Riassunto

L'aggiunta di zimosano al siero normale di cavia ne diminuisce notevolmente il potere opsonico; l'aggiunta di properdina al siero inattivato con zimosano o col calore restaura interamente questo potere. La properdina usata da sola senza siero, non ha potere opsonico. Sembra molto probabile che la frazione termolabile delle opsonine sia da identificare con la properdina.

DDT-Analogs as Synergists for DDT

Diaryl-trifluoromethyl-carbinols $Ar_2C(OH) \cdot CF_3$ have been found to be active as synergists against a strain of moderately resistant houseflies[1]; the most active compound of the series was the di-(*p*-chloro)-derivative. It seemed of interest to investigate analogs containing chlorine and/or fluorine in the methyl group and also to study the importance of the hydroxyl group present in these compounds.

The compounds tested have been described by BERGMANN *et al.*[2], apart from di-(*p*-chlorophenyl)-dichloromethyl-carbinol and 1,1-di-(*p*-chlorophenyl)-2,2-dichloroethane which have been reported by PEPPER and KULKA[3] and by HALLER *et al.*[4], respectively. The housefly species used in these experiments were a susceptible strain ('T') and a 200 times more resistant strain ('R') of *Musca vicina*. Benzene solutions of the chemicals were applied *topically* to groups of 25 flies. Mortality counts were made after 24 and 48 h. Each compound was tested in at least four concentrations with 5 to 8 repetitions. The experimental procedure has been described in detail by TAHORI[1].

The Table summarises the results obtained; it indicates the insecticidal activity of each compound as well as its synergist value (for a ratio of DDT: synergist = 10:1). In order to characterize the slope of the dosage-mortality curves, the LD_{15} and LD_{85} values are given in parenthesis.

Against *susceptible* flies, none of the carbinols approaches DDT in insecticidal activity. It is noteworthy that all those diarylmethyl-carbinols in which the methyl group is fully substituted (CF_3, CF_2Cl, $CFCl_2$) (VI–VIII) are fairly active on topical application, though on tarsal application no effect has been found for di-(*p*-chlorophenyl)-trifluoromethyl-carbinol[5]. It can be predicted that the same will probably hold true for di-(*p*-chlorophenyl)-trichloromethyl-carbinol. This carbinol and the dichloromethyl analog (IX) have been reported by GUNTHER, BLINN, and METCALF[6] to be of low toxicity to *Musca*. The di-(*p*-chlorophenyl)-trichloromethyl carbinol used by these authors, of Rohm and Haas manufacture, appears to be different from the compound used by REUTER and ASCHER, and to which the same formula had been assigned. On the other hand, the diaryl-*di*halomethyl-carbinols (IX–XIII) exhibit lower activity; this activity rises from the difluoro- over the fluorochloro- to the dichloromethyl compound. One is tempted to assume that, as in the case of DDT, the size of the substituents of the methyl group is the decisive factor, causing a distortion of the tetrahedron of the arylated carbon atom into a trihedral configuration[7]; chlorine is effective, fluorine too small. Another observation may be of interest in this connection. The inactive carbinols are easily dehydrated, the active ones not: the trihalogenomethyl compounds can, of course, not be dehydrated at all, but we have noted that also di-(*p*-chlorophenyl)-*di*chloromethyl-carbinol offers a strong resistance to every attempt at dehydration.

All carbinols tested are more active insecticides than DDT for *resistant* houseflies; again the two groups defined above are clearly distinguished.

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	Compound	Insecticidal Activity in µg/fly		Toxicity in µg of DDT/fly + compound at a ratio of 10:1	
		R	T	R	T
I	$(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CCl_3$	140 (80; 250)	0·7 (0·4; 1·1)	140 (80; 250)	0·7 (0·4; 1·1)
II	$(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CF_3$	13 (8; 21)	4 (2; 7)	13 (8; 22)	0·2 (0·1; 0·4)
III	$(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CF_2Cl$	50 (32; 77)	14 (9; 19)	14 (8; 23)	0·4 (0·2; 0·8)
IV	$(p\text{-}ClC_6H_4)_2CH \cdot CFCl_2$	115 (68; 190)	5 (3; 8)	30 (17; 51)	0·4 (0·2; 0·7)
V	$(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CHCl_2$	> 140	4 (2; 7)	140	0·6 (0·3; 1·0)
VI	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CF_3$	6 (3; 11)	5 (3; 7)	9 (5; 18)	0·3 (0·2; 0·5)
VII	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CFCl_2$	5 (3; 9)	6 (4; 11)	13 (7; 21)	0·5 (0·3; 1·0)
VIII	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CFCl_2$	5 (3; 9)	6 (4; 11)	13 (7; 21)	0·5 (0·3; 1·0)
IX	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHCl_2$	4 (2; 7)	1 (0·5; 2)	15 (9; 27)	0·2 (0·1; 0·4)
X	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHFCl$	11 (7; 18)	9 (5; 14)	13 (7; 21)	0·4 (0·3; 0·7)
XI	$(C_6H_5)_2 \cdot C(OH) \cdot CHF_2$	70 (43; 110)	52 (37; 72)	66 (38; 115)	0·4 (0·2; 0·7)
XII	$(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHF_2$	27 (16; 46)	31 (18; 56)	27 (16; 46)	0·4 (0·2; 0·7)
XIII	$(p\text{-}BrC_6H_4)_2 \cdot C(OH) \cdot CHF_2$	31 (19; 49)	27 (17; 45)	31 (20; 48)	0·3 (0·2; 0·7)
XIV	$(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CF_3$	7 (5; 13)	4 (3; 7)	9 (4; 18)	0·2 (0·1; 0·4)
XV	$(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CF_2Cl$	80 (47; 130)	150 (150)	36 (18; 70)	0·4 (0·2; 0·7)
XVI	$(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CFCl_2$	> 200	> 200	39 (15; 100)	0·5 (0·3; 0·8)

Toxicities of some DDT analogs to 'R' and 'T' strains of *M. vicina* (The values in brackets indicate LD_{15} and LD_{85})

A similar picture is obtained from the figures dealing with the *synergist* activities of the carbinols. In the case of *susceptible* flies, their effect is small (lowering the value of 0·7 for DDT to 0·3–0·4), whilst for the *resistant* strain the 'active' carbinols increase the toxicity of DDT about 10 times, the 'inactive' ones much less. The distinction between the two groups is clear only in the latter case.

Replacement of the hydroxyl group in the carbinols by hydrogen (compounds II–V) produces compounds which are less active than DDT in susceptible and somewhat more active than the standard compound in resistant flies. If in the latter case the differences are significant, it appears that the sequence of the activities is the reverse from what one would have expected on the basis of the 'trihedral theory': $CF_3 > CF_2Cl > CFCl_2$, whilst the size of the halogenated methyl group varies accordingly to $CFCl_2 > CF_2Cl > CF_3$. This is true both for the insecticidal power and the synergist activity of the compounds.

Acetylation of the hydroxyl group in the 'active' carbinols (XIV–XVI) destroys the activity for the fluorodichloro and—to a lesser degree—for the difluorochloro compound. The trifluoro compound (XIV) has the same effect as the free carbinol. It can be assumed that in the latter case the esterases of the body are capable of hydrolyzing the acetyl compounds. Indeed, XIV reaches its activity only after 72 h. With increasing size of the methyl substituents, the hydrolysis becomes more difficult—perhaps as a result of steric hindrance. One would then expect that the acetate of di-(*p*-chlorophenyl)-trichloromethyl-carbinol would be completely inactive.

The mode of action of the 'active' carbinols is not quite clear. They may function as unspecific narcotics, as proposed by GAVAUDAN and POUSSEL[8] for DDT. In this respect, it may be recalled that the vapour of di-(*p*-chlorophenyl)-trifluoromethyl-carbinol has a certain depressant effect on flies[9]. Experiments in this direction are now in progress.

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The Medical Research Laboratories, Army Medical Corps, Israel Defence Forces, June 25, 1957.

Résumé

L'influence de la halogénuration du groupement méthyl dans le di-(*p*-chlorophényl)-méthyl-carbinol sur l'activité de ce composé comme insecticide ou synergiste du DDT a été étudiée. On essaye de donner une explication rationnelle des faits observés.

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The Enzymatic Degradation of Yeast Nucleic Acid by Normal Rat Liver Tissue[1]

Using rat liver homogenates and mitochondrial preparations respectively, DELAMIRANDE *et al.*[2] and ROTH[3] found two optima in measuring the pH activity of their preparations against yeast nucleic acids. On the basis of these findings they postulated the presence of an acid as well as of alkaline ribonuclease in normal rat liver tissue. MAVER and GRECO[4] reported similar results. According to ROTH[5] the alkaline ribonuclease is similar or identical with the crystalline pancreatic ribonuclease, whereas the peak on the acid side is due to the action of a non-specific diesterase.

Table I
Enzymatic Hydrolysis of Yeast Nucleic Acid by Rat Liver Ribonuclease Plus Prostate Acid Phosphatase

pH	% *I.P.* Based on the total P of substrate
5·2–8·9	93–100%

Applying to normal liver homogenates the same method of extraction[6] which yielded a soluble enzyme preparation in the case of the cells of the mouse ascites tumor, we confirmed the presence of two peaks in the pH activity curve using 0·14 veronal buffer (1–2 cm^3 rat liver extract corresponding to 1–2 g rat liver; 10 mg yeast nucleic acid: Total volume 10 cm^3). Maximal depolymerization as measured by determination of the acid soluble P, after precipitation with MacFadyen's reagent, was observed at pH 5·6 and 8·6 respectively after 6–8 h at 37° C. Moreover the rate of hydrolysis at these pH optima was found to be approximately the same. After exhaustive digestion (40–48 h) all the diester bonds of yeast nucleic acid were found to be cleaved, as suggested by the amount of *I. P.* after 3 h incubation with purified prostate acid phosphatase at pH 5·6 (Table I). Therefore, it does not seem that this 'alkaline' ribonuclease can be identical with or even similar to pancreatic ribonuclease, since pancreatic ribonuclease is known to hydrolyze linkages in yeast nucleic acid involving pyrimidine nucleotides only, and not all diester bonds. Moreover, purine polynucleotides (prepared by exhaustive digestion of yeast nucleic acid with crystalline ribonuclease) were also found to be cleaved completely by this preparation, and the pH activity curve showed the same two optima at pH 5·6 and 8·6 respectively.

In connection with the above results the observation of HIRS *et al.*[7] are of interest. Their chromatographic analyses of acid extracts of beef liver did not reveal the presence of either of the two enzymes of the pancreas with ribonuclease activity.

Our preparations apparently contain only ribonuclease and no non-specific diesterase activity since glycerolphosphorylcholine was not cleaved at any pH.

Still, the two peaks in the pH activity curve seem to be due to the presence of two ribonucleases. Treatment of the enzyme preparation with sulfuric acid (standing in 0·25 *M* sulfuric acid for 24 h, at 5° C) revealed a difference in its hydrolytic behaviour at pH 5·6 and 8·6 respectively (Table II). There was no appreciable cleavage at pH 8·6. The partial inactivation at pH 5·6 involved reduction to about the same degree of the

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