frigerating for 24 hours, a cup plate assay against *Staphylococcus aureus* A.T.C.C. No. 6538 showed 12 I.U. per ml. After washing with 750 ml of 1 per cent. $NaHCO_3$ buffer adjusted to pH 7.8 followed by 300 ml of water, the assay of the amyl acetate solution was 30 I.U. per ml. The combined wash liquid assayed less than 5 I.U. per ml.

The resulting washed amyl acetate solution was concentrated at 30° C under vacuum to a yellow oil weighing 1.46 g. Theoretically, this oil should have a potential potency of about 1,000 I.U. per mg. It was dissolved in dry methanol to form 30 ml of solution. The theoretical potential potency of the solution was about 50,000 I.U. per ml.

Emulsions were prepared by adding the methanol solution of the ester to distilled water to which were added sodium bicarbonate and Duponol[4] as indicated in Table 1. These mixtures were held at 5° C and were assayed at intervals with the results as indicated in Table 1. The fact that the methyl ester of penicillin can be hydrolyzed *in vitro* to yield active penicillin is evident.

The ethyl ester of penicillin was prepared in the same manner as the preparation of the methyl ester, except that diazoethane[3] was employed as the esterifying agent and the yellow oil concentrate was dissolved in dry ethanol to make 30 ml of solution. This also should have a theoretical potential potency of about 50,000 I.U. per ml.

Suspensions of the ethyl ester were prepared and handled in the same manner as were those of the

TABLE 1

REGENERATION OF ACTIVE PENICILLIN FROM THE METHYL ESTER

Emulsion*	$NaHCO_3$ added mg	Duponol added mg	pH	Assay, I.U. per ml Day: 0	1	2	3	4	Maximum reactivation per cent. of theory
1	0	0	6.0	12	30	17	17	14	4.8
2	20	0	7.3	13	136	161	150	114	25.8
3	0	5	5.8	14	3	1	0.2	.2	2.2
4	20	5	7.1	18	73	119	117	105	19.0

* All emulsions were prepared by adding 0.25 ml of the methanol solution of the methyl ester to 19.75 ml of distilled water. The theoretical potential potency of the emulsion was about 625 I.U. per ml.

methyl ester. The fact that active penicillin may be regenerated by hydrolysis of this ester is shown in Table 2.

The emulsions were physically stable in the presence of Duponol or $NaHCO_3$ or both, but in the absence of these agents (emulsions 1 and 5) some of the oily material collected on the walls of the containing

[3] C. R. Noller, "Organic Syntheses," Vol. XV, pp. 3–5, New York: John Wiley and Sons, Inc. 1935. Diazoethane was prepared by the same general method.

TABLE 2

REGENERATION OF ACTIVE PENICILLIN FROM THE ETHYL ESTER

Emulsion*	$NaHCO_3$ added mg	Duponol added mg	pH	Assay, I.U. per ml Day: 0	1	2	3	4	Maximum reactivation per cent. of theory
5	0	0	6.2	7	6	8	7	6	1.3
6	20	0	7.2	10	34	98	100	66	16.0
7	0	5	6.0	9	1	0.3	.2	.2	1.4
8	20	5	7.3	10	33	59	55	42	9.5

*All emulsions were prepared by adding 0.25 ml of the ethanol solution of the ethyl ester to 19.75 ml of distilled water. The theoretical potential potency of the emulsion was about 625 I.U. per ml.

vessels. The emulsions became increasingly transparent on standing, particularly in the presence of $NaHCO_3$. Hydrolysis was indicated. Duponol[4] appears to exert an inactivating effect on penicillin.

Both the methyl and ethyl esters are slightly soluble in water with the methyl ester being the more soluble of the two. Data in Tables 1 and 2 show that the methyl ester is also the more readily hydrolyzed of the two esters. Methyl esters are generally formed and hydrolyzed more readily than esters of higher alcohols.[5]

Although the theoretical amount of active penicillin has not as yet been obtained from these bacteriostatically inactive esters, the fact that a significant fraction may be regenerated *in vitro* is of interest.

Summary: It has been found that methyl and ethyl esters of penicillin may be hydrolyzed *in vitro* to yield 26 and 16 per cent. respectively of the theoretical bacteriostatically active penicillin.

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THE EFFECT OF ULTRAVIOLET RADIATION ON CYSTS OF ENDAMOEBA HISTOLYTICA[1]

THE object of these experiments was to determine whether or not ultraviolet radiation is lethal to the cysts of the intestinal pathogen, *Endamoeba histolytica.*

Two models of ultraviolet lamps were supplied by the General Electric Company. Both lamps emitted energy of wave-length 2,537A, which lies within the region where the maximum germicidal effect might be expected.[2] Distilled water suspensions of the cysts,

[4] Duponol ME, E. I. du Pont de Nemours and Co., Wilmington, Del.

[5] A. Michael and K. Wolgast, *Ber.*, 42: 3157–3176, 1909.

[1] The material in this article should be construed only as the personal opinion of the writers and not as representing the opinion of the U. S. Navy Department.

in concentrations ranging from 30,000 to 200,000 cysts per liter, were irradiated for various periods of time. Following irradiation, the suspensions were sedimented and the residue planted upon a suitable culture medium. Subsequently, the cultures were examined for the presence of motile trophozoites. A control suspension was run with each test, and handled in the same manner except that irradiation was omitted.

It was found that all *E. histolytica* cysts were destroyed by ten minutes' irradiation in the five tests done upon suspensions of 30,000 cysts per liter of distilled water (Tables 1 and 2). Heavier suspensions were not tested for this time interval, since 30,000 cysts per liter exceeds the concentration likely to be encountered in water purification practice. When heavier suspensions and shorter exposures were used, only a portion of the cysts were destroyed (Tables 1 and 2). The extreme sluggishness and

TABLE 1

CYSTICIDAL EFFECT OF IRRADIATION WITH GERMICIDAL LAMP, MODEL 1

Test (number)	Exposure time (minutes)	*E. histolytica* cysts per liter of distilled water	Results per cent. survival*	
			Test	Control
1	1	200,000	0	100
2	2	200,000	20	100
3	3	200,000	0	100
4	4	200,000	20	100
5	5	100,000	0	100
6	1	58,000	0	100
7	2	58,000	0	100
8	3	58,000	60	100
9	4	58,000	20	100
10	10	30,000	0	100
11	10	30,000	0	100
12	10	30,000	0	100
13	10	30,000	0	100

* Per cent. survival is the ratio of the number of positive cultures to the total number of culture tubes inoculated with the sediment from the cyst suspension.

TABLE 2

CYSTICIDAL EFFECT OF IRRADIATION WITH GERMICIDAL LAMP, MODEL 2

Test (number)	Exposure time (minutes)	*E. histolytica* cysts per 4 liters of distilled water	Results per cent. survival*	
			Test	Control
1	5	216,000	10	100
2	5	200,000	0	100
3	5	120,000	10	100
4	10	120,000	0	100

* Per cent. survival is the ratio of the number of positive cultures to the total number of culture tubes inoculated with the sediment from the cyst suspension.

altered general appearance of the trophozoites which did emerge from the cysts surviving exposure indicated a sublethal effect of ultraviolet radiation upon these organisms; such trophozoites changed shape but slowly and showed none of the explosive pseudopod formation typical of *E. histolytica*. The control cultures showed normally active trophozoites.

Although ozone is produced while the lamps are in use, the amount is small and the effect apparently negligible.[3, 4]

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INSECTICIDAL ACTIVITY OF SOME ALKOXY ANALOGS OF DDT

SEVERAL 2,2-bis(*p*-alkoxyphenyl)-1,1,1-trichloroethanes were synthesized by the general method described[1] for the ethoxy member and were tested in comparison with DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (m.p. 107°).

The results of the tests on houseflies (*Musca domestica* L.), which were obtained by the large Peet-Grady method,[2] are shown in Table 1. Deo-Base, a refined petroleum distillate, was the solvent used for making the spray solutions. The percentage kill given by the Official Test Insecticide (OTI) was determined for each culture of flies to provide an index of the resistance of the culture.

TABLE 1

TOXICITY OF SOME *Para*-SUBSTITUTED DIPHENYLTRICHLOROETHANES OF THE GENERAL FORMULA $(R\text{–}C_6H_4)_2CHCCl_3$ TOWARD HOUSEFLIES AS DETERMINED BY THE PEET-GRADY METHOD

Substituent R is:	Concn. of compound g/100 ml	Pyrethins added g/100 ml	Knock-down in 10 min., per cent.	Kill in 24 hrs., per cent.	OTI kill in 24 hrs., per cent.
Cl (DDT)	.25	..	42	38	49
CH_3–O (Methoxy) ...	.15	..	91	31	51
" " ...	.20	..	99	32	34
" " ...	.40	..	99	85	46
C_2H_5–O (Ethoxy) ...	.20	..	90	86	34
" " ...	.10	.05	97	78	44
" " ...	.15	.05	99	91	44
Cl (DDT)	.10	.05	96	86	44
Control	..	.05	96	34	44
C_2H_5–O + CH_3–O	.15 + .15	..	97	88	44
Cl (DDT) + CH_3–O ..	.10 + .15	..	94	86	44
n–C_3H_7–O (*n*-propoxy)	.50	.025	95	48	54
n–C_4H_9–O (*n*-butoxy).	.50	.025	99	25	54

With DDT used at a concentration of .25 g per 100 ml the knock-down in 10 minutes was very poor, but most of the flies knocked down were dead in 24 hours.

[2] Matthew Luckiesh and Lewis L. Holladay, *General Electric Review*, 47: 4, 45–50, April, 1944.

[3] H. S. Forbes and G. A. Daland, *Am. Jour. Physiol.*, 66: 50, 1923.

[4] L. R. Koller, *Jour. Applied Physics*, 10: 9, 630, September, 1939.

[1] P. Fritsch and F. Feldmann, *Liebigs Ann. Chem.*, 306: 72, 1899.

[2] "Blue Book," p. 177. New York: MacNair-Dorland Co., 1939.