[illegible] resting potential of the *Anisodoris* [illegible] can be separated [illegible] two components: one which can be predicted by the ionic hypothesis and another which depends upon the electrogenicity of active Na transport. This separation has special interest in light of reports that the Na pump not only makes a general contribution to potential but may have more specific functions such as the [illegible] of synaptic effects (15). The [illegible] appears to vary with temperature and provides another mechanism by which the potential is regulated. A similar division of the resting potential into components may apply to excitable cells other than the *Anisodoris* giant neuron. Our data support the hypothesis (6) that the special characteristics of the resting potential in different nerve and muscle cells result from mechanisms, such as the Na pump, that modify a predictable ionic potential common to all excitable cells.

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26 August 1969

Abstract. *The pesticide DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and its metabolite DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] can be photooxidized in methanol. Photolytic generation of free radicals that may abstract hydrogen from solvent, react with oxygen, or abstract hydrogen from unreacted substrate occurs. Further decomposition of short-lived intermediates yields many compounds. Oxidation products include benzoic acids, aromatic ketones, and chlorinated phenols. The DDE also undergoes photocyclization to give dichlorofluorene derivatives.*

The persistent insecticide DDT may be slowly degraded by the action of light and air. Photodecomposition of DDT has been discussed widely (*1*). We now report investigations of the photoxidation of DDT [1,1,1-trichloro-2-2-bis(*p*-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene].

[illegible] in methanol [illegible] radiation, the [illegible] photolysis of ether [illegible] solvent, and [illegible] of loss of chlorine from the [illegible] methyl group of DDT at 2 [illegible] shown in Table 1. Both 1,1-dichloro-2,2-bis(*p*-chlorophenyl)[illegible] and 1-chloro-2,2-bis(*p*-chlorophenyl)ethane are obtained (Eqs. 1–[illegible]). The wavelength of the irradiation determines the nature of the products; at shorter wavelengths chlorine is displaced from the aromatic ring. Products of photodecomposition were identified by combined gas chromatography and mass spectrometry, as well as by other techniques (Table 2). The structures of the products support the sequence of reactions of free radicals postulated by Mosier *et al.* (*1*).

The products formed in the presence of oxygen are complex and result primarily from the reaction of oxygen with radical intermediates. A reaction sequence (Eqs. 5–8) is postulated for the formation of methyl-2,2-bis(*p*-chlorophenyl) acetate, a photooxidation product of DDT in methanol with oxygen (2600 Å).

The reaction sequence (Eqs. 5–7) follows that suggested for pentachloroethane photooxidation (*2*). A similar sequence could give rise to *p,p'*-dichlorobenzophenone (Eqs. 9–12). Solvent molecules may be involved in the reactions and add to the reactive inter-

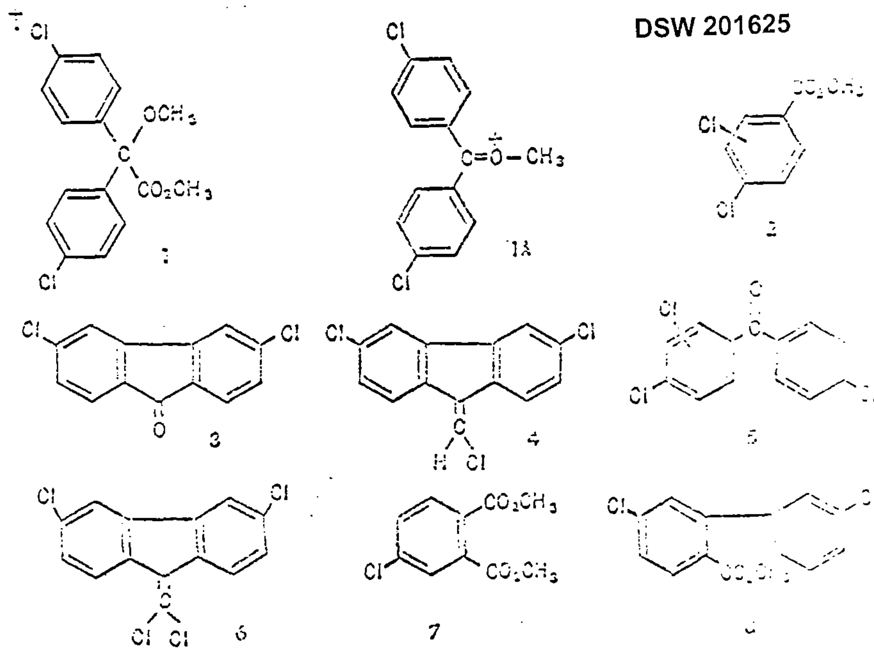

Fig. 1. Formulas of products of photooxidation.

Table 1. Percentage yields of irradiation products of *p,p'*-DDT in methanol (1 g/liter); R is *p*-ClC_6H_4Cl.

Irradiation (hour)	Yield (%)		
	R_2CHCCl_3	$R_2CHCHCl_2$	R_2CHCH_2Cl
1	76	8	+
2	61	11	5
4	44	13	6
8	24	15	6

mediates to form compounds such as (1) (Fig. 1).

When irradiated in methanol under nitrogen, DDE undergoes reductive dechlorination by a free-radical mechanism. In oxygen, DDE also undergoes a photocyclization reaction. We have obtained a 10 percent yield of 3,6-dichlorofluorenone (3) by irradiation of a methanolic solution of DDE (wavelength > 2600 Å) (5). The diketone structure is also postulated for other irradiation products for example, (4) and (6). The planarity of DDE probably facilitates cyclization to a five-membered carbocyclic ring. The photocyclization of 1,1-diphenylethylenes has not previously been recorded.

Quantitatively, p,p'dichlorobenzophenone is an important photodecomposition product of DDE and it is probably formed by a mechanism similar to that shown in Eqs. 9–12. The radical formed in Eq. 11 may alternatively decompose or react with solvent. Such reactions probably account for the formation of chlorobenzoic acid, methyl chlorobenzoate, chlorophenols, and other fragmentation products. Photooxidation of p,p'dichlorobenzophenone gives similar products together with p,p'dichlorobiphenyl, a [illegible] and DDE [illegible]

Further reaction [illegible] products complicates the picture but affords information on [illegible] degradation pathways. Other products of 3,6-dichlorofluorenone (7), and (8) were detected. Polychlorinated phenols, dichlorobenzoic acid, and a trichlorobenzophenone indicate that these compounds react with liberated chlorine radicals.

We used a 450-watt mercury lamp in a water-cooled quartz housing to irradiate methanol solutions of DDE or DDT (about 1 g/liter). A solution filter eliminated light of short wavelengths (Corex > 2800 Å or Pyrex > 3000 Å). Oxygen or nitrogen was bubbled through the solution in separate experiments.

Products were obtained by chromatography of the methanolic solution after it was concentrated in a rotary evaporator. 3,6-Dichlorofluorenone, which crystallized from the reaction mixture, was removed by filtration. The remaining syrup was dissolved in ether. Acids and phenols were removed by extraction with sodium hydroxide. The sodium hydroxide extract was acidified, and the free acids and phenols were extracted into ether. Acids were then extracted from the ether with sodium bicarbonate solution. Portions of the free acids and phenols thus obtained were methylated with diazomethane before being subjected to gas chromatography.

The neutral extract was fractionated by chromatography on a silica column. Infrared spectra of individual fractions were determined. Each fraction was examined by combined gas chromatography and mass spectrometry (4). We identified a number of products by cochromatography and by comparison with authentic specimens of known physical properties. In many cases, structures could be postulated from mass spectral fragmentation patterns. The compounds obtained by reductive loss of chlorine have fragmentation patterns related to their precursors, DDT and DDE. The fragmentation of DDT and DDE has been discussed (5). The number of chlorine atoms present in the molecular ion of an unknown could be deduced from the relative peak heights of the molecular weight (M), the molecular weight plus

Table 2. Photolysis products of DDT and DDE in methanol: R is ClC_6H_4. The compounds listed represent the volatile components of the photolyzate separated by gas chromatography; m, present in moderate amount; +, detected; —, not detectable; Ph, phenyl.

m/e	Structure	DDT		DDE	
		O_2	N_2	O_2	N_2
	Neutral fraction				
136	$PhCO_2CH_3$	+	—	—	—
140	RCHO	+	—	+	
170	RCO_2CH_3	+	+	m	m
180	$Ph_2C=CH_2$	+	+	—	—
184	$RCH_2CO_2CH_3$	—	—	+	+
204	(2)	—	—	+	—
214	$PhRC=CH_2$	—	+	+	+
222	R_2	+	m	+	+
226	$Ph_2CHCO_2CH_3$	—	+	—	—
232	$PhRCHOCH_3$	+	+	—	—
236	R_2CH_2	+	+	+	+
248	$R_2C=CH_2$	—	—	—	—
248	(3)	—	—	m	
250	R_2CO	+	m	+	m
260	$PhRCHCO_2CH_3$	m	+		
266	R_2CHOCH_3	+	+	+	+
280	(4)	—	—	+	
282	$R_2C=CHCl$	+	—	+	m
284	$PhRCHCHCl_2$	m	+	—	—
284	(5)		—	+	
294	$R_2CHCO_2CH_3$	m	+	+	+
314	(6)	—	—	+	
316	$R_2C=CCl_2$ (DDE)	—	m	+	+
318	$R_2CHCHCl_2$ (DDD)	m	+	—	—
324	$R_2C(OCH_3)CO_2CH_3$ (1)	—	—	m	
338	Unknown	—	—	m	
352	R_2CHCCl_3 (DDT)	+	+		
	Acidic and phenolic compounds (methylated)				
142	$PhOCH_3$	—		+	
170	RCO_2CH_3	+		+	+
176	$Cl_2C_6H_3OCH_3$	+		+	
204	$Cl_2C_6H_2CO_2CH_3$	+		+	
210	$Cl_2C_6H_2OCH_3$	+		+	+
228	(7)	—		+	
280	(8)	+		+	
294	$R_2CHCO_2CH_3$	+		+	

Hydrogen abstraction

$$R_2CHCCl_3 \xrightarrow{light} R_2CH\dot{C}Cl_2 + \dot{C}l \qquad (1)$$

$$R_2CH\dot{C}Cl_2 + CH_3OH \longrightarrow R_2CHCHCl_2 + \dot{C}H_2OH \qquad (2)$$

$$R_2CHCHCl_2 \xrightarrow{light} R_2CH\dot{C}HCl + \dot{C}l \qquad (3)$$

$$R_2CH\dot{C}HCl + CH_3OH \longrightarrow R_2CHCH_2Cl + \dot{C}H_2OH \qquad (4)$$

In the presence of oxygen

$$R_2CH\dot{C}Cl_2 + O_2 \longrightarrow R_2CHCCl_2(\dot{O}_2) \qquad (5)$$

$$2R_2CHCCl_2(\dot{O}_2) \longrightarrow 2R_2CHCCl_2\dot{O} + O_2 \qquad (6)$$

$$R_2CHCCl_2\dot{O} \longrightarrow R_2CHCOCl + \dot{C}l \qquad (7)$$

$$R_2CHCOCl + CH_3OH \longrightarrow R_2CHCOOCH_3 + HCl \qquad (8)$$

$$R_2CHCCl_3 + \dot{C}l \longrightarrow R_2\dot{C}CCl_3 + HCl \qquad (9)$$

$$R_2\dot{C}CCl_3 + O_2 \longrightarrow R_2C\dot{O}_2CCl_3 \qquad (10)$$

$$2R_2C\dot{O}_2CCl_3 \longrightarrow 2R_2C\dot{O}CCl_3 + O_2 \qquad (11)$$

$$R_2C\dot{O}CCl_3 \longrightarrow R_2CO + \dot{C}Cl_3 \qquad (12)$$

($R = p\text{-}Cl\cdot C_6H_4$)

2 (M + 2), and so forth, as ^{35}Cl and ^{37}Cl occur in the ratio of 3 : 1. Many of the oxidation products are known; therefore, mass spectral and chromatographic comparisons could be made with authentic samples. Mass spectral studies were extended to model compounds, and the photochemistry of *p,p'*-dichlorobenzophenone was also investigated to obtain information on the further breakdown of photolytic products. Substitution of an oxygen atom at C-1 of the 1,1-diphenylethane system affords abundant fragments at *m/e* 139 ($^{35}ClC_6H_4CO$) and *m/e* 111 ($^{35}ClC_6H_4$).

Molecular ions were obtained in the majority of cases, but difficulty was encountered with (1). An intense base peak at *m/e* 265 suggested the oxonium ion structure (1A), presumably formed by loss of a methoxycarbonyl residue from (1). The presence of this residue in the isolated material was confirmed by the infrared and nuclear magnetic resonance spectra of the isolated fraction.

The compound with *m/e* 338, which was present in significant quantity in the photooxidation products of DDE, could not be assigned a complete structure on the available evidence. However, nuclear magnetic resonance, infrared, and mass spectral data suggest that it contains a methyl dichlorofluorenyl carboxylate moiety.

Mass spectral data alone were used for assignment of structure where trace quantities were obtained and authentic material was unavailable.

Most of the photolysis products could be examined with these techniques. Compounds of higher molecular weight, such as those described by Fleck (*1*), are probably present in small quantity. The seqence of reactions of free radicals postulated by Mosier *et al.* (*1*) explains the photolytic pathway. There are many instances of photolytic fission of a C–Cl bond to form a radical which can subsequently react with solvent by hydrogen abstraction (*6*). We postulate that, in the presence of excess oxygen, photochemically generated free radicals from DDE and DDT add oxygen. Rearrangement and reaction of unstable intermediates account for the formation of the photooxidation products which we have identified.

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29 August 1969

Duplicate [illegible] Immune Cell Products: [illegible] Class Secretion by Single Cells

Abstract. *Antibodies* [illegible] *individual immune cells were* [illegible] *locally in very thin "original" and "replica" layers of agar containing* [illegible] *target antigen, sheep erythrocytes. Identical treatment of both layers led to mirror image patterns of hemolytic plaques. Development of one layer for immunoglobulin M hemolysins and the other for immunoglobulin G hemolysins produced unrelated plaque patterns indicating that few, if any, cells simultaneously release substantial amounts of both γM and γG antibodies.*

A comprehensive understanding of the synthetic capacities of individual immune cells depends upon the facility with which the products of single cells may be collected and analyzed. Single cell isolation procedures permit such sampling, but the numbers of cells that can be examined are relatively few (1). Fluorescent antibody techniques also permit reasonable inferences about immunoglobulin synthesis since they detect residual globulins in or on the cells of fixed tissue specimens (2). Studies of single cells by the isolation procedure showed that up to 19 percent of immune cells may produce two classes of immunoglobulins simultaneously (*1*), while with fluorescent antibody techniques instances of possible double producer cells have ordinarily averaged around 1 percent (2). We now report a new replica-plating procedure which allows duplicate sampling of the products of single viable immune cells without necessitating isolation of single cells.

Adult female New Zealand White rabbits were immunized [illegible] with 10-ml portions of [illegible] 10 percent sheep erythrocytes on days 0, 9, 18, and 24. Suspensions of immune cells (*3*) were obtained by processing the whole spleens of these animals on days 5, 6, 7, 8, and 27. The cells were washed by centrifugation, and those from two rabbit spleens were pooled and suspended in 20 ml of [illegible] Hanks solution. Very thin layers of sheep erythrocytes in agar were prepared as follows. A mixture [illegible] composed of 18 ml of [illegible] percent [illegible] fied Ionagar (Difco) in [illegible] 0.05*M* tris-buffered (pH 7. [illegible]) minimum essential [illegible]

The resting potential of the *Anisodoris* giant neuron can be separated into at least two components: one which can be predicted by the ionic hypothesis and another which depends upon the electrogenicity of active Na transport. This separation has special interest in light of reports that the Na pump not only makes a general contribution to potential but may have more specific functions such as the mediation of synaptic effects (*15*). The ratio P_{Na}/P_K appears to vary with temperature and provides another mechanism by which the potential is regulated. A similar division of the resting potential into components may apply to excitable cells other than the *Anisodoris* giant neuron. Our data support the hypothesis (*6*) that the special characteristics of the resting potential in different nerve and muscle cells result from mechanisms, such as the Na pump, that modify a predictable ionic potential common to all excitable cells.

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26 August 1969

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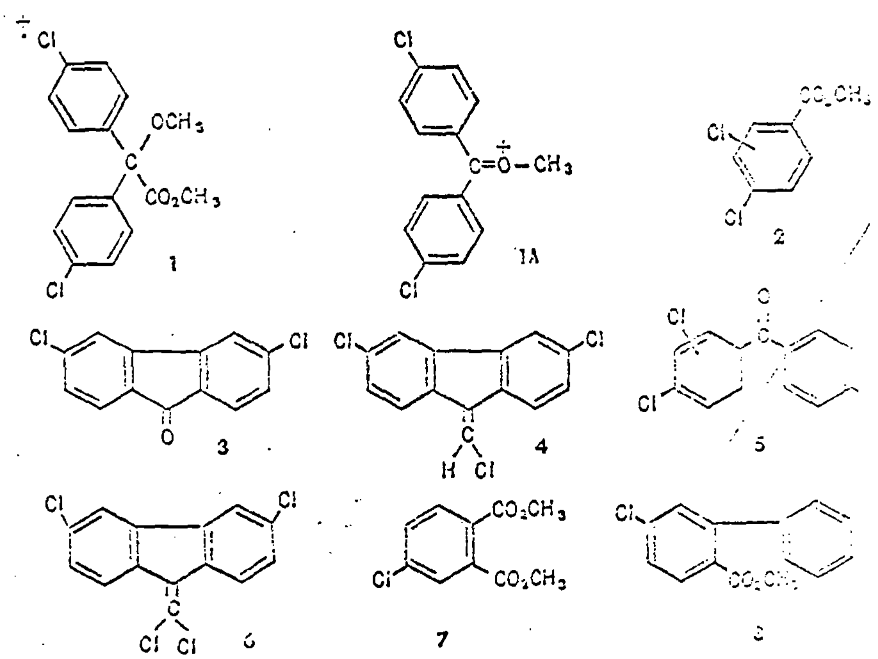

Fig. 1. Formulas of products of photooxidation.