MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXVIII, No. 1, Article 3 (Biology), 1961

$CuSO_4$ Resistance in *Drosophila melanogaster*

IV. Are there Any Cross Resistance Phenomena among Various Chemical Agents ?[1)]

By

Sizue YANAGISHIMA

Zoological Institute, College of Science, University of Kyoto

(Received April 6, 1961)

The present author has already mentioned that when *Drosophila melanogaster* Oregon RS strain was cultured on the medium containing 0.5 mM of $CuSO_4$ for one generation, the flies of the next generation (they are called the Cu-strain in this report) were found to be more resistant to copper than the flies which were cultured on normal PEARL's medium. In this case, there is no need of assuming selection for inducing specific resistant variant to copper, because no significant difference in emergence rate was found out between on the copper culture medium and on the normal culture medium, when the larvae of the normal strain were tested in these media (YANAGISHIMA and SUZUKI, 1959 a, b).

In the third report of the present series, the author has shown that the copper resistant flies were different from the control ones in morphological, ecological and physiological characters (YANAGISHIMA, 1961 a).

This time, the author has studied the following point on the copper resistance built up by subjecting to a sublethal dose of copper : Whether this copper resistance is of specific character only to copper or this has cross resistance and collateral sensitivity to other toxic agents ; in other words, can this copper resistance have a multiple resistance ?

A number of reports on a resistance to insecticides have been published. *Drosophila melanogaster* was used as the experimental material in the following reports : TSUKAMOTO, 1950, 1954, 1955, 1956 ; CROW, 1951, 1954 ; BARTLETT, 1952 ; KIKKAWA, 1953 ; KING, 1954 ; BOCHNIG, 1956 ; HUNTER, 1956 ; TSUKAMOTO *et al.*, 1957. Houseflies and mosquitoes were the experimental materials in the other reports (BARBER and SCHMITT, 1949 a ; KEIDING and VON DEURS, 1949 ; MARCH *et al.*, 1949, 1950, 1956 ; BRUCE, 1950 ; BRUCE and DECKER, 1950, 1951 ; MORRISON, 1950, 1957 ; PERRY *et al.*, 1950, 1951, 1953 ; PIMENTEL and DEWEY, 1950, 1951 ; BUSVINE, 1951, 1953, 1956, 1957 ; HARRISON, 1951 ; WIESMANN and KOCHER, 1951 ;

---

1) Contributions from the Adaptive Variation Research Group, No. 56.

DECKER and BRUCE, 1952; MARCH, 1952; MARIANI, 1953; ASCHER and KOCHER, 1954; KING, 1954; MCKENZIE and HOSKINS, 1954; METCALF, 1955; BROWN, 1956; BROWN and PERRY, 1956; MELTZER, 1956; SUZUKI and TÔYAMA, 1956; UENO and MATSUYAMA, 1956; LA BRECQUE and WILSON, 1957, 1960; WHEELER *et al.*, 1958).

By examining these reports we can find the following two interesting problems: 1) the problem of the acquisition of resistance to toxic agents through selection, 2) the problem of cross resistance and collateral sensitivity. First, the insecticides used in these experiments were DDT and its analogues, BHC and cyclodiene derivatives, nitroparaffins and DANP, pyrethrin, organophosphorus compounds, thiocyanate, and carbamates. And all of these investigators have changed the resistance to insecticides by applying the toxicants in more or less lethal doses, that is to say, the test animals were always subjected to some degrees of selection pressure. On the other hand, no report has been found on the tolerance built up by using a sublethal dose repeatedly or continuously, so that the resistance could be considered to be increased through some adaptive changes. Secondly, the results obtained by those investigators were more or less contradicting one another in regard to cross resistance. Namely, in some cases there were clear cross resistance phenomena among various toxicants, whereas in others we could find no phenomenon of this kind.

In this report, the author wishes to mention about the experiments performed to make clear the problems of cross resistance.

Here the author appreciates very much the valuable suggestions and hearty cooperation given by Prof. D. MIYADI, Assist. Prof. S. MORI, Prof. J. ASHIDA, Mr. H. NAKAMURA (Kônan University) and the members of the "Adaptive Variation Research Group".

Experiments

As mentioned in the previous paper, the fly can acquire copper resistance by living only one generation on copper-containing medium, and this copper resistant variant was denoted as the Cu-strain. Whether or not cross resistance or collateral sensitivity might exist between copper sulfate and other chemical reagents was tested, using the Cu-strain.

1. *Fundamental method of experiment.*

Experiments were performed with *Drosophila melanogaster* Oregon RS strain, derived from the standard stock culture in the Genetics Laboratory of Zoological Institute, Kyoto University.

The control medium was PEARL's synthetic medium, 15 ml of which was put in a culture bottle of 50 ml capacity. When the medium cooled down, two drops of yeast suspension were dropped on the surface of the medium. The test media were made in a culture bottle, containing PEARL's synthetic media with various concentrations of toxic agents, mixed immediately after boiling. After it cooled down, two drops of yeast suspension were dropped on.

Twenty flies which had emerged 2 days before were put in oviposition tube. Slide glass with PEARL's synthetic medium on it, on which yeast suspension had been dropped, was inserted into the tube, and the flies were allowed to deposit eggs on it. Newly hatched larvae were put by 20 individuals into a culture bottle containing PEARL's medium (they are called the normal strain in this report).

The 1st instar larvae of the normal strain were transferred to the medium containing 0.5 mM of $CuSO_4$. The adult flies that had emerged from this copper containing medium were transferred to the normal medium for oviposition. The larvae which had hatched from these eggs were the larvae of the Cu-strain.

The larvae of the Cu- and control strains to be tested were transferred to the test media containing 4 mM of $CuSO_4$. Rearing temperature was 25°±0.1°C throughout the experiment. After the formation of pupae, each bottle was examined every 12 hours in order to know developmental rates and the numbers of adults emerged were counted, males and females separately.

2. *Preliminary emergence test.*

With the methods described above, the median emergence dose (ED_{50}), serving as indicator of the average tolerance, was examined in connection with various toxic agents. PEARL's synthetic media containing various concentrations of BHC, NaCl, $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$ were poured into culture bottles, 15 ml to a bottle. After cooling, two drops of yeast suspension were put in a culture bottle and then 20 individuals of the control strain which had newly hatched were transferred to it. After rearing at 25°±0.1°C, the emerged flies were counted, males and females separately.

The experimental results are shown in Tables 1—7. The concentrations of ED_{50} (the median emergence dose) were 0.7 γ/cc for BHC, 15 mM for $MnSO_4$, 5 mM for $NiSO_4$, 20 mM for $ZnSO_4$ and 0.07 mM for $CdSO_4$. In the case of NaCl as shown in Table 2, the emergence-dosage curve went down between 1.2 and 1.25 M so rapidly that it was difficult to decide the median emergence dose

Table 1. Relations between emergence rates and concentrations of BHC. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration γ/cc	No. of larvae	Emerged No.	Emerged % to larvae
0	200 (10)	196	98.0
0.25	200 (10)	176	88.0
0.5	200 (10)	150	75.0
0.7	200 (10)	98	*49.0*
1.0	200 (10)	25	12.5
Total	1000 (50)		

Table 2. Relations between emergence rates and concentrations of NaCl. The concentrations in italics indicate the doses used in the test of resistance.

Concentration M	No. of larvae	Emerged No.	Emerged % to larvae
0	200 (10)	186	93.0
0.5	240 (12)	216	90.0
0.7	240 (12)	214	89.1
0.8	240 (12)	186	77.5
0.9	240 (12)	186	77.5
1.0	240 (12)	172	71.6
1.1	260 (13)	189	*72.6*
1.2	200 (10)	149	*74.5*
1.25	380 (19)	80	*21.0*
1.3	200 (10)	43	21.5
Total	2240 (112)		

Table 3. Relations between emergence rates and concentrations of $MnSO_4$. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration m Mol	No. of larvae	Emerged No.	Emerged % to larvae
0.25	280 (14)	270	96.4
0.5	280 (14)	242	86.4
1.0	280 (14)	254	90.7
1.5	280 (14)	260	92.9
2.0	280 (14)	256	91.4
2.5	280 (14)	261	93.2
3.0	280 (14)	248	88.6
4.0	280 (14)	250	89.3
5.0	280 (14)	242	86.4
7.0	280 (14)	252	90.0
9.0	280 (14)	244	87.1
11	280 (14)	256	91.4
15	280 (14)	148	*52.9*
20	280 (14)	23	8.2
30	280 (14)	3	1.1
40	280 (14)	0	0
Total	4480 (224)		

Table 4. Relations between emergence rates and concentrations of $CoSO_4$. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration m Mol	No. of larvae	Emerged No.	Emerged % to larvae
0.05	280 (14)	242	86.4
0.1	280 (14)	232	82.9
0.15	280 (14)	238	85.0
0.2	280 (14)	214	76.4
0.25	200 (10)	149	74.5
0.5	200 (10)	111	*55.5*
0.75	200 (10)	34	17.0
1.0	200 (10)	19	9.5
1.25	200 (10)	14	7.0
1.5	200 (10)	5	2.5
1.75	200 (10)	2	1.0
2.0	200 (10)	2	1.0
3.0	140 (7)	1	0.7
4.0	140 (7)	0	0
Total	3000 (150)		

Table 5. Relations between emergence rates and concentrations of $NiSO_4$. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration m Mol	No. of larvae	Emerged No.	Emerged % to larvae
0.25	200 (10)	182	91.0
0.5	200 (10)	180	90.0
0.75	200 (10)	188	94.0
1.0	200 (10)	179	89.5
1.25	200 (10)	171	85.5
1.5	200 (10)	171	85.5
1.75	200 (10)	179	89.5
2.0	200 (10)	179	89.5
3.0	200 (10)	167	83.5
4.0	200 (10)	151	75.5
5.0	320 (16)	167	*52.2*
6.0	280 (14)	98	35.0
8.0	200 (10)	16	8.0
10	200 (10)	4	2.0
Total	3000 (150)		

Table 6. Relations between emergence rates and concentrations of $ZnSO_4$. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration m Mol	No. of larvae	Emerged No.	Emerged % to larvae
0.25	200 (10)	192	96.0
0.5	180 (9)	163	90.6
0.75	360 (18)	336	93.3
1.0	200 (10)	188	94.0
1.5	200 (10)	182	91.0
2.0	200 (10)	187	93.5
4.0	200 (10)	175	87.5
6.0	200 (10)	168	84.0
8.0	200 (10)	177	88.5
10	200 (10)	149	74.5
15	280 (14)	230	82.1
20	280 (14)	154	*55.0*
25	280 (14)	90	32.1
30	320 (16)	66	20.6
40	280 (14)	25	8.9
Total	3580 (179)		

Table 7. Relations between emergence rates and concentrations of $CdSO_4$. The concentration in italics indicates the dose used in the test of resistance as 50% emergence dose (ED_{50}).

Concentration m Mol	No. of larvae	Emerged No.	Emerged % to larvae
0.007	200 (10)	190	95.0
0.05	220 (11)	181	82.3
0.07	200 (10)	106	*53.0*
0.1	200 (10)	50	25.0
0.15	200 (10)	21	10.5
0.2	200 (10)	13	6.5
0.4	200 (10)	3	1.5
Total	1420 (71)		

exactly; accordingly the author prepared to use three kinds of NaCl concentrations (1.1, 1.2 and 1.25 M) as the doses of emergence test.

3. *Larval resistance to various kinds of toxic agents (Emergence test).*

Method: Each test medium contained the median emergence dose of the toxic agent as mentioned above. Fifteen ml of each medium was poured into a culture bottle. Two drops of yeast suspension were dropped on the surface of the test medium in the bottle. Numbers of bottles used are shown in Tables 8 and 9. The larvae of the Cu- and control strains were transferred to the test media and the emergence rates were measured.

Results: The first experiments were performed in 1958, the test media employed being BHC, NaCl, $CuSO_4$ and $CdSO_4$, and the next ones were made in 1960, using the media containing various kinds of bivalent metallic salts

Table 8. Results of the emergence test performed in 1958 on the media containing 4 mM of $CuSO_4$, 0.07 mM of $CdSO_4$, 1.1, 1.2 and 1.25 M of NaCl and 0.7 γ/cc of BHC. The 1st instar larvae hatched from eggs deposited by the flies, which had spent their larval period in 0.5 mM of $CuSO_4$-containing medium and in the normal PEARL's medium, were put in the above test media and the emergence rates were examined. Except for the case of NaCl, the experiments were repeated twice or thrice as for each toxicant. Numbers in parentheses show numbers of bottles used.

Strain	Control strain					Cu-strain				
Agents	No. of larvae	Pupated		Emerged		No. of larvae	Pupated		Emerged	
		No.	%	No.	%		No.	%	No.	%
	200 (10)	120	60.0	112	56.0	200 (10)	140	70.0	137	68.5
$CuSO_4$	200 (10)	110	55.0	110	55.0	200 (10)	142	71.0	135	67.5
	200 (10)	111	55.5	97	48.5	200 (10)	123	61.5	123	61.5
Total	600 (30)	341	56.8	319	53.1	600 (30)	405	67.5	395	65.8
BHC	300 (15)	198	66.0	190	63.3	300 (15)	198	66.0	193	64.3
	300 (15)	147	49.0	147	49.0	300 (15)	142	47.3	142	47.3
Total	600 (30)	345	57.5	337	56.1	600 (30)	340	56.6	335	55.8
NaCl 1.1 M	280 (14)	214	76.4	206	73.6	280 (14)	216	77.1	210	75.0
NaCl 1.2 M	400 (20)	298	74.5	298	74.5	400 (20)	298	74.5	298	74.5
NaCl 1.25 M	400 (20)	92	23.0	84	21.0	400 (20)	96	24.0	91	22.8
Total	1080 (54)					1080 (54)				
	200 (10)	173	86.5	142	71.0	200 (10)	164	82.0	138	69.0
$CdSO_4$	240 (12)	190	79.1	136	56.6	240 (12)	180	75.0	130	54.2
	280 (14)	196	70.0	143	51.1	280 (14)	212	75.7	132	47.1
Total	720 (36)	559	77.6	421	58.4	720 (36)	556	77.2	400	55.6

Examinations of differences between two strains (χ^2-test).

		Pupation	Emergence
$CuSO_4$		$0.001 > \alpha$*	$0.001 > \alpha$*
BHC		$0.90 > \alpha > 0.80$	$0.98 > \alpha > 0.95$
NaCl	1.1 M	$0.70 > \alpha > 0.50$	$0.70 > \alpha > 0.50$
	1.25 M	$0.70 > \alpha > 0.50$	$0.70 > \alpha > 0.50$
$CdSO_4$		$\alpha = 0.90$	$0.50 > \alpha > 0.30$

* Significant.

Table 9. The results of similar experiments as Table 8, using various kinds of toxic agents, performed in 1960.

Strain	Control strain					Cu-strain				
Test medium	No. of larvae	Pupated No.	Pupated %	Emerged No.	Emerged %	No. of larvae	Pupated No.	Pupated %	Emerged No.	Emerged %
$CuSO_4$	200 (10)			112	56.0	200 (10)			137	68.5
	200 (10)			110	55.0	200 (10)			133	66.5
	200 (10)			97	48.5	200 (10)			127	63.5
	280 (14)			163	58.2	280 (14)			175	62.5
	280 (14)			123	43.9	280 (14)			176	62.8
Total	1160 (58)			605	52.1	1160 (58)			748	64.4
$MnSO_4$	320 (16)	238	74.2	169	52.9	320 (16)	244	76.2	170	53.1
	200 (10)	142	71.0	94	47.0	200 (10)	148	74.0	120	60.0
	280 (14)	216	77.1	148	52.8	280 (14)	240	85.7	148	52.8
	280 (14)	220	78.5	149	53.0	280 (14)	212	75.7	162	57.8
Total	1080 (54)	816	75.5	560	51.8	1080 (54)	844	78.1	600	55.5
$CoSO_4$	200 (10)	124	62.0	104	52.0	200 (10)	102	51.0	97	48.5
	400 (20)	230	57.5	220	55.0	450 (20)	172	43.0	165	41.2
	400 (20)	224	56.0	209	52.2	400 (20)	181	45.2	169	42.2
	200 (10)			142	71.0	200 (10)			122	61.0
	160 (8)			117	65.0	160 (8)			99	61.8
Total	1360 (68)			792	58.2	1360 (68)			652	47.9
$NiSO_4$	200 (10)			98	49.0	200 (10)			114	57.0
	200 (10)			92	46.0	200 (10)			110	55.0
	200 (10)			95	47.5	200 (10)			112	56.0
	200 (10)			89	44.5	200 (10)			117	58.5
	200 (10)			93	46.5	200 (10)			112	56.0
Total	1000 (50)			467	46.7	1000 (50)			565	56.5
$ZnSO_4$	200 (10)			96	48.0	200 (10)			92	46.0
	200 (10)			110	55.0	200 (10)			115	57.5
	280 (14)			144	51.4	280 (14)			174	62.1
	200 (10)			122	61.0	200 (10)			124	62.0
Total	880 (44)			472	53.6	880 (44)			505	57.3
$CdSO_4$	200 (10)	130	65.0	112	56.0	200 (10)	143	71.5	116	58.0
	200 (10)	141	70.5	120	60.0	200 (10)	139	69.5	128	64.0
	200 (10)	142	71.0	121	60.5	200 (10)	154	77.0	111	55.5
	200 (10)	159	79.5	142	71.0	200 (10)	164	82.0	139	69.5
	280 (14)	196	70.0	144	51.4	280 (14)	212	75.7	132	47.1
Total	1080 (54)	768	71.1	639	59.1	1080 (54)	812	75.1	626	57.9

Examinations of differences between two strains (χ^2-test).

$CuSO_4$	$0.001 > \alpha$*
$MnSO_4$	$0.10 > \alpha > 0.05$
$CoSO_4$	$0.001 > \alpha$*
$NiSO_4$	$0.001 > \alpha$*
$ZnSO_4$	$0.10 > \alpha > 0.05$
$CdSO_4$	$0.70 > \alpha > 0.50$

* Significant.

such as $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$. The results in 1958 are shown in Table 8 and those in 1960 in Table 9.

It is understood from Table 8 that, normally, the Cu-strain showed higher pupation and emergence rates in $CuSO_4$ test medium than the control strain. The differences are statistically highly significant ($0.001 > \alpha$).

The experimental results with $CdSO_4$ showed no difference in pupation rate between the two strains ($\alpha = 0.90$). But the results of the emergence test were lower in the Cu-strain than in the control strain, although the statistical examination showed no significant difference ($0.50 > \alpha > 0.30$). This difference, however, seemed to have some biological meaning, because the same tendency was always found in the repeated experiments.

Then the experiments were conducted with three kinds of concentrations of NaCl; and it was found that there were no significant differences in pupation ($0.70 > \alpha > 0.50$) and emergence rates between the two strains ($0.70 > \alpha > 0.50$). The same result could be obtained by the experiments with BHC test medium. No difference was observed (Pupation $0.90 > \alpha > 0.80$, Emergence $0.98 > \alpha > 0.95$).

In short, the Cu-resistance acquired as mentioned above did not show the cross resistance to BHC and NaCl, but it was considered to have some kinds of resistance to $CdSO_4$ (the same bivalent metallic salts with $CuSO_4$).

In the next series of experiments (1960), the author has studied about cross resistance phenomena of the copper resistant variant to some bivalent matallic salts than those used in the former series of experiments. The experimental results are shown in Table 9. The emergence rate of the Cu-strain is lower than that of the control strain on the $CoSO_4$ test medium, though higher on the $NiSO_4$ test medium. In both cases, the differences are highly significant statistically ($0.001 > \alpha$). And there are no statistically significant differences in emergence rates between the two strains on the $MnSO_4$, $ZnSO_4$ and $CdSO_4$ test media ($MnSO_4$ $0.10 > \alpha > 0.05$, $ZnSO_4$ $0.10 > \alpha > 0.05$, $CdSO_4$ $0.70 > \alpha > 0.50$).

It is very interesting to note that in comparison with the pupation rates on both $MnSO_4$ and $CdSO_4$ test media, the emergence rates are remarkably lower, the cause of which is attributable to the failure of ecdysis.

The copper resistance of the Cu-strain is thought to be a result of some physiological changes caused by copper during its parent generation. This acquired resistance observed in the Cu-strain is not rigidly specific to copper. The Cu-strain is more resistant to $NiSO_4$ and less resistant to $CoSO_4$ than the normal strain. It is interesting that there exist the relations of cross resistance and collateral sensitivity among bivalent metallic salts.

4. *Developmental rate.*

When the emergence test was performed, it was noticed that some differences in the developmental time existed between the normal and Cu-strains, namely the developmental rate was delayed or accelerated on each test medium. Acceleration or retardation in the developmental rate may be taken as one of

Table 10. Results of the test of developmental rate of Cu- and normal strains in the various kinds of toxic agents, performed in 1958. The test with BHC repeated twice, represented by I and II. Figures show average periods.

Medium	Control strain				Cu-strain			
	Average periods from hatching to				Average periods from hatching to			
	Pupation		Emergence		Pupation		Emergence	
	days	s.d.	days	s.d.	days	s.d.	days	s.d.
$CuSO_4$	9.33	0.64	13.33	0.37	7.65	0.34	12.05	0.32
BHC I	6.34	0.21	9.95	0.21	5.53	0.39	9.99	0.29
II	5.97	0.46	9.85	0.62	6.04	0.57	9.18	0.75
NaCl 1.1 M	5.45	0.18	9.68	0.14	5.36	0.31	9.65	0.15
1.25 M	6.86	0.47	10.60	0.67	6.95	0.46	10.70	0.40
$CdSO_4$	8.18	0.30	12.35	0.17	7.70	0.28	11.86	0.10
Control	4.66	0.05	8.72	0.11	4.56	0.08	8.64	0.10

Examinations of differences between two strains (t-test).

	Pupation	Emergence
$CuSO_4$	$0.01 > \alpha > 0.001$*	$0.001 > \alpha$*
BHC I	$0.001 > \alpha$*	—
II	—	$0.02 > \alpha > 0.01$*
NaCl 1.1 M	$0.5 > \alpha > 0.4$	—
1.25 M	$0.6 > \alpha > 0.5$	$0.6 > \alpha > 0.5$
$CdSO_4$	$0.02 > \alpha > 0.01$*	$0.01 > \alpha > 0.001$*
Control	$0.01 > \alpha > 0.001$*	$0.7 > \alpha > 0.6$

* Significant.

Table 11. The results of similar experiments as shown in Table 10, performed in 1960. Developmental rates of the Cu- and normal strains on the media containing various kinds of metallic salts.

Medium	Control strain				Cu-strain			
	Average periods from hatching to				Average periods from hatching to			
	Pupation		Emergence		Pupation		Emergence	
	days	s.d.	days	s.d.	days	s.d.	days	s.d.
$CuSO_4$	8.19	(0.63)	13.33	(3.04)	7.20	(0.73)	12.16	(0.42)
$MnSO_4$	5.33	(0.54)	8.18	(0.48)	5.37	(0.61)	8.22	(0.56)
$CoSO_4$	9.81	(1.12)	13.97	(1.52)	9.97	(0.99)	14.37	(1.85)
$NiSO_4$	5.55	(0.68)	9.09	(0.68)	4.87	(0.55)	9.01	(0.13)
$ZnSO_4$	7.40	(0.99)	10.45	(1.51)	7.63	(1.14)	11.83	(2.40)
$CdSO_4$	9.54	(0.97)	14.78	(1.05)	9.21	(0.88)	13.37	(0.78)
Control	4.65	(0.05)	8.68	(0.11)	4.55	(0.08)	8.65	(0.12)

Examinations of differences between two strains (t-test).

	Pupation	Emergence
$CuSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*
$MnSO_4$	$0.40 > \alpha > 0.30$	$0.70 > \alpha > 0.60$
$CoSO_4$	$0.30 > \alpha > 0.20$	$0.10 > \alpha > 0.05$
$NiSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*
$ZnSO_4$	$0.10 > \alpha > 0.05$	$0.001 > \alpha$*
$CdSO_4$	$0.01 > \alpha > 0.001$*	$0.01 > \alpha > 0.001$*

* Significant.

the indices showing the degree of resistance to toxic agents.

Method: The same method as adopted in the first report (YANAGISHIMA and SUZUKI, 1959 a) was used.

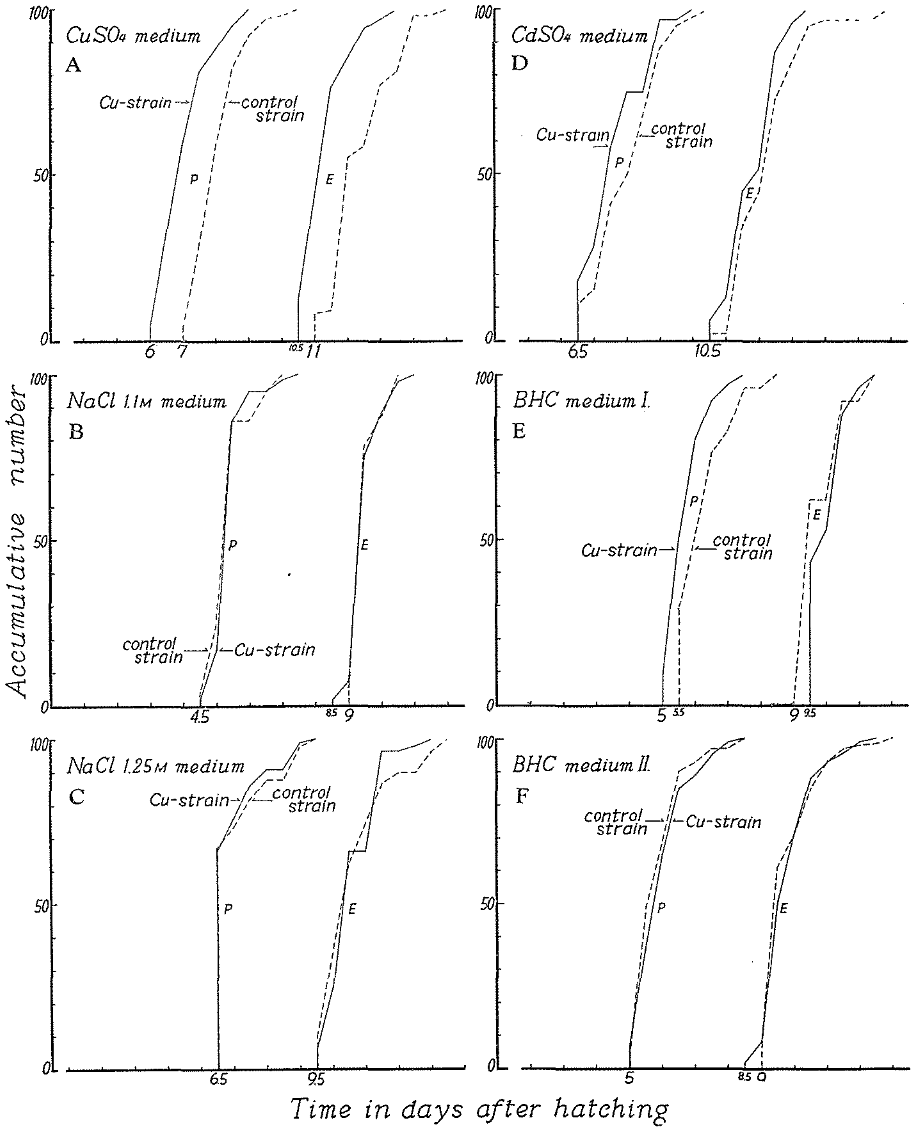

Fig. 1. Developmental rates of the Cu- and control strains on the media containing BHC (0.7γ/cc), NaCl (1.1 and 1.25 M), $CuSO_4$ (4 mM), and $CdSO_4$ (0.07 mM). P: pupation. E: emergence. The experiments were performed in 1958.

Results: The experimental results are shown in Tables 10—11 and Figs. 1—2. There are two cases: one is that the Cu-strain developed faster than the control strain, and the other is that the Cu-strain developed more slowly than the control strain.

As for the larval period, the Cu-strain developed faster than the control strain on $CuSO_4$, $NiSO_4$ and $CdSO_4$ test media, and these differences were highly significant ($CuSO_4$ $0.001 > \alpha$, $NiSO_4$ $0.001 > \alpha$, $CdSO_4$ $0.01 > \alpha > 0.001$). No

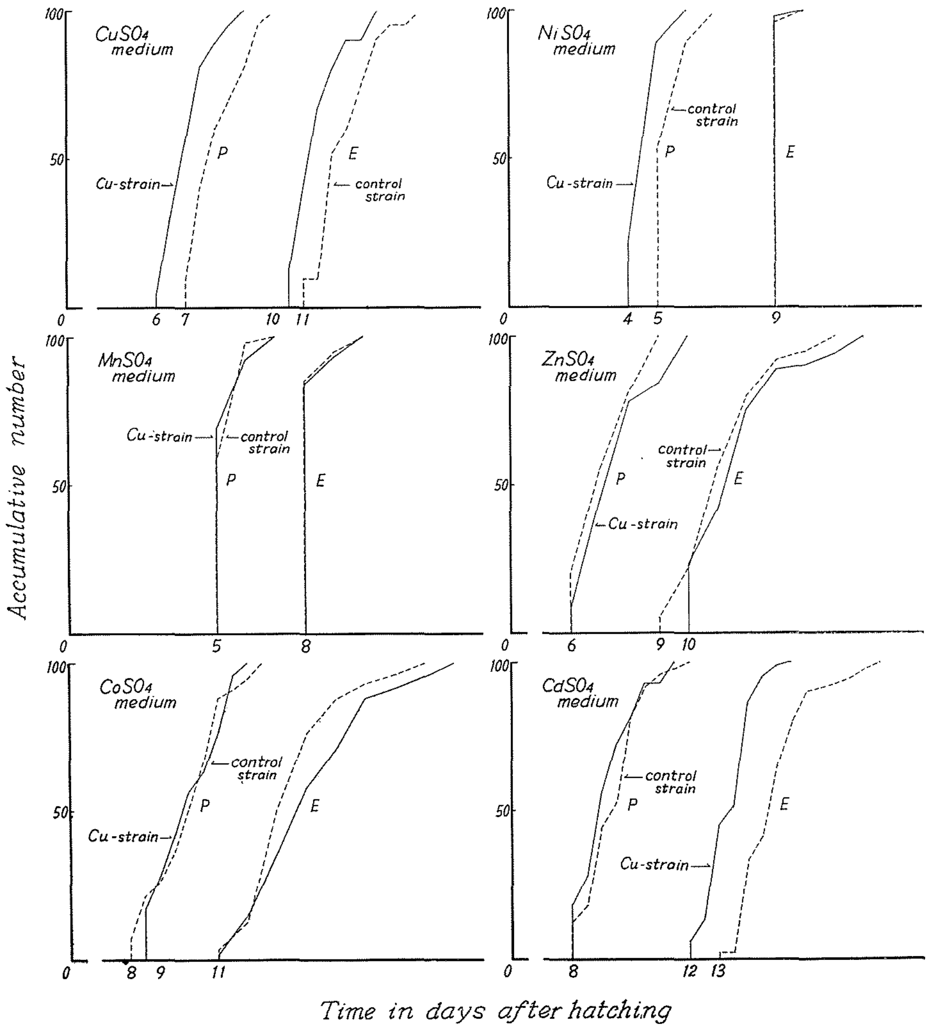

Fig. 2. The results of the similar experiments as Fig. 1, performed in 1960. $MnSO_4$ (15 mM), $CoSO_4$ (0.5 mM), $NiSO_4$ (5 mM), $ZnSO_4$ (20 mM), $CdSO_4$ (0.07 mM).

significant difference was seen in the time of larval development between the Cu- and control strains on the other media except on BHC.

The experiments with BHC test medium were repeated four times, in all of which two instances are cited in Table 10. The results were sorted into two kinds. In the first kind, the larval period of the Cu-strain was shorter than the control strain, whereas the period from hatching to emergence was not different. The statistical examination of the first experiment showed that the differences were highly significant only in relation to the larval period between the two strains (I $0.001 > \alpha$). In the second kind, the period from hatching to emergence was shorter in the Cu-strain than in the control strain, whereas the larval period was not different. Difference, therefore, was statistically significant in the period from hatching to emergence (II $0.02 > \alpha > 0.01$). After all, the different data were obtained from two experiments. Too scarce observation, being conducted only every 12 hours, may be the cause of this phenomenon, and more frequent observations must be carried out in future. But it must clearly be noticed here that, in both cases, the Cu-strain grew up earlier than the control strain.

After all, it can be said from the present experimental results that the Cu-strain develops faster than the control one not only on $CuSO_4$ medium but also on $NiSO_4$, $CdSO_4$ and BHC media; but its slow development at the pupal stage results in a slowdown of development as a whole on $ZnSO_4$ medium. The relation of these results to the degree of resistance will be discussed later on.

5. *Body length.*

Body length measurements were made on the flies of one day old after emergence from various test media. The comparisons of these measurements will show the metabolic differences and so will be able to take as one of the indices representing the degree of resistance to various toxic agents. The results of the measurements are shown in Tables 12 and 13.

In the case of male flies, the Cu-strain was smaller than the control strain on $MnSO_4$ and $ZnSO_4$ test media but larger on $NiSO_4$ test medium and control medium. In the case of female flies, the Cu-strain was smaller than the control strain on $ZnSO_4$ test medium, but larger on $CoSO_4$ and $NiSO_4$ test media. On the other media tested, no statistically significant difference in body length of emerged flies was observed between the two strains.

Putting together these results concerning both male and female flies it can be said that the Cu-strain grows larger than the control strain on $NiSO_4$ test medium, but the result is reversal on $ZnSO_4$ test medium. And then the difference was seen only in male flies on $MnSO_4$ test medium or in female flies on $CoSO_4$ test medium. No significant difference was observed in body length between the two strains on the other media tested.

Table 12. Body lengths of the Cu- and control strains cultured on the media containing $CuSO_4$, BHC, NaCl and $CdSO_4$. The results of the experiments performed in 1958. N: numbers of adults used in the measurement. $\bar{x}$: mean values. s.d.: standard deviations.

Medium	Control strain						Cu-strain					
	♂ mm			♀ mm			♂ mm			♀ mm		
	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.
$CuSO_4$	46	2.61	0.10	72	3.01	0.12	52	2.59	0.08	80	3.02	0.17
BHC	62	2.60	0.13	68	3.04	0.16	48	2.60	0.09	62	3.00	0.15
NaCl 1.1M	68	2.67	0.90	76	3.03	0.15	49	2.71	0.09	63	3.06	0.10
$CdSO_4$	28	2.41	0.14	26	2.45	0.08	30	2.39	0.15	22	2.52	0.17
Control	80	2.69	0.09	70	3.09	0.12	80	2.73	0.07	85	3.11	0.18

Examinations of differences in body lengths (t-test).

	♂	♀
Control	$0.05 > \alpha > 0.02$*	$0.6 > \alpha > 0.5$
$CuSO_4$	$0.7 > \alpha > 0.6$	$0.4 > \alpha > 0.3$
BHC	$0.9 > \alpha > 0.8$	$0.2 > \alpha > 0.1$
NaCl (1.1M)	$0.3 > \alpha > 0.2$	$0.3 > \alpha > 0.2$
$CdSO_4$	$0.6 > \alpha > 0.5$	$0.2 > \alpha > 0.1$

* Significant.

Table 13. Body lengths of the Cu- and control strains cultured on the media containing various kinds of toxic agents. The results of similar experiments using various kinds of toxic agents, performed in 1960.

Medium	Control strain						Cu-strain					
	♂ mm			♀ mm			♂ mm			♀ mm		
	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.
Control	78	2.69	0.10	65	3.01	0.12	89	2.72	0.11	80	3.10	0.14
$CuSO_4$	92	2.60	0.10	140	3.00	0.12	104	2.59	0.08	80	3.22	0.17
$MnSO_4$	70	2.58	0.07	54	2.84	0.12	96	2.50	0.12	50	2.85	0.11
$CoSO_4$	118	2.28	0.13	106	2.50	0.16	60	2.27	0.12	96	2.69	0.08
$NiSO_4$	71	2.50	0.08	87	2.95	0.14	86	2.55	0.12	136	2.99	0.09
$ZnSO_4$	112	2.44	0.14	78	2.59	0.15	119	2.35	0.16	47	2.51	0.03
$CdSO_4$	56	2.41	0.14	26	2.45	0.08	60	2.39	0.15	44	2.52	0.17

Examinations of differences in body lengths (t-test).

	♂	♀
Control	$0.05 > \alpha > 0.02$*	$0.6 > \alpha > 0.5$
$CuSO_4$	$0.7 > \alpha > 0.6$	$0.4 > \alpha > 0.3$
$MnSO_4$	$0.001 > \alpha$*	$0.2 > \alpha > 0.1$
$CoSO_4$	$0.7 > \alpha > 0.6$	$0.001 > \alpha$*
$NiSO_4$	$0.01 > \alpha > 0.001$*	$0.01 > \alpha > 0.001$*
$ZnSO_4$	$0.001 > \alpha$*	$0.01 > \alpha > 0.001$*
$CdSO_4$	$0.6 > \alpha > 0.5$	$0.2 > \alpha > 0.1$

* Significant.

Discussion

As described above, the Cu-strain, whose parents had been cultured in 0.5 mM $CuSO_4$-containing medium during their larval period, did not show the cross resistance to BHC and NaCl media, though it showed the cross resistance or collateral sensitivity to some kinds of bivalent metallic salts. Now let us examine some literatures.

CROW (1954) reported that the DDT-tolerant strains of housefly, which had been cultured in DDT medium for one year, could strengthen tolerance not only in regard to DDT medium but also to gamma-BHC, methoxychlor, aldrin and toxaphene; but no change in tolerance could be shown to non-chlorinated insecticides (paration, nicotine and pyrethrine). KIKKAWA (1953), TSUKAMOTO (1955), TSUKAMOTO *et al.* (1957) obtained the same results using the DDT-tolerant strain collected in the field. BARTLETT (1952) showed that there was some relation between DDT-tolerance and, DFDT, HCN and tartar emetic-tolerance.

BRUCE (1950), as well as DECKER and BRUCE (1952), showed that the resistant strain of housefly developed in DDT and methoxychlor pressure had higher tolerance than the non-resistant ones to the same media, but that it had little tolerance to gamma-BHC, chlordane and dieldrin media. On the other hand, the resistant strain produced by subjecting to the selection pressures such as gamma-BHC, chlordane and dieldrin had little tolerance to DDT and methoxychlor, and higher tolerance to gamma-BHC, chlordane and dieldrin than the non-tolerant ones. Judging from the data described above, it can be understood that BHC and chlordane resistances were independent of DDT resistance.

According to the data obtained by METCALF (1955), the resistant strain induced by DDT and its analogous compound had a little resistance to BHC and cyclodiene derivatives. MARCH (1952) and PIMENTEL *et al.* (1953) reported that the BHC-resistant strain cultured in gamma-BHC could acquire some cross resistance against DDT. MARCH *et al.* (1956) and MELTZER (1956) showed that the resistant strain treated with insecticides could show some resistance not only to the same insecticides but also to the other toxicants.

The examples of collateral sensitivity are found in the reports made by BARTLETT (1952), ASCHER and KOCHER (1954) and BOCCACCI and BETTINI (1956). BARTLETT (1952) reported that the HCN-tolerant strain produced under HCN-pressure was susceptible to DDT and tartar emetic. ASCHER and KOCHER (1954) and ASCHER (1957) showed that DDT-resistant strain of housefly produced by DDT treatment was susceptible to potassium bromide medium.

As shown above, many reports have treated phenomena of cross resistance or collateral sensitivity among various kinds of insecticides; but the author may as well add here that we have not yet found coincidence and definite correlation among the results obtained. This conclusion is also applicable to the case of the Cu-strain and we can not find any definite correlation through the whole results obtained with various metallic salts.

In the cases of $CdSO_4$ and $MnSO_4$ test media, it is conspicuous that the emergence rate was found to be very low in comparison with pupation rate. This low emergence rate was due to the frequent occurrence of the abnormal pupae that could not emerge. Considerable researches have been done on the abnormal pupae found in toxic medium. UENO and MATSUYAMA (1956) reported that the pupae of houseflies, which had been cultured in lindane medium during the 3rd larval instar, became abnormal and the emergence rate decreased than the control strain. In this case, the abnormal pupae were different in form from the normal pupae. But the present results are rather different, i.e., the form of the abnormal pupae was not different from the normal one.

Concerning the developmental rate of the housefly, we can find some reports which show the existence of more or less difference between the DDT-resistant strain and the normal strain. Some authors reported that the former had longer larval period than the latter (PERRY *et al.*, 1951; PIMENTEL *et al.*, 1951; McKENZIE *et al.*, 1954; BOCHNIG, 1956). The different data, on the other hand, could be found in the reports (LA FACE, 1948; NORTON, 1953), which had shown that the larval period of the DDT-resistant strain was shortened than the non-resistant strain. It has been found by BARBER *et al.* (1949), MARCH and LEWELLEN (1950) and UENO and MATSUYAMA (1956) that there is no relationship between the resistance and length of life cycle in housefly. As for *Drosophila melanogaster*, HUNTER (1956) showed that the larval period was longer in the DDT-resistant strain than in the susceptible one, and TAMURA (1958) reported that there was no significant difference in the length of the larval and pupal periods when comparison was made between the parathion-resistant strain and non-resistant one. GREIFF (1943) mentioned that there was no significant difference in the developmental rate between the line selected for $ZnSO_4$ and the control one in *D. melanogaster*.

In the result obtained by the present author, the copper resistant strain (Cu-strain) developed faster than the control strain on the $CuSO_4$ test medium. And this tendency of shortening the life cycle was observed, when the Cu-strain was cultured on the $NiSO_4$, $CdSO_4$ and BHC test media, but the reverse tendency was seen on $ZnSO_4$ test medium. As stated above, there is no general relationship between the resistance and length of life cycle of *Drosophila* as seen in the housefly.

The reports on the change of body weight of the resistant strain have been given by GREIFF (1943), PIMENTEL *et al.* (1951), BOCHNIG (1956) and SUZUKI and TÔYAMA (1956). GREIFF (1943) has shown that the body weight of imago in $ZnSO_4$ selected line is smaller than that of the control line in *D. melanogaster*. On the other hand, BOCHNIG (1956) showed that in houseflies the body weight of the DDT-tolerant strain was heavier than that of the non-tolerant one. PIMENTEL *et al.* (1951) and SUZUKI and TÔYAMA (1956) found no difference in the adult body size between the DDT-tolerant strain and the non-tolerant strain.

As for body length, the Cu-strain becomes larger than the control one on $NiSO_4$ test medium, but the results are reversed on $ZnSO_4$ test medium (in the case of female) and smaller on $MnSO_4$ medium (in the case of male). The Cu-strain showed no difference in the body length from that of the control strain both on $CuSO_4$ and $CdSO_4$ test media. And the Cu-strain had the same tendency on $CuSO_4$ test medium as that shown by PIMENTEL *et al.* (1951) and the body length of males of the Cu-strain was found to be larger than the control strain on the control medium.

Now let us consider the relations among body length, developmental rate and larval tolerance to toxicants. The data obtained in the present investigation are summarized in Table 14. The Cu-strain showed higher emergence

Table 14. Summarizing table showing relations among emergence rate, developmental rate and body length on various toxic agents, which were seen between the Cu- and the control strains.

Test medium	Emergence rate (higher > lower)	Developmental rate from hatch till emergence (faster > slower)	Body length (larger > smaller)	
			♂	♀
Control	Cu > cont.	Cu > cont.	Cu > cont.	n. no dif.
$CuSO_4$ (4 mM)	Cu > cont.	Cu > cont.	n. no dif.	n. no dif.
$CdSO_4$ (0.07 mM)	insignif. (Cu < cont.)	Cu > cont.	n. no dif.	insignif. (Cu > cont.)
$MnSO_4$ (15 mM)	insignif. (Cu > cont.)	n. no dif.	Cu < cont.	n. no dif.
$CoSO_4$ (0.5 mM)	Cu < cont.	insignif. (Cu < cont.)	n. no dif.	Cu > cont.
$NiSO_4$ (5 mM)	Cu > cont.	Cu > cont.	Cu > cont.	Cu > cont.
$ZnSO_4$ (20 mM)	insignif. (Cu > cont.)	Cu < cont.	Cu < cont.	Cu < cont.
NaCl (1.25 M)	n. no dif.	n. no dif.	n. no dif.	n. no dif.
BHC (0.7γ/cc)	n. no dif.	Cu > cont.	n. no dif.	insignif. (Cu < cont.)

Cu : Cu-strain. Cont.: Control strain.
n. no dif.: nearly no difference is seen between both strains.
> or <: difference is seen at less than 5% significance level.
insignif.: a fair difference is seen but no significant statistically.

rate and faster development than the control strain but no difference in body length was seen on $CuSO_4$ test medium. In the case of $CdSO_4$ test medium, the Cu-strain developed faster than the control strain but no significant difference in emergence rate and body length was found between the two strains. On the contrary, in the case of $MnSO_4$ test medium, though there was no statistically significant difference in emergence rate and developmental rate between the two strains, the male flies of the Cu-strain had smaller bodies than the control strain. The Cu-strain showed lower emergence rate than the control strain on $CoSO_4$ test medium, but no difference in developmental rate was seen between the two. In the case of $NiSO_4$ test medium, the Cu-strain emerged in higher rate and developed faster than the control strain, and both male and female flies of the former type had larger bodies. On $ZnSO_4$ test medium, the Cu-strain developed more slowly and had smaller bodies than the control, but there was no difference in emergence rate between the two strains.

Judging from the results mentioned above, some interesting relations between developmental rate and body length will be brought forth. Namely, we can recognize the following 4 types. First of all, $NiSO_4$ medium type, in which the Cu-strain develops faster than the control strain and the bodies of the flies of former type become larger than those of the latter. Secondly, $ZnSO_4$ medium type, in which the slow development of the Cu-strain results in small body length. Thirdly, $CuSO_4$ and $CdSO_4$ media type, in which developmental rate is faster in the Cu-strain but body length does not become smaller. Fourthly, $MnSO_4$ and $CoSO_4$ media type, in which no difference in developmental rate can be observed between the two strains, though the body length of male or female becomes larger or smaller respectively.

Then, the author wishes to consider whether there are any correlations among developmental rate, body length and emergence rate. When examining Table 14, we can find two toxic agents which show a conspicuous contrast, that is, $NiSO_4$ and $ZnSO_4$. On $NiSO_4$ medium, the Cu-strain is shorter in developmental period and larger in body length than the control strain, and these characteristics are quite consistent with the higher resistant character to copper of the Cu-strain. Namely, in this case, the Cu-strain manifests all-round higher vitality. On the other hand, the circumstances are a little more complicated in the case of $ZnSO_4$, but the general tendency reveals somewhat opposite trend to the case of $NiSO_4$, and also it shows that the developmental rate is slower and the body lengths of both sexes are smaller in the Cu-strain as mentioned previously; nevertheless the emergence rate is little higher in the Cu-strain. As for other toxic agents than these two agents, the matters are much complicated, and we can not draw any consistent conclusion through their data.

Summary

When the larvae of *Drosophila melanogaster* Oregon RS strain were cultured on PEARL's medium containing 0.5 mM of $CuSO_4$ which was sublethal dose to the fly, the larvae of the next generation became tolerant to copper. This copper resistant strain was denoted as the Cu-strain.

The experiments were conducted so as to see whether the Cu-resistance thus acquired through perhaps some adaptive changes in physiological processes could show cross resistance or collateral sensitivity to other toxic agents, such as BHC, NaCl, $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$.

The main results described in this paper are as follows:

1. When the larvae were put into NaCl or BHC test media, no difference in emergence rate was found between the Cu- and control strains. However, the differences in the emergence rates between the two strains were significantly seen on the media containing other bivalent metallic salts. As a rule, the Cu-strain showed cross resistance to such bivalent metallic salts as $NiSO_4$, $MnSO_4$ and $ZnSO_4$, and collateral sensitivity to $CoSO_4$ and $CdSO_4$.

2. The Cu-strain developed faster than the control strain on the copper medium. The same tendency of fast development was also observed on the media containing BHC, $NiSO_4$ and $CdSO_4$. On the contrary, the Cu-strain developed more slowly than the control strain on $ZnSO_4$ test medium.

3. The body length of the Cu-strain was larger than the control strain on $NiSO_4$ test medium, but smaller on $ZnSO_4$, in both cases regardless of sex. In the case of $MnSO_4$ or $CoSO_4$ test medium, the difference was seen either in male or in female flies respectively.

4. Significant correlation was found among the larval tolerance for toxic agent, developmental time and body length, in the case of $NiSO_4$ test medium; but these correlations were not found in the cases of other test media.

References

ASCHER, K. R. S., 1957. Riv. Parassit., **18**: 113.
——— & C. KOCHER, 1954. Experientia, **10**: 465.
BARBER, G. W., & J. B. SCHMITT, 1949 a. J. econ. Ent., **42**: 287.
——— & ——— 1949 b. Ibid., **42**: 844.
BARTLETT, B. R., 1952. Meis. Canad. Ent., **84**: 189.
BOCCACCI, M., & S. BETTINI, 1956. Sanita., **19**: 1239.
BOCHNIG, V., 1956. Z. indukt. Abstamm.— u. Vererblehre., **87**: 694.
BROWN, A. W. A., 1956. Bull. Wld. Hlth. Org., **14**: 807.
——— & A. S. PERRY, 1956. Nature (Lond.), **178**: 368.
BRUCE, W. N., 1950. Pest Control, **18**: 9.
——— & G. C. DECKER, 1950. Soap, **26**: 122, 145.
BUSVINE, J. R., 1951. Nature (Lond.), **168**: 193.
——— 1953. Ibid., **171**: 118.
——— 1956. Ibid., **177**: 533.
——— 1957. Bull. Wld. Hlth. Org., **16**: 205.
CROW, J. F., 1954. J. econ. Ent., **47**: 393.
——— 1957. Ann. Rev. Ent., **2**: 227.
DECKER, G. C., & W. N. BRUCE, 1952. Amer. J. trop. Med. Hyg., **1**: 395.
GREIFF, D., 1940. Am. Nat., **74**: 363.
——— 1943. Ibid., **77**: 426.
HARRISON, C. M., 1951. Nature (Lond.), **167**: 855.
HUNTER, P. E., 1956: J. econ. Ent., **49**: 671.
KEIDING, J., & H. VAN DEURS, 1949. Nature (Lond.), **163**: 964.
KIKKAWA, H., 1953. Jap. J. Genet., **28**: 171.

KING, J. C., 1954. J. econ. Ent., **47**: 387.
LA BRECQUE, G. C., & H. G. WILSON, 1957. Agric. Chemic., **12**: 46.
——— & ——— 1960. J. econ. Ent., **53**: 320.
LA FACE, L., 1948. Riv. Parassit., **9**: 199.
McKENZIE, R. E., & W. M. HOSKINS, 1954. J. econ. Ent., **47**: 984.
MARCH, R. B., 1952. National Research Council Publication, No. 219.
——— & L. L. LEWALLEN, 1950. J. econ. Ent., **43**: 722.
——— & ——— 1956. Ann. ent. Soc. Amer., **49**: 371.
——— & R. L. METCALF, 1949. J. econ. Ent., **42**: 990.
MARIANI, M., 1953. Boll. Soc. Ital. Biol. Sper., **29**: 1774.
MELTZER, J., 1956. Med. Rijks. Opzoek. Staat Gent., **31**: 459.
METCALF, R. L., 1955. Physiol. Rev., **35**: 197.
MORRISON, F. O., 1957. J. econ. Ent., **50**: 554.
MORRISON, H. E., *et al.*, 1950. Ibid., **43**: 846.
NORTON, R. J., 1953. Contrib. Boyce-Thompson Inst., **17**: 105.
PERRY, A. S., R. W. FAY & A. J. BUCKNER, 1953. J. econ. Ent., **46**: 972.
——— & W. M. HOSKINS, 1950. Science, **111**: 600.
——— & ——— 1951. J. econ. Ent., **44**: 850.
PIMENTEL, D., & J. E. DEWEY, 1950. Ibid., **43**: 105.
———, ——— & H. H. SCHWARDT, 1951. Ibid., **44**: 477.
———, ——— & ——— 1953. Ibid., **46**: 295.
SUZUKI, T., & T. TÔYAMA, 1956. Botyu-Kagaku, **21**: 43.
TAMURA, S., 1958. Jap. J. Genet., **33**: 192.
TSUKAMOTO, M., 1955. Botyu-Kagaku, **20**: 73.
——— & T. HIROYOSHI, 1956. Ibid., **21**: 71.
———, M. OGAKI & H. KOBAYASHI, 1957. Jap. J. san. Zool., **8**: 118.
UENO, H., & Y. MATSUYAMA, 1956. Botyu-Kagaku, **21**: 117.
WHEELER, C. M., H. D. NEWSOM & T. E. BLAKESLEE, 1958. U. S. Armed Forces med. J., **9**: 68.
WIESMANN, R., & C. KOCHER, 1951. Z. angew. Ent., **33**: 297.
YANAGISHIMA, S., 1961 a. Mem. Coll. Sci. Univ. Kyoto, (B), **28**: 9.
——— & N. SUZUKI, 1959 a. Zool. Mag. Tokyo, **68**: 231.
——— & ——— 1959 b. Ibid., **68**: 419.