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LETHAL EFFECTS OF AQUEOUS FORMULATIONS CONTAINING FATTY AMINES OR ACIDS AGAINST EGGS AND LARVAE OF AEDES AEGYPTI

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16. Abstracts

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Fatty amines, ranging widely in chain length and polarity, were evaluated against both larvae and eggs. Relative larvicidal activities correlated roughly with ovicidal activities. The least larvicidal of these amines, 6-amino-1-hexanol, used at 0.6% in water with 0.1% decyl alcohol and 6% urea, provided a good ovicide. Of all the ovicides tested, it is presumably the least hazardous to higher organisms.

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Lethal Effects of Aqueous Formulations Containing Fatty Amines or Acids Against Eggs and Larvae of *Aedes aegypti*[1,2,3]

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ABSTRACT

Various aqueous ovicidal formulations, some of which pose minimum hazards for higher organisms, are described for use against *Aedes aegypti* (L.). These ovicides appear to attack mainly layers of the eggshell which resist water permeability, an attack which causes the egg to dehydrate and collapse under ambient conditions. Humidity effects were studied before and after treatment. Basic and acidic formulations containing both nonpolar and polar compounds were found effective against either dry or moist eggs exposed to ambient conditions after treatment. The basic mixture contained a nonpolar long-chain aliphatic amine such as octylamine emulsified in an aqueous solution of a polar compound such as ethanolamine or urea, and the acidic mixture contained a nonpolar fatty acid such as octanoic acid in aqueous polar mercapto acid. Nonpolar compounds alone in water were ovicidal under special conditions. An emulsion of fatty acid in water was effective against eggs exposed to high humidity before or after treatment, and aqueous fatty amines were ovicidal for eggs exposed to high humidity after treatment.

Fatty amines, ranging widely in chain length and polarity, were evaluated against both larvae and eggs. Relative larvicidal activities correlated roughly with ovicidal activities. The least larvicidal of these amines, 6-amino-1-hexanol, used at 0.6% in water with 0.1% decyl alcohol and 6% urea, provided a good ovicide. Of all the ovicides tested, it is presumably the least hazardous to higher organisms.

Eggs of the yellowfever mosquito, *Aedes aegypti* (L.), which are not laid on water and can survive for months under dry conditions, have shells which are effectively water-proofed with lipid and tanned protein (Clements 1963). Against the more permeable eggs of mosquitoes which lay their eggs on water, nonpolar aliphatic amines were good ovicides (Mulla and Chaudhury 1968), but these amines were ineffective in aqueous sprays for aedine eggs unless they were supplemented with a polar amine, such as ethanolamine, hydrazine, or ethylenediamine (Wilton et al. 1968, Cline et al. 1969, Wilton and Fay 1969). These formulations then were found effective in field trials (Jakob 1969, Jakob et al. 1970). When eggs were exposed to ambient conditions for several hours after such treatment, they characteristically collapsed and some showed dehiscence, indicative of hatch stimulation, such as Judson et al. (1962) described for other ovicidal agents. Present evidence shows that such amines damage primarily the eggshell and thus reduce the resistance of the egg to water loss under ambient conditions. Aedine eggshell protein resembles larval cuticle protein on the basis of X-ray diffraction data (Harwood 1958), and cuticular protein resembles collagen in some respects (Fraenkel and Rudall 1947). Since collagen has labile ester (Gallop et al. 1959) or cyclic imide (Bornstein 1970), linkages which can be broken by mild treatment with hydrazine or hydroxylamine, similar linkages possibly may be broken in the ovicidal action of polar amines on aedine eggs. Also, strong hydrogen bonding protein denaturants, such as urea in ovicides, might be expected to damage the shell. Some correlation was found between larvicidal and ovicidal activities of amines, which suggests that the attack on the larval cuticle is similar to the attack on the eggshell.

MATERIALS AND METHODS.—Duomeen® L-11 (β-propylenediamine with aliphatic chain of 11 carbons) was furnished by Armour Industrial Chemical Co., Chicago, Ill., and (2-ethylhexoxy)-3-propylamine by BASF Corp., Paramus, N.J. The anionic surfactant, Aerosol® TR-70% (sodium bis(tridecyl)sulfosuccinate), was supplied by American Cyanamid Co., Wayne, N.J. The nonionic surfactant, T-det® N-6 (nonylphenol ethylene oxide adduct), was furnished by Thompson-Hayward Chemical Co., Kansas City, Mo., and the nonionic Pluronic® L101 (polyoxypropylene-polyoxyethylene condensate) by BASF Wyandotte Corp., Wyandotte, Mich.

Aminoalcohols were synthesized by heating an alkyl halide with excess amine. Most of these products were solids and were recrystallized from ethanol and water. Products were purified until they showed only traces of byproducts on silica gel thin layer chromatograms developed in n-propanol-34% ammonium hydroxide (7:3).

The amines, tested as larvicides, were emulsified or dissolved in tap water by shaking and in some cases by heating. About 25 larvae were added to each 100 ml of mixture in a 400-ml beaker. Average mortalities were derived from tests performed on 2 or more different weeks.

Details for screening aqueous ovicidal formulations by the dip technique were described previously (Cline et al. 1969). Briefly, the eggs on paper tabs were dipped quickly in the formulation, excess fluid was removed, and tabs were exposed on a shelf at ambient 74–78°F and 55–70% RH for 24 hr. Eggs were then washed, and hatchability was determined after submerging the tab in hatching medium consisting of day-old culture of brewer's yeast and pulverized lab chow contained in the center well of a culture microslide. Replicate determinations of ovicidal activity were done on different weeks.

To condition eggs at 100% RH, a paper strip with its eggs was placed in a beaker resting on a wet paper towel in the bottom of a loosely sealed quart jar in a room kept at 80°F and 65–75% RH.

In radiocarbon studies, the treated egg tab was

[1] Diptera: Culicidae.
[2] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education and Welfare.
[3] Received for publication Apr. 27, 1971.

placed in a quart jar ca. 4 in. from 5 μliters of ^{14}C-ethanolamine (1.25×10^8 counts/min), and the jar was sealed. After a 3-hr exposure, the tab was washed with ethanol, and 200 eggs were brushed from the paper. These eggs were burned in a modified Coleman Carbon-Hydrogen Analyzer, the $^{14}CO_2$ was trapped in ethanolamine, and the trap solution was used for scintillation counting as described previously (Cline and Pearce 1966).

RESULTS AND DISCUSSION.—*Fatty-Amine Formulations.*—After eggs of *A. aegypti* were given a quick dip in aqueous formulations of 0.1% decylamine or Duomeen L-11 (*N*-alkylpropylenediamine) in 1% ethanolamine and exposed to air at ambient 74–78°F and 55–70% RH for 24 hr, they characteristically lost water, collapsed, and gave a 0–4% hatch on submergence in hatching media. However, hatches were fairly high after treated eggs were exposed for many days to very moist rather than ambient conditions. For the 2 nonpolar amines in combination with ethanolamine at 1%, hatches after exposure for various lengths of time in a moist jar (100% RH) were: 0.1% decylamine (1 day 56% hatch; 8 days 50%; 15 days 43%; 22 days 57%; 29 days 50%), 0.1% Duomeen L-11 (8 days 64%; 22 days 42%; 29 days 68%). With untreated eggs, hatches were 70–90% after storage for 1–4 weeks at 100% RH. The very moist atmosphere of the jar promoted the partial hatching and mortality of some of the eggs. Eggs stored at 65–75% RH for several weeks gave hatches of 90–100%. When treated eggs were removed from the very moist jar at any time and exposed to ambient conditions for 24 hr, they collapsed and gave a negligible hatch on submergence. Results of these experiments indicate that the ovicidal amines attacked primarily the eggshell rather than the larva inside the shell. After destruction of water-proofing layers of the shell, the larva would be killed by dehydration if the egg were exposed to slightly less than 100% RH.

An alternative mode of action of the amines, involving a hatching response of the larva, was explored with use of eggs killed by freezing for 2 days. When these dead intact eggs were dipped quickly in aqueous 0.2% octylamine or 0.1% Duomeen L-11 in 1% ethanolamine and exposed to ambient conditions for 24 hr, most of them collapsed more than 50%. Most of the controls treated only with water remained intact. These observations provide further evidence of eggshell corrosion by the amines, since dead eggs could not dehydrate as a result of a shell-cracking hatching response of the larva. Moreover, as expected, freeze-killed eggs did not collapse after treatment with formulations not found previously (Cline et al. 1969) to cause collapse or mortality in live eggs. These formulations consisted of nonpolar or polar amines used alone in water, combinations of 0.2% of a surfactant, such as Aerosol TR-70% or T-det N-6, with aqueous 1% ethanolamine, or 0.1% of a nonpolar amine with 1% aqueous tertiary amine, such as dimethylaminoethanol, tetramethylethylenediamine, or triethanolamine.

Different types of amines also produced different effects on eggs killed by heating in water. Collapse of aedine eggs in saturated NaCl at 50–55°C has been attributed to disorganization of the wax layer of the shell (Christophers 1960). When eggs were heated in water at 90°C for 1 min, a procedure described by Beckel (1958), they collapsed almost completely shortly after submergence in hypertonic M solutions of NaCl, urea, or certain amines (Table 1). These data show a permanent collapse in solutions of NaCl, urea, or tertiary amines but only a transitory collapse in solutions of the primary amines, ethanolamine and ethylenediamine. Addition of only 0.1% of the nonpolar decylamine to the primary amine solutions essentially prevented collapse, and when added to solutions of the other compounds it shortened the duration of the collapse. From these results, it appears that the primary polar amines and the nonpolar decylamine, in contrast with other compounds, react with the heated eggshell to further increase permeability.

The high ovicidal activity of primary amines, such as ethanolamine, hydrazine, and ethylenediamine, in contrast with tertiary amines, such as dimethylaminoethanol, tetramethylethylenediamine, and triethanolamine (Cline et al. 1969), might be explained on the basis of a greater reactivity of the primary amine with hydrogen or other bonds of the shell protein. The outstanding solvent action of polar amines for protein was explained partly on the basis of hydrogen bonding (Singer 1962), and collagen was shown to be degraded by mild treatment with dilute aqueous hydrazine or hydroxylamine at 40°C (Gallop et al. 1959, Bornstein 1970). Well-known protein denaturants, such as glycerol and urea, were also effective in ovicides.

Many nonpolar to moderately polar amines, found effective in ovicides containing 1% ethanolamine, also were tested for 24-hr activity against 4th-stage larvae. Table 2 lists these amines in the order of

Table 1.—Collapse of heat-killed eggs in hypertonic solutions of various compounds.

1 M compound	Avg % collapse at intervals after submergence					
	1 M compound			1 M compound + 0.1% decylamine		
	1 hr	3 hr	5 hr	1 hr	3 hr	5 hr
NaCl (5.8%)	70	80	80	80	70	30
Ethanolamine (6.1%)	40	30	10	10	0	0
Dimethylaminoethanol (8.9%)	50	50	50	50	30	10
Ethylenediamine (6.0%)	50	30	10	5	0	0
N,N,N',N'-Tetramethylethylenediamine (11%)	80	80	80	70	30	10
Triethanolamine (14.9%)	70	80	80	70	60	50
Urea (6.0%)	60	80	80	70	40	10

Table 2.—Larvicidal activities of amines used with 1% ethanolamine ovicides.

Amine	Larvicide: Avg LC_{50}, ppm	Ovicide: % Amine	Ovicide: Avg %[a] hatch
N,N-Dimethyldodecylamine	20	0.1	0.5
N-Dodecyl-3-amino-1,2-propanediol	20	.1	5
N-Dodecyl-*N*-methylamino-ethanol	20	.1	1
N-Decyl-1-amino-2-propanol	25	.1	0
2-Octylaminoethanethiol	30	.1	6
N-Dodecyldiethylenetriamine	30	.1	2
Duomeen L-11	35	.1	1
N-Decylaminoethanol	40	.1	2
N-Decyl-2-amino-1-butanol	40	.1	0.2
N-Dodecyliminodiethanol	40	.1	3
Cyclododecylamine	40	.1	7
Decylamine	45	.1	2
N-Octyl-3-amino-1-propanol	45	.1	3
N-Hexyl-1,3-diamino-2-propanol	60	.1	4
N-Dodecyl-2-(2-aminoethoxy) ethanol	75	.1	5
Octylamine	80	.1	2
N-Octyl-6-amino-1-hexanol	80	.1	3
Trihexylamine	80	.1	4
N-Octyl-2-amino-1-methoxypropane	90	.1	0
(2-Ethylhexoxy) -3-propylamine	100	.1	5
Heptylamine	150	.3	1
N-Octyl-1-amino-2-propanol	170	.1	6
2-Ethylhexylamine	250	.2	12
Cyclooctylamine	350	.4	3
N-Hexyl-3-amino-1-propanol	360	.2	4
Hexylamine[b]	370	.4	4
N-Hexyl-6-amino-1-hexanol	440	.4	13
N,*N*-Dibutylaminoethanol[b]	1050	1	4
Cyclohexylamine	1500	2	10
6-Amino-1-hexanol	6000	3	10

[a] Avg of 2 replicates. Old dry eggs as in Table 4.
[b] 0.025% Aerosol TR-70% added.

decreasing larvicidal activity, which also seems to be roughly the order of increasing polarity based on chain length and number and types of polar groups. The concentration of nonpolar amine required for effective ovicidal action, which varied from 0.1 to 3%, was roughly proportional to the larvicidal LC_{50}, which varied from 20 to 6000. Therefore, it seems possible that the fatty amine had a destructive action on the larval cuticle similar to that on the eggshell.

When eggs were treated with nonpolar amine alone in water, effective ovicidal action never resulted, although evidence was obtained which indicated appreciable uptake of the amine by the shell.

Table 3.—Uptake of ^{14}C-ethanolamine vapor after treatment of eggs with various aqueous surfactants.

0.2% surfactant	Counts/min/100 eggs: Controls	Counts/min/100 eggs: Treated
Decylamine	418	1190
Duomeen L-11	524	1399
Aerosol TR-70%	492	572
T-det N-6	1172	1204

Table 4.—Aqueous ovicidal formulations containing compounds with low mammalian toxicities.

Amine (%)	Polar compound: Name	Polar compound: %	Avg % hatch (3 replicates): Young moist eggs[a]	Avg % hatch (3 replicates): Old dry eggs[b]
Duomeen L-11 (0.1)	Urea	8	4	4
Decylamine (0.1)	Urea	7	8	4
Octylamine (0.2)	Urea	5	2	7
	Glycerol	3	2	8
	Water alone		8	10
Heptylamine (0.2)	Water alone		4	3
N-Octyl-1-amino-2-propanol (0.2)	Urea	5	3	2
	Glycerol	5	0	10
2-Ethylhexylamine (0.4)[c]	Urea	6	11	10
Cyclooctylamine (0.2)[c]	Ethanolamine	1	1	8
	Urea	6	2	1
N-Hexyl-3-amino-1-propanol (0.3)	Urea	6	4	4
	Glycerol	5	1	2
Hexylamine (0.3)	Water alone		15	17
N-Hexyl-6-amino-1-hexanol (0.3)[c]	Urea	6	2	3
N,*N*-Dibutylamino-ethanol (0.4)[c]	Ethanolamine	1	1	1
	Urea	6	3	13
Cyclohexylamine (0.4)[c]	Ethanolamine	1	6	8
	Urea	6	1	2
6-Amino-1-hexanol (0.8)[c]	Ethanolamine	1	3	8
(0.6)[c]	Urea	6	2	2

Conditioning between oviposition and treatment: [a] 100% RH for 6 days, control hatch rates 90–100%; [b] 100% RH for 4 days followed by 65–75% RH for 25 days, control hatch rates 95–100%; [c] 0.1% Decyl alcohol added.

Thus, eggs were killed by a combination of amines even when a period of 4 hr separated dipping first in aqueous nonpolar amine and second in aqueous polar amine (Wilton and Fay 1969). Table 3 shows that eggs dipped quickly in aqueous decylamine or Duomeen L-11 and exposed to the vapor of ^{14}C-ethanolamine for 3 hr absorbed ca. 3 times as much radioactivity as controls dipped in water. Two commercial nonnitrogenous surfactants, Aerosol TR-70% and T-det N-6, which were ovicidally ineffective at 0.2% in 1% ethanolamine, did not facilitate radiocarbon uptake. These data indicate that after the shell absorbs nonpolar amine it becomes more permeable to polar amines or has a greater affinity for them.

In further ovicidal formulation studies, efforts were made to avoid or decrease the use of amines, especially fatty amines, because of the potential hazard of such compounds to higher organisms. With the fatty diamine, Duomeen L-11, tested in the adult male rat, Gaines[4] found LD_{50} values of 122 mg/kg oral and 1140 dermal, and a severe skin reaction with a single dosage of only 25 mg/kg. The more polar ethanolamine gave much higher LD_{50} values of 1100 oral and > 6000 dermal. Ebeling (1964) showed that anionic and nonionic surfactants greatly increased the rate of penetration of water through a layer of beeswax. However, such nonnitrogenous surfactants gave poor results when tested as fatty amine

[4] Personal communication (1970). Thomas B. Gaines, Supervisory Research Pharmacologist, Atlanta Toxicology Branch, Environmental Protection Agency, Chamblee, Ga. 30341.

Table 5.—Ovicidal action of aqueous emulsions of fatty acids on moist and dry eggs.

Aqueous formulations	Avg % hatch (4 replicates)				
	Moist eggs			Dry eggs	
	Young	Old	Rehydrated	Young	Old
0.4% Octanoic acid, 0.01% A[a]	6	0.3	9	39	76
0.2% Nonanoic acid, 0.01% A	9	.1	6	48	75
0.2% Undecylenic acid, 0.01% A	1	.1	7	47	69
0.01% A	70	54	48	56	69

[a] A = Aerosol TR-70%.

substitutes in ovicides. Table 4 shows that good results were obtained with Table 2 formulations modified by substitution with various relatively nontoxic compounds, such as decyl alcohol for some of the fatty amine, and urea or glycerol for ethanolamine. In Table 4, the fatty amines are listed in the order of decreasing larvicidal activity, which also may be the order of decreasing mammalian toxicity. Table 4 tests were made with 2 types of eggs which might be expected to differ in susceptibility, young eggs stored moist and old eggs stored comparatively dry. Although most of the old eggs collapsed after these treatments, many of the young moist eggs remained intact. With the latter, it therefore appears that some other mechanism of ovicidal action may be involved in addition to shell destruction. However, when killed by freezing, most of both types of eggs collapsed after treatment with Table 4 formulations and exposure to ambient conditions for a week. Control dead eggs treated only with water remained largely intact.

In many tests, eggs were treated with 1 amine alone in water, allowed to dry, stored in a jar at 100% RH for 24 hr, exposed to ambient conditions for 24 hr, and then hatched. Table 4 shows that good results were obtained with octylamine, heptylamine, and hexylamine. In an experiment with octylamine, mortality was high even though exposure to high relative humidity was begun 24 hr after treatment. These treatments were characterized by much collapse of the eggs. Control eggs treated with water alone gave hatches of 95–100%.

Fatty-Acid Formulations.—Simple aqueous emulsions of fatty acids were found effective against eggs preconditioned in a water-saturated atmosphere but were ineffective against dry eggs. Shell protein seems likely to be involved in this effect, for Richards (1958) found wet arthropodin to be more permeable to solvents than the dry protein. Table 5 shows that moist eggs gave very low hatches after treatment with 0.2% of the 9-carbon nonanoic acid, or the 11-carbon undecylenic acid, or 0.4% of the 8-carbon octanoic acid. The anionic surfactant, Aerosol TR-70%, which was added to facilitate emulsification, had insignificant activity when used alone. Between oviposition and treatment, moist eggs were subjected to relative humidities as follows: young eggs (5 days 100%), old eggs (28 days 100%), rehydrated eggs (4 days 100%, then 24 days 65–75%, and then 7 days 100%). With dry eggs, conditions of relative humidity were: young eggs (4 days 100%, then 1 day in a sealed jar with drierite), old eggs (4 days 100%, then 24 days 65–75%). Susceptibility is clearly dependent on moisture rather than on age, because young moist eggs lost susceptibility after only 1 day in a jar with desiccant; old dry eggs gained susceptibility after exposure to moist conditions for 1 week; and young and old eggs were equally susceptible after moist storage.

The ovicidal action of fatty acids, emulsified in water, against moist eggs appeared to involve a smothering effect or interference with respiration, rather than destruction of waterproofing layers, because such action was not accompanied by much egg collapse, and the action was reversed by exposure to oxygen after treatment. In 3 experiments in which moist eggs were dipped in 0.2% of a fatty acid emulsified in aqueous 0.01% Aerosol TR-70%, allowed to dry, and exposed to oxygen or air in a sealed jar for 24 hr, average hatches were: nonanoic acid (oxygen 5%; air 0.4%) and undecylenic acid (oxygen 57%; air 0.3%).

Exposure to very high relative humidity after treatment led to fairly good results only with 0.4% octanoic acid in 0.1% Pluronic L101. In 4 experiments in which young moist eggs were dipped in this formulation, allowed nearly to dry, stored in a moist jar for 24 hr, and exposed to ambient conditions for 24 hr, the average hatch was 1%. Old dry eggs subjected to this treatment gave an average hatch of 8%. Control young and old eggs dipped in 0.1% Pluronic L101 gave hatches of 64 and 91%, respectively.

Addition of mercapto acids and urea to aqueous fatty acids provided formulations effective against both dry and moist eggs (Table 6). The action of

Table 6.—Acidic ovicides containing mercapto acids and urea.

Components in addition to 0.2% nonanoic acid and 0.01% Aerosol TR-70%	Avg % hatch (3 replicates)	
	Young moist eggs[a]	Old dry eggs[b]
None	12	72
8% Urea	6	80
1% Thioglycollic acid	10	14
1% Thioglycollic acid, 2% urea	3	4
0.5% Thioglycollic acid, 4% urea	14	21
1% 3-Mercaptopropionic acid	14	26
1% 3-Mercaptopropionic acid, 2% urea	5	5
0.5% 3-Mercaptopropionic acid, 4% urea	8	10

[a, b] See Table 4.

such formulations resembled that of the amine formulations in giving evidence of damage primarily to the eggshell. Thus, old dry eggs when dipped in aqueous 0.2% nonanoic acid, 1% 3-mercaptopropionic acid, 2% urea, and 0.01% Aerosol TR-70%, and stored in a moist jar for 2 weeks gave a 79% hatch on direct submergence but a hatch of only 9% when exposed to ambient conditions for 24 hr between moist storage and submergence. Most of the eggs with low hatch were collapsed badly. Freeze-killed eggs also collapsed after treatment with the mercapto acid formulation. The effectiveness of mercapto acids in ovicidal formulations suggests the cleavage of disulfide bonds of the shell protein. These bonds could not be very numerous, because Christophers (1960) found the sulfur content of the aedine eggshell to be very low. Small amounts of sulfur amino acids were found in insect cuticles, and it was concluded that the disulfide bond has a role in the hardening of the cuticle (Hackman and Goldberg 1971). A variety of sulfur compounds, tested as substitutes for the mercapto acids, gave poor results. Mercapto acids are highly toxic and corrosive to mammals.

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