as compared with the percentage attracted by no arm (check trap). For the bioassays of isolated fractions, the olfactometer was modified; the rubber sleeves were replaced with two Plexiglas disks, each having a centrally located, polyethylene tube (1.25 cm, outside diameter) through which an auxiliary flow of 2.5 liters of dry, tank nitrogen and 2.5 ml of carbon dioxide per minute was maintained to introduce the sample (see Fig. 1). The sample tube was an assembled male and female 24/40 ground glass joint with the outside diameter of the unground ends reduced to 0.9 cm to fit easily into the line. Before the test, the sample, usually in 0.5 ml of acidified acetone, was plated out onto the inner walls of the sample tube by allowing the solvent to evapo-

Table 1. Percentage of female *A. aegypti* attracted by tube containing 10 μg of L-lactic acid as a stream of nitrogen (2.5 liter/min) passed through, with and without the addition of 2.5 ml of CO_2 per minute.

Cage	Mosquitoes attracted (%)			
	No CO_2 added		CO_2 added	
	Lactic acid	No lactic acid	Lactic acid	No lactic acid
A	0.1	0.0	13	0.1
B	0.0	0.0	9	0.0
E	0.0	0.0	20	0.2
F	0.0	0.0	32	0.3
G	0.0	0.0	15	0.0
H	0.1	0.1	20	5.5

Table 2. Amount of L-lactic acid found in acetone rinses of skin of individuals compared with the percentage of female *A. aegypti* attracted by the rinses.

Subject	L-Lactic acid found (μg/ml)	Attracted by 0.5 ml of rinse (%)
	Test No. 1	
HG	30	33
NS	54	52
TN	15	26
	Test No. 2	
HG	44	53
NS	25	41
TN	12	41

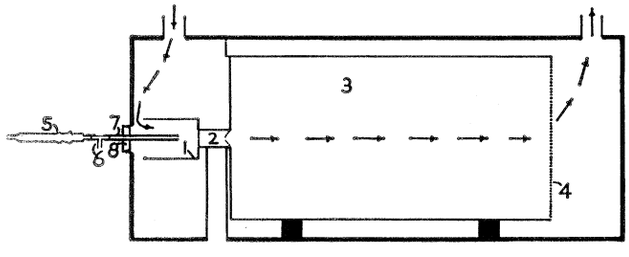

Fig. 1. Schematic side view of olfactometer with arrows showing flow of purified outside air through (1) plastic sleeve, (2) mosquito trap, (3) mosquito chamber, and (4) aluminum screening. An auxiliary flow of 2.5 liters of N_2 per minute passes through (5) sample tube, (6) T-tube (through which 2.5 ml of CO_2 per minute is added), (7) polyethylene tube in (8) Plexiglas disk and then into (3) via (1) and (2). A duplicate sample inlet system (5, 6, 7, 8, 1, 2), behind the one shown, also leads into the mosquito chamber (3). Mosquitoes attracted by vapors from sample tube enter trap (2) from chamber (3) through opening in screen funnel and cannot return.

rate as the tube was rotated and tilted back and forth. Attractiveness of samples was based on the percentage of insects trapped in 3 minutes after the sample tube was connected into one of the two lines (the other was the check). All parts were handled with disposable gloves to avoid contamination of the system. Before each test, mosquitoes were exposed to the nitrogen–carbon dioxide mixture; if more than 2.5 percent of the mosquitoes responded, the sleeves and traps were replaced.

With mosquitoes of average avidity, 10 μg of L-lactic acid attracted 29 to 75 percent of the mosquitoes in 3 minutes, and three to four consecutive tests could be made (subjected to air flow for 9 to 12 minutes) before the sample tube lost its attractiveness. The attractiveness of lactic acid did not appear to be enhanced by the addition of any other component isolated from the acid fraction, including the unidentified minor attractant. However, the presence of carbon dioxide is essential (Table 1). Carbon dioxide has long been recognized as a mosquito activator (*4*), but alone in nitrogen or in purified air it does not attract mosquitoes.

Other carboxylic acids containing as many as five carbon atoms were tested for attractiveness, but the response was never more than feeble compared with the responses to L-lactic acid.

The quantity of lactic acid in the acetone rinses of the hands of individuals who differed in their attractiveness to mosquitoes was determined by enzymatic assay. Overall there was good correlation between the amount of lactic acid found in the rinses of these subjects and the percentage of mosquitoes attracted by the rinses from each (Table 2). The rinses showed the same order of attractiveness between subjects that had been shown in many previous tests of the hands of the same individuals.

Lactic acid occurs in sweat and on skin (*5*); as a product of animal muscle metabolism it is invariably in the L(+)-configuration (*6*) (sometimes referred to as "sarcolactic acid"). An old report (*7*) indicated that the compound is slightly attractive to *A. aegypti*. The compound appears to be more volatile than is generally recognized, and conversion to the less volatile ammonium salt was required to obtain satisfactory recoveries in the evaporations of biological material and eluates from chromatograms.

A good attractant in traps is an invaluable aid to the detection of insect infestations. Such an attractant is needed for the worldwide eradication of the yellow-fever mosquito; lactic acid may be useful for this purpose.

Note added in proof: A recent report (*8*) stated that formic, acetic, propionic, and lactic acids attracted *A. aegypti* and that lactic acid (L- or D- not specified) was the most attractive. The acids tested were not isolated from humans, and the concurrent need of carbon dioxide for attraction was not noted.

FRED ACREE, JR., R. B. TURNER
H. K. GOUCK, MORTON BEROZA*
NELSON SMITH
Entomology Research Division, Agricultural Research Service, Gainesville, Florida 32601

References and Notes

1. The gas chromatography was carried out on an F & M Model 810 instrument equipped with a flame-ionization detector and stainless steel columns, 180 by 0.3 cm (OD), packed with 5 percent Carbowax 20M on 60/80 mesh Diatoport S (F & M Scientific Corp.) and maintained at 100°C. The nitrogen flow rate was 45 ml/minute.
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* Agricultural Research Center, Beltsville, Md. 20705.

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Gaucher's Disease: A Genetic Disease Detected in Skin Fibroblast Cultures

Abstract. *Skin fibroblasts from three adult patients with chronic noncerebral Gaucher's disease, three children of one of the patients, three parents, and six normal individuals were grown in cell culture. Giant fibroblasts containing metachromatic material were seen in all cultures derived from affected individuals and heterozygous carriers but not in those derived from normal individuals.*

Gaucher's disease is a rare genetic disorder of cerebroside metabolism which results in the accumulation of glucocerebroside in various tissues of the body (*1*). The metabolic defect appears to be a deficiency of the enzyme which catalyzes the cleavage of glucose from glucocerebroside (*2*). Although there are two known clinical forms, infantile cerebral and adult chronic noncerebral, no biochemical difference between the two has been found (*3*). Family studies suggest an autosomal recessive mode of inheritance (*4*). The clinical manifestations are presumed secondary to organ infiltration of a large storage cell, the Gaucher cell, resulting in hepatosplenomegaly, lymphadenopathy, and bone lesions. The cytoplasm of the Gaucher cell has a unique reticular appearance and permits diagnosis to be made by light microscopy (*5*).

It has become evident as a result of studies in cell culture that diseases associated with the intracellular accumulation of a metabolite and which can be detected either morphologically or chemically should be amenable for cell culture studies.

Skin biopsies were obtained from three patients with the adult form of Gaucher's disease, three clinically unaffected offspring from one patient with the disease, and three parents of affected individuals. Six normal unrelated individuals also were studied. Four different biopsies were taken from one patient on four different occasions over a 12-month period. The establishment of cell lines from biopsy specimens by standard culture methods has been described (*6*). The cell lines were grown as monolayer cultures from 2 to 5 months in culture in modified Eagle's medium (*7*) with 10 percent newborn calf serum. The preparation of slides and subsequent staining of the cells with the metachromatic dye toluidine blue O have been described elsewhere (*6*).

Cultures of skin fibroblasts from patients with Gaucher's disease contained large cells with marked metachromasia (Fig. 1). These cells were observed in the cultures from the first subculture (2 months after establishment of explant culture) through the 20th subculture (9 months in culture). The number of metachromatic fibroblasts was approximately 20 to 40 percent and was relatively constant in replicate cultures de-