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Relationship between the Structure of Compounds and Negatively Correlated Activity. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster*. II. Zenichi OGITA (Department of Genetics, Faculty of Medicine, Osaka University, Osaka). Received Jan. 20, 1961. *Botyu-Kagaku*, **26**, 18, 1961.

4. 化学構造と negatively correlated activity との関係 キイロショウジョウバエにおける negatively correlated cross-resistance の遺伝生化学的研究.

荻田善一 (大阪大学医学部 遺伝学教室) 36. 1. 20 受理

DDT, BHC, parathion に対して逆相関交叉抵抗性 (negatively correlated cross-resistance) を示す phenylthiourea (PTU) と，その対応酸素化合物である phenylurea (PU) の化学構造を種々の置換基でかえることによって PTU のもっている negatively correlated activity (N.C. activity) や PU のもっている positively correlated activity (P.C. activity) がどのように変化するかを遺伝学的解析によってしらべた．その結果，化学構造とこれらの activity との間の関係を示すことができた．

すなわち，alkylthiourea や thiourea は，いずれも毒性は arylthiourea 誘導体よりもはるかに高いけれども，N.C. activity を示さない．また，urea は毒性が殆んどなくP.C. activity も示さなかった．そして arylthiourea 誘導体の中で PTU に含まれるベンゼン核のパラ位置の水素のハロゲン置換体のみが PTU よりも N.C. activity 及び毒性もともに高い．これと同様に arylurea 誘導体の中で PU に含まれるベンゼン核のパラ位置の水素のハロゲン置換体のみが PU よりも P.C. activity 及び毒性もともに高い．しかし，オルソ位置の水素の塩素による置換体は PTU や PU の毒性を高めなかった．その外，他の基による置換体は毒性が低下し N.C. activity や P.C. activity を失なう．更に PTU や *p*-chlorophenylthiourea に含まれる thioureido 基のメチル基による N-置換体や S-置換体は毒性の低下とともに N.C. activity をも減少させる．したがって，PTU が N.C. activity を示すためには構造中に含まれるベンゼン核，そのパラ位置の水素の置換基および thioureido 基が大きな役割をはたしていると結論された．

The cross-resistance pattern to DDT, BHC and parathion observed in *Drosophila melanogaster* is negatively correlated with the resistance to phenylthiourea (PTU), but is positively correlated with phenylurea (PU)-resistance.

In order to understand the biochemical mechanism of the negatively correlated cross-resistance, and to find substances having toxicities stronger than PTU, the author has investigated the relationship between molecular structure and negatively correlated activity in a number of compounds, using genetical analyses.

Introduction

In previous papers[1,2,3], it was reported that DDT-, BHC-, and parathion-resistant strains of *Drosophila melanogaster* were consistently susceptible to phenylthiourea (PTU), and *vice versa*. On the other hand, phenylurea (PU)-resistant strains were resistant to DDT, BHC, parathion and nicotine sulfate. Genetical analyses showed that resistance to PTU and PU was due to a polygenic system consisting of two main genes on the 2nd and 3rd chromosomes respectively. One of them was a dominant gene conferring susceptibility to PTU and resistance to PU; namely it resulted from the pleiotropic expression of the dominant gene on the 2nd chromosome responsible for resistance to DDT, BHC and parathion.

The other was a dominant gene conferring resistance to both PTU and PU; it resulted from a pleiotropic expression of the dominant gene on the 3rd chromosome responsible for resistance to nicotine sulfate.

To understand the biochemical mechanism of the negatively-correlated cross-resistance, and also to find substances having toxicities stronger than PTU, genetical analyses were carried out for certain derivatives and relatives of thiourea and of urea.

Materials and Methods

I. Biological materials

The following strains of *D. melanogaster* maintained in the laboratory of Osaka University were employed;

Hikone-R_{31}: Selected with DDT in Japan, and resistant to various insecticides.

HL2-Q: Obtained from Dr. J. Bennett in U. S. A. and used in the present study after selection with DDT.

KSL: Highly parathion-resistant, selected by Dr. B. Rasmuson in Sweden.

+ ; + ; *HR_3*: A synthetic strain having the 1st and 2nd chromosomes from *Canton-S* and the 3rd chromosome from *Hikone-R*. This strain was DDT-susceptible but nicotine-resistant, and was used in the present study as a PTU-resistant strain.

PTUa-S_{10}: A highly PTU-resistant strain obtained by applying PTU selection pressure to a mixture of the *bw;st ss* and *Hikone-R_{31}* strains.

Canton-S: Extraordinary DDT-susceptible.

bw ; st ss: A multi-chromosomal strain used for the genetical analyses. This is susceptible to DDT, BHC, parathion and nicotine sulfate, and the 2nd and 3rd chromosomes are marked with recessive morphological mutant genes, *bw* (*brown*) on the 2nd chromosome, *st* (*scarlet*) and *ss* (*spineless*) on the 3rd chromosome respectively.

II. Chemical substances and medium

PTU, PU and the *p*-halogeno-derivatives were synthesized and purified by the author. Certain other chemicals were obtained from the Japan Agricultural Chemicals and Insecticides Company. The chemicals used are listed in Table 1.

The medium was composed of 2g. of agar, 3g. of dry yeast powder, and 4g. of sugar in 100ml. of distilled water. The soluble chemicals were dissolved in hot water and added to the dry yeast medium; insoluble chemicals were used as ethanolic solutions. Chemicals which were insoluble even in hot ethanol were added to the dry yeast medium immediately after boiling as crystal powders or talc powders containing the insecticides, and the medium was thoroughly mixed in a Waring Blendor. Parathion was used as an ethanolic solution and nicotine sulfate was used as an aqueous solution.

III. Methods

The "larval test" method was employed, as described in the previous papers; 50 or 100 first-instar larvae hatched from eggs on agar plates were reared on the medium containing each chemical, and the number of flies emerged from these media was compared with that from untreated normal media. The concentrations of chemicals were expressed in millimols per liter of the medium.

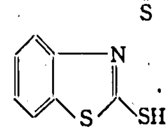
All tests were performed at approximately 25°C.

Experimental Results

I. Significance of para-position of benzene ring of PTU or PU on negatively or positively correlated activity and on toxicity.

The effect of addition of a halogen atom or

Table 1. Relationship between molecular structure and negatively or positively correlated activity in substituted thioureas and ureas.

Chemicals	Chemical constitution	N. C. A.
Phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot NH_2$	+
p-Chlorophenylthiourea	$p\text{-}Cl\cdot C_6H_4\cdot NH\cdot C(=S)\cdot NH_2$	++
p-Bromophenylthiourea	$p\text{-}Br\cdot C_6H_4\cdot NH\cdot C(=S)\cdot NH_2$	++
p-Methylphenylthiourea	$p\text{-}CH_3\cdot C_6H_4NH\cdot C(=S)\cdot NH_2$	−
p-Methoxyphenylthiourea	$p\text{-}CH_3\cdot O\cdot C_6H_4\cdot NH\cdot C(=S)\cdot NH_2$	−
p-Ethoxyphenylthiourea	$p\text{-}C_2H_5\cdot O\cdot C_6H_4\cdot NH\cdot C(=S)\cdot NH_2$	−
o-Chlorophenylthiourea	$o\text{-}Cl\cdot C_6H_4\cdot NH\cdot C(=S)\cdot NH_2$	+
α-Naphthylthiourea	$C_{10}H_7\cdot NH\cdot C(=S)\cdot NH_2$	−
N-Methyl-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot N(CH_3)(H)$	−
N-Dimethyl-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot N(CH_3)(CH_3)$	(±)
S-Methyl-phenylisothiourea	$C_6H_5\cdot NH\cdot C(=NH)\cdot S\text{-}CH_3$	−
N-Methyl-N′-(*p*-chlorophenyl)-thiourea	$p\text{-}Cl\cdot C_6H_4\cdot NH\cdot C(=S)\cdot N(CH_2)(H)$	(±)
N-Dimethyl-N′-(*p*-chlorophenyl)-thiourea	$p\text{-}Cl\cdot C_6H_4\cdot NH\cdot C(=S)\cdot N(CH_3)(CH_3)$	(±)
N-Acetyl-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot N(C(=O)\cdot CH_3)(H)$	(±)
N-Propionyl-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot N(C(=O)\cdot C_2H_5)(H)$	−
N-Allyl-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot N(CH_2\cdot CH{:}CH_2)(H)$	−
N-(Phenyl)-N′-carboethoxythiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot NH\cdot C(=O)\cdot OC_2H_5$	−
N-(*p*-Chlorophenyl)-N′-carboethoxythiourea	$p\text{-}Cl\text{-}C_6H_4\cdot NH\cdot C(=S)\cdot NH\cdot C(=O)\cdot OC_2H_5$	−
N, N′-Diphenylthiourea	$C_6H_5\cdot NH\cdot C(=S)\cdot NH\cdot C_6H_5$	−
N-(*p*-Nitrophenyl)-N′-phenylthiourea	$p\text{-}NO_2\text{-}C_6H_4\cdot NH\cdot C(=S)\cdot NH\cdot C_6H_5$	−
N-Diethoxythiophospho-N′-phenylthiourea	$C_6H_5\cdot NH\cdot C\cdot NH\cdot P(=S)(OC_2H_5)(OC_2H_5)$	−
Phenylthiosemicarbazide	$C_6H_5\cdot NH\cdot C(=S)\cdot NH\cdot NH_2$	−
Methyl-N-phenylthionocarbamate	$C_6H_5\cdot NH\cdot C(=S)\cdot O\cdot CH_3$	−
Ethyl-N-phenylthionocarbamate	$C_3H_5\cdot NH\cdot C(=S)\cdot O\cdot C_2H_5$	−
Mercaptobenzothiazol		−
Thiourea	$_2HN\cdot C(=S)\cdot NH_2$	−
N-Methylthiourea	$(CH_3)(H)N\cdot C(=S)\cdot NH_2$	−
N, N′-trimethylthiourea	$(CH_3)(H)N\cdot C(=S)\cdot N(CH_3)(CH_3)$	−
N-Allylthiourea	$(CH_2{:}CH\cdot CH_2)(H)N\cdot C(=S)\cdot NH_2$	−
Ethylenethiourea	$(CH\text{-}NH)(CH\text{-}NH)>C=S$ (CH=CH)	−
Sodium-diethyldithiocarbamate	$(C_2H_5)(C_2H_5)N\cdot C(=S)\cdot S\cdot Na$	−

Chemicals	Chemical constitution	P. C. A.	Chemicals	Chemical constitution	P. C. A.
Phenylurea	$C_6H_5{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	+	*o*-Chlorophenylurea	$o\text{-}Cl\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	+
p-Chlorophenylurea	$p\text{-}Cl\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	++	*p*-Methylphenylurea	$p\text{-}CH_3\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	−
p-Bromophenylurea	$p\text{-}Br\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	++	*p*-Methoxyphenylurea	$p\text{-}CH_3O\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	−
p-Iodophenylurea	$p\text{-}I\text{-}C_6H_4{\cdot}NH{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	++	Urea	${}_2HN{\cdot}\underset{\underset{O}{\|}}{C}{\cdot}NH_2$	−

The following abbrevations are used : N. C. A., Negatively correlated activity ; P. C. A., Positively correlated activity ; +, Degree of activity similar to that of PTU or PU. ; ++, Degree of activity higher than that of PTU or PU ; ±, Degree of activity lower than that of PTU or PU. ; −, Activity not shown.

several radicals on the negatively correlated activity of PTU was investigated by comparing the toxicity of similarly substituted phenylthioureas and phenylureas to insecticide-resistant and susceptible strains, and by subsequent genetical analyses.

Toxicities determined are shown in Figs. 1, 2 and 3. Here the percentage survival of the various strains, to the lowest concentration causing 100 percent mortality to the most susceptible strain, are presented as histograms.

1. Toxicity of arylthioureas and arylureas to insecticide-resistant and susceptible strains

Among the N-arylthioureas, *p*-chlorophenylthiourea (Fig. 1-d) and *p*-bromophenylthiourea (Fig. 1-f) were highly toxic to DDT-, BHC-, parathion- and nicotine-resistant strains such as *Hikone-R*$_{31}$, *HL2-Q* and *KSL*, but they were almost non-toxic to the strains which were susceptible to DDT,

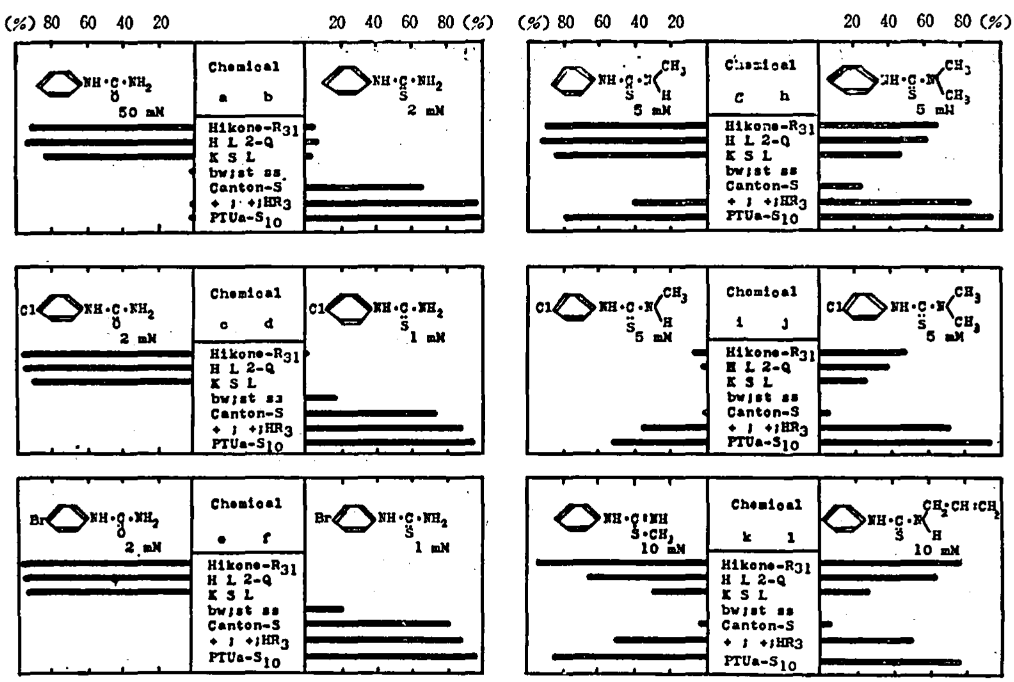
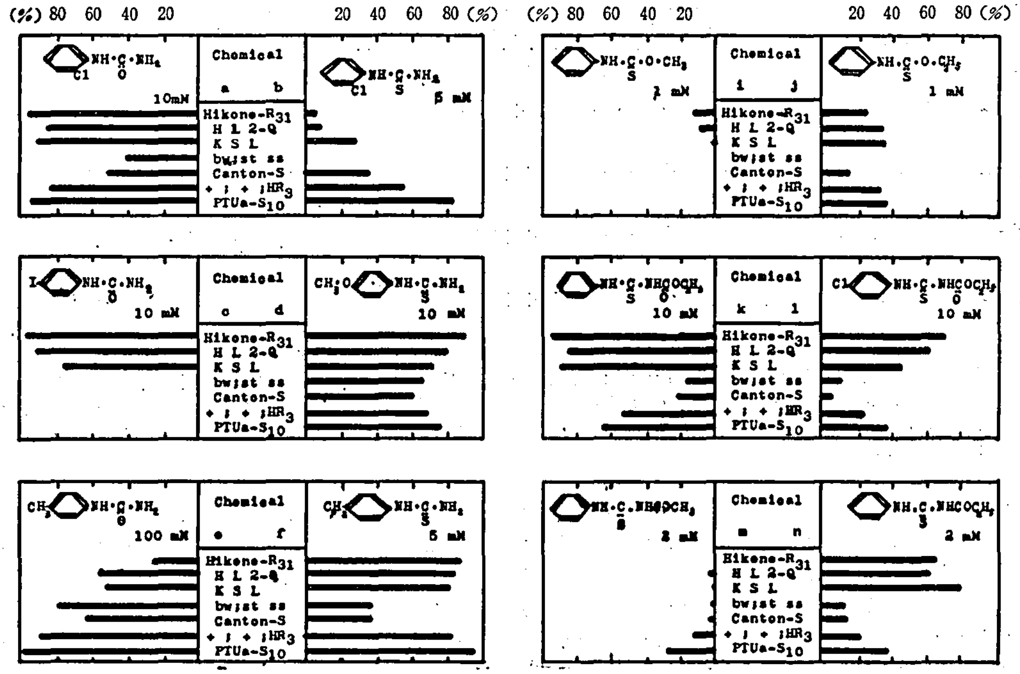


Fig. 1. Percentage survival of various strains at a concentration giving the LD$_{100}$ of the most susceptible of the strains. (Based on 500 larvae by the larval test method.)



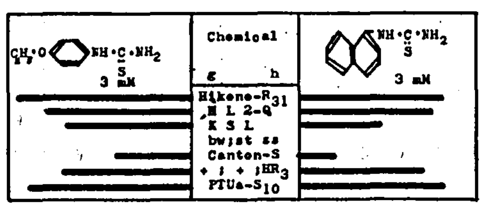


Fig. 2. Parcentage survival of various strains at a concentration giving the LD_{100} of the most susceptible of the strains. (Based on 500 larvae by the larval test method.)

BHC and parathion, and simultaneously resistant to nicotine sulfate, such as + ; + ; *HR_3* and *PTUa-S_{10}* ; that is, the insecticidal action of *p*-chlorophenylthiourea and of *p*-bromophenylthiourea resembled PTU (Fig. 1-b), and the activity seemed to be higher than that of PTU.

On the other hand, among the N-arylurea, *p*-halogenophenylureas such as *p*-chlorophenylurea (Fig. 1-c), *p*-bromophenylurea (Fig. 1-e) and *p*-iodophenylurea (Fig. 2-c) showed high toxicities to DDT-, BHC-, parathion- and nicotine-susceptible strains but not to the resistant strains. They seemed to be positively correlated with DDT and other insecticides, more clearly so than PU (Fig. 1-a), and they were more toxic than PU. The levels of resistance (LD_{50} and LD_{100}) of the various strains to such chemicals as PTU, PU and these halogeno-derivatives are shown in Table 2.

These results suggest that *p*-halogeno-substituents of PTU and of PU are more toxic and characteristic in activity than their parent compounds.

However, other para-substituted derivatives such as *p*-methylphenylthiourea (Fig. 2-f), *p*-methoxyphenylthiourea (Fig. 2-d), *p*-ethoxyphenylthiourea (Fig. 2-g) and *p*-methylphenylurea (Fig. 2-e) etc., seemed to lose the peculiar activity characteristic of the parent compounds. However, *p*-ethoxyphenylthiourea might have a positively correlated activity with nicotine sulfate. The mechanism of insecticidal action of *p*-methylphenylurea (Fig. 2-e) was not clear, since it was toxic to *Hikone-R_{31}* but not to other strains.

Of the N-arylthioureas with the thioureido radical, α-naphthylthiourea (Fig. 2-h) proved somewhat toxic to nicotine-susceptible strains, but not to nicotine-resistant strains.

Of the ortho-halogenated derivatives, *o*-chloro-

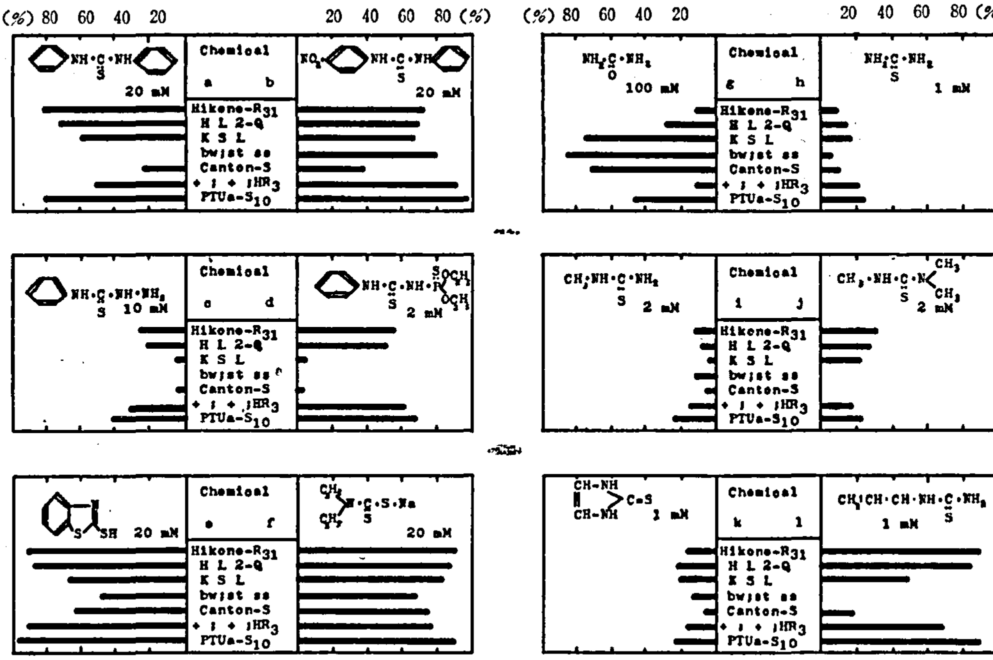


Fig. 3. Percentage survival of various strains at a concentration giving the LD_{100} of the most susceptible of the strains. (Based on 500 larvae by the larval test method.)

Table 2. Levels of resistance to various chemicals in some strains of *D. melanogaster*. (LD_{50} or LD_{100} figures expressed in mM concentration larval medium, and based on tests with 500 larvae)

Chemical	PTU		*p*-Cl-PTU		*p*-Br-PTU		PU		*p*-Cl-PU		*p*-Br-PU	
Strain	LD_{50}	LD_{100}	LD_{50}	LD_{100}	LD_{50}	LD_{100}	LD_{50}	LD_{100}	LD_{50}	LD_{100}	LD_{50}	LD_{100}
	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)
Hikone-R_{31}	1.0	2.5	0.3	1.2	0.2	0.8	100	——	100	——	100	——
KSL	0.8	2.0	0.4	1.0	0.4	1.0	70	100	65	100	65	100
HL2-Q	0.7	1.5	0.8	1.3	0.5	1.2	85	100	85	100	85	100
bw ; st ss	1.2	1.5	0.6	1.5	0.6	1.5	20	30	1.2	2.0	1.0	1.5
Canton-S	2.7	7.0	1.5	5.0	1.7	5.0	25	40	0.2	1.2	0.3	1.2
+ ; + ; *HR_3*	10.0	25.0	8.2	15.0	8.0	15.0	35	45	0.5	1.5	0.4	1.4
PTUa-s_{10}	25.0	55.0	18.0	25.0	18.5	25.0	35	45	1.4	2.2	1.0	1.5

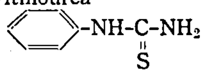
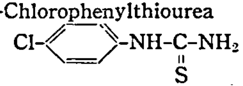
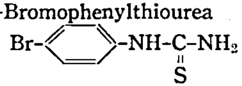
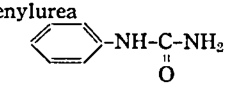
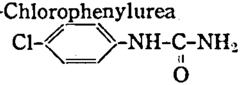
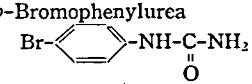
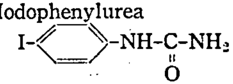
phenylthiourea (Fig. 2-b) was non-toxic to PTU-resistant strains, but it was relatively toxic to DDT-, BHC- and parathion-resistant strains or nicotine-susceptible strains in 5mM concentration, and only PTU-resistant strains such as *PTUa-S_{10}* could emerge from a medium containing 10mM of this chemical. The chemical was no more toxic than PTU, although it did not seem to lose the negatively correlated activity completely. *o*-Chlorophenylurea (Fig. 2-a) was non-toxic to all strains in 10mM concentration. Thus neither *o*-chloro-PTU nor *o*-chloro-PU were more toxic than their parent compounds.

2. Genetical analyses on the insecticidal action of arylthioureas and arylureas

The multi-chromosomal mutant strain, *bw ; st ss* which was susceptible to various insecticides, was combined with *Hikone-R_{31}*, *HL2-Q* and *KSL*

Table 3. Numbers of each phenotype in F_2 progeny from backcross (1), (2) and (3) emerging from treated media, expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by the larval test method.)

Backcross		(1) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *Hikone-R*$_{31}$♂)♂				(2) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *KSL*♂)♂				(3) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *HL2-Q*♂)♂			
Phenotype		*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +	*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +	*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +
DDT	10mM	0	0	81.0	89.5	0	0	82.4	95.2	0	0	76.8	83.2
BHC	3×10⁻²mM	0	0	53.7	84.3	0	0	54.4	86.4	0	0	44.0	54.4
Parathion	1×10⁻²mM	0	0	16.5	38.6	0	0	23.2	41.6	0	0	10.4	33.6
Nicotine sulfate	2mM	0	83.3	0	94.4	0	15.2	1.6	46.4	0	60.8	0	67.2
Phenylthiourea 	3mM	0	91.2	0	0	0	23.2	0	0	0	46.4	0	0
p-Chlorophenylthiourea 	3mM	0	94.4	0	0	0	20.0	0	0	0	32.0	0	0
p-Bromophenylthiourea 	3mM	0	84.8	0	0	0	25.6	0	0	0	28.8	0	0
Phenylurea 	70mM	0	0	0	87.2	0	0	0	62.4	0	0	0	55.2
p-Chlorophenylurea 	5mM	0	0	31.2	84.8	0	0	14.4	69.6	0	0	21.6	76.0
	10mM	0	0	0	76.0	0	0	0	22.4	0	0	0	36.0
p-Bromophenylurea 	5mM	0	0	20.0	78.4	0	0	9.6	26.0	0	0	15.2	53.6
	10mM	0	0	0	68.8	0	0	0	24.0	0	0	0	28.8
p-Iodophenylurea 	10mM	0	0	48.8	102.0	0	0	42.4	92.8	0	0	34.4	78.4

strains which were resistant to DDT, BHC, parathion and nicotine sulfate.

(1) *bw* ; *st ss*♀ ×F_1(*bw*; *st ss*♀ ×*Hikone-R_{31}*♂)♂

(2) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*HL2-Q*♂)♂

(3) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*KSL*♂)♂

One hundred first-instar F_2 larvae resulting from each of these backcrosses were put into a 60ml. glass vial containing 15ml. of dry yeast medium with or without insecticides or chemicals. In each experiment, 500 first-instar larvae obtained from the backcross (1), (2) and (3) were used respectively. The percentages of phenotypes of the flies emerged from the medium containing a chemical as compared to those from the normal medium were calculated (Table 3).

Only + ; *st ss* and + ; + + (wild type) flies emerged from the media containing DDT, BHC and parathion. These flies had the 2nd chromosome carrying the DDT resistant gene derived from *Hikone-R_{31}*, *KSL* or *HL2-Q* in the heterozygous condition. There were more + ; + + flies than + ; *st ss* flies, since the former were heterozygous for the nicotine-resistant gene on the 3rd chromosome.

On the other hand, only *bw* ; + + flies emerged from the media containing PTU, *p*-chlorophenylthiourea, or *p*-bromophenylthiourea. These flies were homozygous for the susceptible gene to DDT, BHC and parathion located on the 2nd chromosome, and were heterozygous for the nicotine-resistant gene on the 3rd chromosome. Consequently, these flies were susceptible to DDT, BHC and parathion and resistant to nicotine sulfate. The flies having the resistant gene to DDT, BHC and parathion on the 2nd chromosome did not emerge. These results suggested that the insecticidal action of *p*-chlorophenylthiourea and of *p*-bromophenylthiourea was negatively correlated to that of DDT, BHC or parathion in the same way as PTU.

On the other hand, only + ; + + and + ; *st ss* flies emerged from media containing 5mM of *p*-chlorophenylurea, 5mM of *p*-bromophenylurea or 10mM of *p*-iodophenylurea, the oxides corresponding to their thioureido compounds. These flies had the resistant gene to DDT, BHC, parathion on the 2nd chromosome. Only wild-type flies emerged from media containing 70 mM of phenylurea, 10 mM of *p*-chlorophenylurea or 10 mM of *p*-bromophenylurea. These results suggested that the *p*-halogeno-substituents of phenylurea were positively correlated to the DDT, BHC, parathion and nicotine sulfate.

As shown in Table 4, the number of *bw* ; + + flies which emerged from media containing 5mM *o*-chlorophenylthiourea was more than that of other phenotypes. The insecticidal action of this chemical was therefore negatively correlated with that of DDT, BHC and parathion.

Nevertheless, at the high concentration of 10mM of this chemical, even the *bw* ; + + flies were almost lacking. This may indicate that a polygenic system requiring many factors might be involved.

The results obtained with *p*-methylphenylthiourea, *p*-methoxyphenylthiourea, *p*-ethoxyphenylthiourea, *α*-naphthylthiourea and *p*-methylphenylurea were not so clear because of their lower insecticidal action. But the action of *p*-ethoxyphenylthiourea or *α*-naphthylthiourea might be similar to that of nicotine sulfate rather than that of PTU (Table 4). The action of *p*-methylphenylurea may be controlled by some other factors than the genes discussed in present paper (Table 4).

II. Significance of the thioureido radical in the negatively correlated activity and toxicity of PTU and *p*-chlorophenylthiourea

To understand the significance of thioureido radical in the negatively correlated activity, toxicities of compound in which the hydrogen atoms of the thioureido radical of PTU or *p*-chlorophenylthiourea were replaced by several radicals were tested on susceptible and resistant-strains.

1. Toxicities of N-substituted phenylthioureas and S-substituted phenylthioureas

Of the N-monomethyl substituted phenylthioureas, N-methyl-N′-phenylthiourea (Fig. 1-g) was toxic to DDT-, BHC-, parathion- and nicotine-susceptible strains and non-toxic to the resistant strains, and at a concentration of 10mM this chemical was non-toxic to DDT-, BHC-, parathion- and nicotine-resistant strains more than PTU-resistant strain (DDT-, BHC-, and parathion-susceptible, nicotine-resistant strains). But N-methyl-N′-(*p*-chlorophenyl) thiourea (Fig. 1-i) was toxic to the nicotine-susceptible strains, and relatively non-toxic

Table 4. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *Hikone-R*$_{31}$♂)♂ emerging from treated media, expressed as a percentage of those emerging from untreated media. (Based on 500 F_2 larvae by the larval test method.)

Phenotype			*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +
Thiourea	$NH_2 \cdot C(=S) \cdot NH_2$	1.0mM	0	1.6	3.3	6.3
		1.5mM	0	0	0	0
Ethylenethiourea	(CH-NH)$_2$>C=S	1.0mM	0	4.1	6.6	18.2
		1.5mM	0	0	0	0
Allylthiourea	$CH_2 \cdot CH \cdot CH_2 \cdot NH \cdot C(=S) \cdot NH_2$	1.0mM	56.4	101.6	52.5	101.5
		1.5mM	3.2	100.0	5.2	99.2
		3.0mM	0	61.5	0	75.4
		5.0mM	0	49.1	0	83.3
p-Ethoxyphenylthiourea	$C_2H_5 \cdot O$-C_6H_4-$NH \cdot C(=S) \cdot NH_2$	5.0mM	0	56.8	62.5	93.6
α-Naphtylthiourea	$C_{10}H_7$-$NH \cdot C(=S) \cdot NH_2$	2.5mM	0	87.6	84.3	92.4
Urea	$NH_2 \cdot C(=O) \cdot NH_2$	150mM	36.2	40.9	45.8	55.5
p-Methylphenylurea	CH_3-C_6H_4-$NH \cdot C(=O) \cdot NH_2$	60mM	96.7	94.2	41.6	43.6
o-Chlorophenylthiourea	Cl-C_6H_4-$NH \cdot C(=S) \cdot NH_2$	3.0mM	0	55.5	23.4	40.8
		5.0mM	0	22.1	6.2	5.1
		10.0mM	0	1.2	0	0

to the PTU-resistant strains more than DDT-, BHC-, parathion- and nicotine-resistant strains. In a concentration of 10mM of this chemical, the relation was clearer than the former case. This chemical seemed to have negatively correlated activity, though it was less than its parent chemical, *p*-chlorophenylthiourea. Of the N-dimethyl-substituted phenylthioureas, N-dimethyl-N′-phenylthiourea (Fig. 1-h) was toxic to nicotine susceptible strains and non-toxic to nicotine resistant strains at a concentration of 5mM, but PTU-resistant strains were somewhat more resistant than DDT-, BHC-, parathion- and nicotine-resistant strains at a concentration of 10mM. N-Dimethyl-N′-(*p*-chlorophenyl) thiourea (Fig. 1-j) was toxic to a nicotine-susceptible strain, but non-toxic to the resistant strains. It seemed to have negatively correlated activity, though it was not so clear as that of N-methyl-N′-(*p*-chlorophenyl)-thiourea, at a concentration of 10mM.

On the other hand, among the S-methyl-substituted phenylthioureas, S-methyl-phenylisothiourea (S-methyl-phenylthiopseudourea) (Fig. 1-k) showed a relatively high toxicity to the nicotine-susceptible strains such as *bw* ; *st ss* and *Canton-S*, and was relatively non-toxic to the resistant strains. These results suggested that S-methylation resulted in a loss of toxicity to DDT-, BHC- and parathion-resistant strains. On the other hand, N-methyl derivatives of *p*-chlorophenylthiourea (Fig. 1-i, j) which had the clearest negatively correlated activity, seemed to retain this activity.

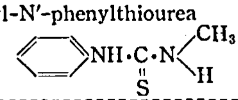
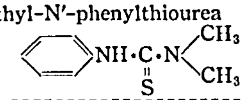
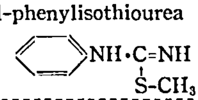
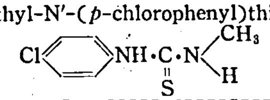
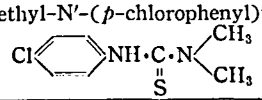
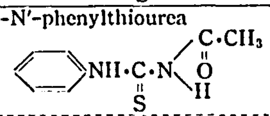
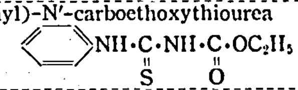
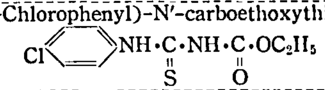
Of the other N-substituted phenylthioureas, N-allyl-N′-phenylthiourea (Fig. 1-l) proved toxic to nicotine-susceptible strains such as *bw ; st ss* and *Canton-S* in a concentration of 10mM. N-(*p*-nitrophenyl)-N′-phenylthiourea (Fig. 3-b) was non-toxic to any of the strains. N, N′-Diphenylthiourea (Fig. 3-a) was toxic to nicotine-susceptible strains in a concentration of 20mM, but it was non-toxic to nicotine-resistant strains. N-(phenyl)-N′-carboethoxythiourea (Fig. 2-k) and N-(*p*-chlorophenyl)-N′-carboethoxythiourea (Fig. 2-l) were toxic to nicotine-susceptible strains and relatively non-toxic to nicotine-resistant strains. Phenylthiosemicarbazide (Fig. 3-c) and N-diethoxythiophospho-N′-phenylthiourea (Fig. 3-d) were toxic to nicotine-susceptible strains, and non-toxic to nicotine-resistant strains. Insecticidal action of these chemicals was not negatively correlated with that of DDT, BHC and parathion, but it seemed to be positively correlated with that of nicotine sulfate.

N-acetyl-N′-phenylthiourea (Fig. 2-m) was highly toxic to almost all strains, but it was not relatively non-toxic to *PTUa-S*$_{10}$ strain. It seemed to have negatively correlated activity. But N-propionyl-N′-phenylthiourea (Fig. 2-n) was relatively non-toxic to DDT-, BHC- and parathion-resistant strains in a concentration of 2mM.

2. Genetical analyses on the insecticidal action of N-substituted phenylthioureas and S-substituted PTU

Similar genetical analyses were performed with seven N-substituted phenylthioureas and

Table 5. Numbers of each phenotype in F_2 progeny from backcross (1). *bw ; st ss*♀ × *F*$_1$(*bw ; st ss*♀ × *Hikone-R*$_{31}$♂)♂ emerging from media containing N-substituents or S-substituent of phenylthioureas, expressed as a percentage of those emerging from untreated media. (Based on 500 F_2 larvae by the larval test method.)

Phenotype		*bw ; st ss*	*bw ; + +*	*+ ; st ss*	*+ ; + +*
N-Methyl-N′-phenylthiourea	1.5mM	26.0	100.8	103.3	102.2
	3.0mM	1.6	89.6	99.1	90.5
	5.0mM	0	49.6	35.5	82.6
	10.0mM*	0	18.5	41.8	61.1
N-Dimethyl-N′-phenylthiourea	1.5mM	60.9	88.0	99.1	94.4
	3.0mM	0	76.0	49.5	100.7
	5.0mM	0	96.8	1.6	90.5
	10.0mM	0	54.4	0	17.3
S-Methyl-phenylisothiourea	3.0mM	85.3	96.8	103.3	100.7
	5.0mM	73.1	90.4	66.1	86.6
	10.0mM	4.0	71.2	17.3	85.0
N-Methyl-N′-(*p*-chlorophenyl)thiourea	5.0mM*	0	33.2	0	3.0
	10.0mM*	0	16.4	0	1.0
N-Dimethyl-N′-(*p*-chlorophenyl)thiourea	5.0mM*	0	77.3	14.8	40.2
	10.0mM*	0	56.5	6.7	22.6
N-Acetyl-N′-phenylthiourea	0.5mM	79.7	100.0	36.3	51.4
	1.0mM	3.4	48.6	0	0.7
	2.0mM	0	7.3	0	0
N-(Phenyl)-N′-carboethoxythiourea	5.0mM	89.8	84.4	78.8	99.3
	10.0mM	5.1	27.5	8.8	37.3
N-(*p*-Chlorophenyl)-N′-carboethoxythiourea	5.0mM	3.4	51.4	11.3	43.0
	10.0mM	0	1.8	0	9.9

*; 1000 F_2-larvae were used for test by larval test method.

S-substituted PTU (Table 5).

+ ; + + and + ; *st ss* flies, that carried the resistant gene for DDT, BHC, parathion on the 2nd chromosome, emerged from media containing 10mM of N-methyl-N′-phenylthiourea in greater numbers than the other phenotypes. Therefore, the insecticidal action of N-methyl-N′-phenylthiourea is probably similar to that of DDT, BHC, parathion, PU or *p*-halogenophenylureas.

On the other hand, *bw* ; + + and + ; + + flies emerged from media containing 5mM of N-dimethyl-N′-phenylthiourea or 10mM of S-methylphenylisothiourea more than flies of other phenotypes. Consequently, the insecticidal action of these chemicals was similar to that of nicotine sulfate. Nevertheless, in a concentration of 10mM N-dimethyl-N′-phenylthioures, *bw* ; + + flies emerged from this medium in greater numbers than + ; + + flies. This result indicated that at high concentrations, the insecticidal action of this chemical seemed to be negatively correlated, to a certain extent, with that of DDT, BHC and parathion.

Only *bw* ; + + flies, and a few + ; + + flies, emerged from the medium containing 5mM N-methyl-N′-(*p*-chlorophenyl)thiourea. The insecticidal action of this chemical, at least at 5mM concentration, was negatively correlated with that of DDT, BHC and parathion.

With regard to N-dimethyl-N′-(*p*-chlorophenyl) thiourea, flies which emerged from media containing 5mM or 10mM of this chemical, were almost all *bw* ; + + and + ; + + type, and the percentage emergence of *bw* ; + + was higher than that of + ; + + flies. These results suggest that this chemical might have somewhat negatively correlated activity.

With regard to N-acetyl-N′-phenylthiourea, flies which emerged from media containing 1mM of this chemical, were almost all *bw* ; + +. The insecticidal action of this chemical was therefore negatively correlated with that of DDT, BHC and parathion.

Nevertheless at the high concentration of 2mM of this chemical, even the *bw* ; + + flies were almost lacking. This may indicate that a polygenic system requiring many factors might be involved.

On the other hand, *bw* ; + + and + ; + + flies emerged from media containing 10 mM of N-(phenyl)-N′-carboethoxythiourea or 5 mM of N-(*p*-chlorophenyl)-N′-carboethoxythiourea more than flies of other phenotypes. Consequently, the insecticidal action of these chemicals was similar to that of nicotine sulfate.

The addition of substituents into the thioureido radical of PTU and *p*-chlorophenylthiourea reduced their toxicities to insecticide-resistant strains as well as their negatively correlated activity to the insecticidal action of DDT, BHC and parathion, but the positively correlated activity to that of nicotine sulfate remained. The insecticidal action of S-methyl-phenylisothiourea was similar to that of nicotine sulfate.

III. Insecticidal action and toxicity of alkylthioureas and related compounds

To understand the effect of the benzene ring on negatively correlated activity, the toxicities of thiourea and alkylthioureas to susceptible and resistant strains were examined.

Thiourea (Fig. 3-h) proved to be highly toxic to both insecticide-resistant and susceptible strains; that is, no larvae of any strain could emerge from a medium containing 2mM thiourea, while the type of insecticidal action of thiourea could not be determined in the medium containing 1mM.

Of the N-alkylthiourea derivatives, N-methylthiourea (Fig. 3-i) showed toxicity to both insecticide-resistant and susceptible strains. N-trimethylthiourea (Fig. 3-j) and allylthiourea (Fig. 3-l) showed high toxicities to nicotine-susceptible strains, but not to nicotine-resistant strains, and their larvicidal action seemed to be positively correlated with that of nicotine sulfate.

Ethylenethiourea (Fig. 3-k), which contained a five-membered ring, was very toxic to all strains even in the concentration of 1mM. In general the N-alkylthioureas showed higher toxicities than the N-arylthioureas.

As shown in Table 4, thiourea and ethylenethiourea were so toxic to both insecticide-resistant and susceptible strains that it was difficult to perform the genetical analyses. These results suggest that resistance to these chemicals may be controlled by certain factors other than the resistant genes discussed in the present study. The insecticidal action of allylthiourea, according to the genetical analyses, seemed to be similar to that of nicotine sulfate.

Thus, alkylthioureas failed to show negative correlation with DDT, BHC and parathion, but most of them had a positively correlated activity to nicotine sulfate.

Urea (Fig. 3-g) was non-toxic to any of the strains. The result of the genetical analysis was therefore not clear. (Table 4).

Methyl-N-phenylthionocarbamate (Fig. 2-i) was highly toxic to all strains, and it seemed to be positively correlated with DDT, BHC and parathion. Ethyl-N-phenylthionocarbamate (Fig. 2-j) was toxic to nicotine-susceptible strains, but relatively non-toxic to nicotine-resistant strains.

Of other compounds tested, sodium diethyldithiocarbamate (Fig. 3-f) and mercaptobenzothiazol (Fig. 3-e) were almost non-toxic to all strains.

Discussion and Conclusion

In the previous papers[1,2,3], the following hypothesis was introduced : "the dominant gene at *II-65*± which confers resistance to DDT, BHC and parathion also confers resistance to phenylurea and abnormal susceptibility to phenylthiourea, and the dominant gene at *III-50*± which confers resistance to nicotine sulfate, also confers resistance to phenylthiourea as well as phenylurea".

The present investigations showed that not only PTU but also *p*-halogenophenylthioureas were negatively correlated with DDT, BHC and parathion, and that their corresponding oxides, not only PU but also *p*-halogenophenylureas, were positively correlated with DDT, BHC, parathion and nicotine sulfate. These *p*-halogenated derivatives were more toxic than their parent compounds, and increased their negatively or positively correlated activity. While *o*-chloro-substituted derivatives of PTU or PU were no more toxic than their parent compounds, though they did not completely lose their negatively or positively correlated activitiy.

However the N-arylthioureas, in which a benzene ring was directly attached to the thioureido radical ($\text{-NH}\cdot\underset{\overset{\|}{\text{S}}}{\text{C}}\cdot\text{NH}_2$), did not always have negatively correlated activity ; *p*-ethoxyphenylthiourea, *p*-methylphenylthiourea, *p*-methoxyphenylthiourea and *α*-naphthylthiourea were of reduced toxicity, and they did not show a negative correlation with DDT and other insecticides. Similarly the N-arylureas which contained the ureido radical ($\text{-NH}\cdot\underset{\overset{\|}{\text{O}}}{\text{C}}\cdot\text{NH}_2$), did not always have positively correlated activity ; the weak toxicity of *p*-methylphenylurea seemed to have no relation to that of general insecticides. Derivatives of PTU or PU with radicals such as methyl, methoxy and ethoxy substituted in the para position were less toxic than their parent compounds, and seemed to have lost their negatively or positively correlated activity to the insecticidal action of DDT, BHC and parathion.

As shown in Table 5, substitution on the thioreido radical of PTU and *p*-chlorophenylthiourea resulted in a reduction of the toxicity and negatively correlated activity ; indeed, the insecticidal action of N-methyl-N′-phenylthiourea appears to be positively correlated to that of DDT, BHC, parathion, PU and *p*-halogenophenylureas, and may be derived from a polygenic system consisting of two factors on the 2nd and 3rd chromosomes, rather than from the nicotine-resistant gene only. On the other hand, resistance to N-dimethyl-N′-phenylthiourea and S-methylphenylisothiourea appeared to be similar to that to nicotine sulfate, i. e., resistance to these chemicals was controlled by the gene on the 3rd chromosome rather than that on the 2nd chromosome. But at high concentration, N-dimethyl-N′-phenylthiourea seemed to have a slight negatively-correlated activity. The N-methyl derivatives of *p*-chlorophenylthiourea however retained a certain degree of negative correlation to DDT, BHC and parathion. These results suggest that the negatively correlated activity shown by PTU and the *p*-halogenophenylthioureas is partly due to the thioureido radical they contained.

Other N-derivatives of PTU showed a reduction in toxicity and negatively correlated activity, in almost all cases, and most of them seemed to be positively correlated with nicotine sulfate. N-Acetyl-N′-phenylthiourea was highly toxic to all strains, but it did retain a certain negative correlation to DDT, BHC and parathion.

It was difficult to perform genetical analyses on thiourea and ethylenethiourea, because they were almost equally toxic to all strains of *D. melanoagster*. They showed a slight positive correlation with nicotine sulfate. Thiourea was the most insecticidal among all the thiourea derivatives. N-Allylthiourea, not so insecticidal, showed a definitely positive correlation with nicotine sulfate.

With regard to the insecticidal action of urea, the result of analysis was not clear and failed to indicate a positive correlation to DDT and other insecticides.

These results obtained lead to the conclusion that the benzene ring and the thioureido radical contained in PTU, and the *p*-position of benzene ring as seen in the *p*-halogenophenylthioureas, each has a significant role in their peculiar negative correlation with DDT and other insecticides.

Summary

Genetical analyses of the larvicidal action of derivatives of thiourea and of urea were performed with 7 different resistant or susceptible strains of *Drosophila melanogaster*:

1) Resistance to phenylthiourea (PTU) and its *p*-halogenated derivatives was negatively correlated with that to DDT, BHC and parathion, whereas resistance to phenylurea (PU) and its *p*-halogenated derivatives was positively correlated with that to DDT, BHC and parathion.

2) Although the *p*-halogeno-substitution of PTU or PU resulted in an increase of toxicity, the *o*-chloro derivatives of PTU and PU were no more toxic than their parent compounds, but they did not completely lose their negatively or positively correlated activity. Other para-substituted derivatives of PTU or PU seemed to lose the toxicity and negatively or positively correlated activity which their parent compounds had.

3) The addition of substituents to the thioureido radical of PTU and *p*-chlorophenylthiourea reduced their toxicity and negatively correlated activity.

4) Certain other para-substituted or N-substituted derivatives of PTU, as well as the alkylthioureas tested, failed to show negative correlation with DDT, BHC and parathion. However most of them appeared to be positively correlated with nicotine sulfate.

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