(*5*) reported successful results with preflooding treatments applied to breeding areas of *Aëdes* mosquitoes along the Columbia River in Oregon. The present paper contains a brief report of tests made with DDT applied as preflooding treatments in 1946. Laboratory tests compared different formulations, and a field test was made in which a dust was applied with hand dusters against salt-marsh mosquito larvae.

In the laboratory tests, four series of two dishes each, one containing a layer of sand and the other a layer of muck, were treated with DDT at a rate equivalent to 0.2 lb/acre. One series of dishes was treated with a solution of DDT in deodorized kerosene, one with a xylene-Triton X-100[2] emulsion containing 25% of DDT, one with a suspension made with 50% water-dispersible DDT powder, and the fourth with a dust containing 5% of DDT in talc. To obtain uniform distribution of the insecticide, ¼″ of water was placed in the dishes and allowed to evaporate. The dishes were reflooded at the end of the 3rd, 8th, and 12th weeks, in each case after complete evaporation of the water. The water was added carefully to avoid disturbing the soil. Immediately afterward, late third-instar larvae of *Anopheles quadrimaculatus* Say, *Aëdes aegypti* (L.), and *Culex quinquefasciatus* Say (20 of each species) were introduced. The results of these tests are shown in Table 1.

TABLE 1

RESIDUAL TOXICITY OF DIFFERENT DDT FORMULATIONS APPLIED AS PREFLOODING TREATMENTS FOR THE CONTROL OF MOSQUITOES AT A RATE EQUIVALENT TO 0.2 LB OF DDT/ACRE (3 REPLICATIONS)

Species	Kind of soil	% mortality 48 hrs after application of: Oil solution	Emulsion	Suspension	Dust
First flooding, 3rd week after treatment					
A. quadrimaculatus	Muck	100	73	100	100
	Sand	93	47	100	90
Ae. aegypti	Muck	90	60	88	98
	Sand	87	97	100	100
C. quinquefasciatus	Muck	80	35	15	36
	Sand	32	40	97	68
Second flooding, 8th week after treatment					
A. quadrimaculatus	Muck	72	98	100	100
	Sand	100	38	100	100
Ae. aegypti	Muck	87	38	95	88
	Sand	72	10	100	100
C. quinquefasciatus	Muck	17	7	20	32
	Sand	58	0	92	53
Third flooding, 12th week after treatment					
A. quadrimaculatus	Muck	58	32	93	50
	Sand	93	3	95	85
Ae. aegypti	Muck	52	5	22	90
	Sand	72	7	92	100
C. quinquefasciatus	Muck	10	2	8	17
	Sand	50	5	52	53

The mortality decreased with successive floodings in nearly all the dishes, and, except in those treated with the emulsion, the decrease was usually greater on muck soil than on sand. At the end of 12 weeks the highest mortality of *A. quadrimaculatus* (more than 90%) followed treatment with the suspension, whereas the highest kill of *Ae. aegypti* was obtained with the dust. The mortality of *C. quinquefasciatus* was low in all the dishes. The emulsion showed the poorest lasting qualities.

A field test was made with a 10% DDT dust, at 1 lb of DDT/acre, on 20 acres of a salt-marsh island off the east coast of Florida. The remainder of the island was untreated and served as a check area. The application was made on May 13, and the island became flooded by rainfall or high tides on May 21, June 4, July 11, and August 1. The treatment gave complete control of the larvae that hatched after the first three floodings. After the fourth flooding a few larvae were found, but the number was less than 1/dip in the treated area as compared with 500 or more/dip in the untreated area. The larvae that did develop in the treated area were retarded in growth and either died in the pupal stage or became weak adults.

These results indicate that preflooding treatments with DDT dust may be very effective for use against salt-marsh mosquitoes.

[2] An aralkyl polyether alcohol.

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The Presence of an Alcoholic, Ketonic Derivative of Estrone in Human and Rabbit Blood[1]

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The existence of intermediates in the metabolism of the estrogenic steroid hormones other than the three so-called natural estrogens (estradiol, estrone, and estriol) has long been suspected. The poor recoveries of injected material (averaging 20% of the administered dose) in the urine of both human and animal subjects has given this hypothesis much weight. The course of estrogen metabolism has been studied intensively by many methods. Doisy, Thayer, and Van Bruggen (*1*), Pincus and Pearlman (*5*), and Heard (*2*) have reviewed the field thoroughly.

[1] Supported in part by grants from the American Cancer Society (Massachusetts Division, Inc.), the Foundation of Applied Research, and the Donner Foundation.

The work to be presented here indicates the presence of an intermediate which has not been noticed in the past. The studies were made in part on human blood, and on human and rabbit blood diluted by White's solution (*7*). The diluted bloods were used as the perfusing media for human and rabbit organs maintained on a perfusion pump for varying lengths of time. Each perfusion study lasted 24 hrs.

The fluids were extracted by a technique originally described by Schiller and Pincus (*6*) and Pincus and Pearlman (*3*). The separations of the component estrogens involved the use of the Girard and succinic anhydride reactions in the order listed. We have added to the technique only the following:

(1) All ether employed in the extractions was decanted from a saturated ferrous sulfate solution not more than 1 hr before use.

(2) All evaporations and distillations were conducted in an atmosphere of nitrogen.

According to previous experience with this technique, the following partitions should occur:

(1) Estradiol and estriol should appear only in the nonketonic fraction.

(2) Estrone should appear only in the nonalcoholic fraction.

Errors in manipulation in these techniques would permit ketonic material to enter the nonketonic fraction and alcoholic substances to enter the nonalcoholic fraction. Thus, the appearance of activity in the ketonic alcoholic fraction can hardly be ascribed to improper separation.

The method of bioassay employed was a modification of the Allen-Doisy technique as previously described (*4*).

TABLE 1*

Fluid	Exp. No.	Volume in cc combined fluids	Perfused through	Estrone added (mg)	Day of organ perfusion	Estrone equivalents (as μg) in		
						non-ketonic fraction	ketonic alcoholic	ketonic non-alcoholic
Rabbit† blood and 50% White's solution	1	250	Rabbit thigh muscle	0	1	40	110	10
	1a	250		2.3	2	750	710	560
Rabbit blood and 50% White's solution	2	100	Rabbit thigh muscle	0	1	0	0	0
	2a	275		2.3	2	175	23	30
Human blood (male)	3	200		Not perfused		25	700	0
Human blood (male) and 50% White's solution	4	420	Human skin rope graft	2.3	1	240	300	40
Human blood (male) and 50% White's solution	5	282	Human testicle	2.6	1	25	400	20
	6	232		2.3	2	0	200	0
	7	210	"	0	3	0	Trace	0
	8	146	"	0	5	40	20	70
	9	325	"	2.6	7	72	400	0
Human blood and 50% White's solution	10	150	Blank run using fine cannula instead of organ	2.6	1	10	42	426
Human blood and 50% White's solution	11	250	"	2.6	1	26	40	106

* Values given in the table are in estrone equivalents as determined by Allen-Doisy assay. Error of assay was ±25%. Listed also are the fluids from which extractions were made, the day of the perfusion run on which the experiment was made, and the organ perfused. Only one specimen, human blood, was not perfused but extracted as received from the blood bank. Total volume extracted in each instance.

† Blood taken from follicle stimulating hormone-treated female rabbits.

As seen in Table 1, biological activity was found in the alcoholic fraction whether or not estrone had been added to the fluids. Several extractions not listed in the table, which were run on untreated blood, demonstrated estrogen titers comparable to Experiment 7.

The addition of estrone to the medium during a perfusion appeared to increase the amount of material found in the alcoholic fraction (see Experiments 1, 1a, 2, 2a, 5, 6, 7, 8, 9).

The cause of the decrement in estrone concentration in the experiment where a blank run on added estrone was made (Experiments 10 and 11) is not understood at present. Each perfusion including the blank was free of fungi or bacteria throughout the run. This raises the

possibility that simple incubation of estrone in blood will lead to degradation to inactive compounds as well as conversion to the new compound suggested. Previous determinations on short-time incubation of estrone in blood have led to recoveries approaching 100% of the activity added. Such tests were made both by us and by Pincus and Schiller (*6*).

It therefore appears reasonable to assume from these data that an alcoholic ketone derivative of estrone is normally present in human and rabbit blood.

It may be added that our experience with the compound indicates an extreme degree of lability. To this, more than to differences in metabolic activity, we ascribe the deviations in results such as those seen between the two experiments using rabbit muscle (Experiments 1 and 2).

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Increase of Herbicidal Action of Concentrate 40 and Oil Emulsion by 2,4-D

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It is well known that 2,4-D is ineffective as a herbicide in the control of grasses. Nonselective herbicides such as Concentrate 40 and oil emulsions are often used to control grass weeds. Preliminary experiments conducted at the Federal Experiment Station in Puerto Rico have shown that Concentrate 40 + 2,4-D[1] and oil emulsion fortified with Santophen 20 + 2,4-D[2] both suppressed the population of "cohitre," or day flower (*Commelina longicaulis* Jacq.), and "bejuco de puerco" (*Ipomoea* spp.) (broadleaf plants easily eradicated with 2,4-D sprays) more than the same nonselective sprays without 2,4-D. The combination sprays also suppressed more weeds than 2,4-D alone. The results indicated that Concentrate 40 and oil emulsion fortified with Santophen 20, when used as a combination spray with 2,4-D, did not inhibit the lethal effects of 2,4-D and that it may be more effective than either nonselective herbicides when used alone on grass control.

In another experiment, an area completely covered with Bermuda grass (*Cynodon dactylon* (L.) Pers.), which is unaffected by 2,4-D and very resistant to arsenical, was divided into 10 equal plots. Five plots were treated with Concentrate 40 and 5 with 0.1% sodium salt of 2,4-D in Concentrate 40 at the rate of 175 gal/acre. Two uniform applications of both spray treatments were made at 4-week intervals, and the results recorded 20 days after the last application. The addition of 2,4-D to Concentrate 40 increased its herbicidal action against Bermuda grass by 50%. Plots sprayed with Concentrate 40 alone were completely covered with weeds, 60% Bermuda grass and 40% nutgrass (*Cyperus rotundus* (L.)). In plots sprayed with 0.10% 2,4-D in Concentrate 40 the area was covered with only 40% Bermuda grass and 5% nutgrass.

The results indicate that 2,4-D possibly activated the constituents in Concentrate 40, or vice versa, with a resulting synergistic reaction. The increased herbicidal effectiveness of the combination sprays may also be due to the injury caused by the constituents of Concentrate 40 (arsenic trioxide, Santobrite, and sodium chlorate), which enables the 2,4-D to enter the plant and exert its physiological effect.

[1] Consisting of 0.42% arsenic trioxide, 0.25% Santobrite (sodium pentachlorophenate), and 0.25% sodium chlorate plus 0.10% 2,4-D.

[2] Consisting of 10% diesel oil emulsion fortified with 0.7% Santophen 20 (pentachlorophenol).

Importance of the Methoxy Group in Antifibrillatory Compounds[1]

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It is of singular interest that all the potent compounds now in clinical use for their antifibrillatory activity possess a methoxy group. These include quinine (*7*), quinidine (*4*), α-fagarine (*3*), and recently atabrine (*5*). The methoxy group is present in a number of other drugs —notably, the antimalarial drugs, certain of the opiates, and colchicine. Of these later drugs, only to papaverine has antifibrillatory activity been attributed (*6*). In a preliminary study to clarify this point, measurements were made of the antifibrillatory activity of cinchonine and N-methyl-dibenzyl-amine with quinidine and α-fagarine as controls. Cinchonine was selected because it has the exact structure of quinidine minus the methoxy group. Similarly, N-methyl-dibenzyl-amine is closely related in structure (one less carbon in the amine chain) to α-fagarine, but lacks two methoxy groups and one dioxymethylenic group.[3] To aid in the comparison of these drugs the changes induced on blood pressure, pulse, electrocardiogram, and the acute fatal toxicity were also studied.

Cats anesthetized with Dial-urethane given intraperitoneally were used. The chest was opened and a pericardial cradle made. Electrodes were attached to the right auricle about 8 mm apart, always in the same location. The stimulating current was generated by a thyra-

[1] Work done under a grant from the Sterling-Winthrop Research Institute.

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[3] N-methyl-dibenzyl-amine and α-fagarine were obtained from the Sterling-Winthrop Research Institute; quinidine and cinchonine, from the Fisher Scientific Company.