$CuSO_4$ Resistance in *Drosophila melanogaster*

VI. Comparative Studies in Resistant Variants Induced by Various Kinds of Bivalent Metallic Salts

By

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Introduction

The present author has already demonstrated that *Drosophila melanogaster* Oregon RS strain, whose parents were cultured in PEARL's medium containing 0.5 mM of $CuSO_4$ during their larval stage, shows markedly resistance to the test medium containing 4 mM of $CuSO_4$, which is known to be 50% emergence dose (ED_{50}) (YANAGISHIMA and SUZUKI 1959 a). The copper resistant strain mentioned above was called the Cu-strain by the author. The author has also reported that the Cu-strain differs from the control one not only in copper resistance but also in longevity, photokinetic activity and metabolic pattern (YANAGISHIMA 1961 a). The cu-strain also shows cross resistance or collateral sensitivity to other bivalent metallic salts than copper (YANAGISHIMA 1961 b). Furthermore, it is worth pointing out that the copper resistance of the Cu-strain can be transmitted through sexual reproduction and degree of resistance neither increases nor decreases, at least phenomenally, during culture on the normal culture medium or on the medium containing 0. 5 mM of $CuSO_4$ for successive generations (YANAGISHIMA 1961 c).

In order to throw some light on mechanisms by which the Cu-strain can acquire a resistance to copper, the present experiments described in this paper were performed. It is important to know whether the acquisition of resistance under conditions where no selection is considered is observable only with copper and whether acquired resistance is specific to a given agent if resistance can be acquired with some other agents than copper. If a resistance induced by a given agent is specific to it, some specific changes of mechanisms must be considered. The agents used in this experiment were such bivalent metallic salts as $CuSO_4$, $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$, some of which had been known to be mutagenic in gene and cytoplasmic mutations (LAW 1938, MAGRAZHIKOVSKAYA 1938, YANAGISHIMA 1956, LINDEGREN et al.

1958, NAKAMURA 1960).

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Experiments

I. Resistant Strains Induced by Various Kinds of Bivalent Metallic Salts

Whether resistance to a given metallic salt might increase, when flies were cultured in a medium containing sublethal dose of a metallic salt, was tested, using the same method as in the case of $CuSO_4$ (YANAGISHIMA and SUZUKI 1959 a, YANAGISHIMA 1961 a). Care was taken specially to avoid affects of selection during the training in media containing metallic salts. The training was made in the larval stage and the acquired resistance was tested in a culture medium containing ED_{50} of each metallic salt. All experiments were performed at 25° ±0. 1°C.

a. *Preliminary experiments*

$CuSO_4$, $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$ were used as metallic salts. As the first step of the experiment, concentrations of metallic salts to be added to the media for the training were determined. The emergence-dose curve was made for each metallic salt and a concentration at which no difference in emergence rate was observed between the media with and without metallic salt supplement was determined, and the training of larvae was performed at this concentration. This concentration was called sublethal dose. The sublethal dose of each salt was : for $MnSO_4$, 2. 5 mM ; $CoSO_4$, 0. 10 mM ; $NiSO_4$, 0. 75 mM ; $ZnSO_4$, 4. 0 mM and $CdSO_4$, 0. 007 mM. The medium used in the emergence test contained 50% emergence dose (ED_{50}) of each metallic salt. ED_{50} of each metallic salt was : for $CuSO_4$, 4 mM ; $MnSO_4$, 15 mM ; $CoSO_4$, 0. 5 mM ; $NiSO_4$, 5 mM ; $ZnSO_4$, 20 mM and $CdSO_4$, 0. 07 mM.

The flies used were originated from the same strain of *Drosophila melanogaster* Oregon RS as used previously by the author. The basic culture medium was PEARL's synthetic medium. Other experimental methods were fundamentally same as those used previously.

b. *Larval resistance to various kinds of bivalent metallic salts* (*the emergence test*)

First instar larvae of the normal strain stocked in PEARL's synthetic medium were transferred to culture media supplemented with sublethal doses of metallic salts, 20 individuals for a culture bottle. Larvae hatched from the eggs laid by the

Table 1. Emergence rates of the variant strains in the media containing ED_{50} of various kinds of metallic salts (Emergence test). Figures in parentheses show numbers of bottles.

Test medium \ Strain	Control					Cu-strain					Mn-strain					Co-strain				
	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%
Control	200 (10)			196	98.0	100 (5)			97	97.0	100 (5)			97	97.0	100 (5)			92	92.0
	200 (10)			192	96.0	100 (5)			89	89.0	100 (5)			90	90.0	100 (5)			96	96.0
Total	400 (20)			388	97.0	200 (10)			186	93.0	200 (10)			187	93.5	200 (10)			188	94.0
$CuSO_4$	200 (10)			112	56.0	280 (14)			175	62.5	140 (7)	77	55.0	77	55.0	280 (14)			184	65.7
	280 (14)			163	58.2	200 (10)			133	66.5	300 (15)	155	51.6	155	51.6	280 (14)			171	61.0
											200 (10)			132	66.0	200 (10)			123	61.5
Total	480 (24)			275	57.2	480 (24)			308	64.1	640 (32)	232	52.7	364	56.8	760 (38)			478	62.8
$MnSO_4$	200 (10)			94	47.0	200 (10)			120	60.0	280 (14)	226	80.7	186	66.4	300 (15)	238	79.3	90	30.0
	240 (12)			148	61.6	280 (14)			148	52.8	280 (14)	220	79.2	203	72.5	140 (7)	87	62.1	43	30.7
						280 (14)			162	57.8										
Total	440 (22)			242	55.0	760 (38)			430	56.5	560 (28)	446	79.6	389	69.4	440 (22)	325	73.8	133	30.2
$CoSO_4$	200 (10)			130	65.0	160 (8)			99	61.8	280 (14)	227	81.0	227	81.0	280 (14)	166	71.0	197	70.3
	200 (10)			142	71.0	200 (10)			122	61.0	240 (12)			178	74.1	200 (10)			152	76.0
	200 (10)	115	57.5	110	55.0	200 (10)			83	41.5						120 (16)			91	75.8
Total	600 (30)			382	63.6	560 (28)			304	54.2	520 (26)			405	77.8	600 (30)			440	73.3
$NiSO_4$	200 (10)			98	49.0	200 (10)			114	57.0	280 (14)	148	52.8	124	44.2	300 (15)	158	52.6	135	45.0
	100 (5)			51	51.0	200 (10)	114	57.0	112	56.0	420 (21)			162	38.5	280 (14)	144	51.4	124	44.2
											200 (10)	80	40.0	72	36.0					
Total	300 (15)			149	49.6	400 (20)			226	56.5	900 (45)	228	47.5	358	39.7	580 (29)	302	52.0	259	44.6
$ZnSO_4$	200 (10)			110	55.0	200 (10)			115	57.5	280 (14)	138	49.2	138	49.2	280 (14)			118	42.1
	200 (10)			97	48.5	200 (10)			139	69.5	280 (14)	139	49.6	120	42.8	280 (14)			138	49.2
	140 (7)			72	51.4	200 (10)			124	62.0	200 (10)	98	49.0	97	48.5	240 (12)			138	57.5
Total	540 (27)			279	51.6	600 (30)			378	63.0	760 (38)	375	49.3	355	46.7	800 (40)			394	49.2
$CdSO_4$	200 (10)	141	70.5	120	60.0	200 (10)	119	59.5	109	54.5	340 (17)	232	68.2	195	57.3	200 (10)	82	41.0	22	11.0
	200 (10)	161	80.5	92	46.0	200 (10)	128	64.0	87	43.5	200 (10)	110	55.0	85	42.5	240 (12)	100	41.6	31	12.9
											140 (7)	85	60.7	77	55.0	200 (10)	83	41.5	19	9.5
Total	400 (20)	302	75.5	212	53.0	400 (20)	247	61.7	196	49.0	680 (34)	427	62.7	357	52.5	640 (32)	265	41.5	72	11.2

Test medium \ Strain	Ni-strain					Zn-strain					Cd-strain				
	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%
Control	100 (5)			93	93.0	100 (5)			96	96.0	200 (10)			194	97.0
	100 (5)			97	97.0	100 (5)			91	91.0	100 (5)			92	92.0
Total	200 (10)			190	95.0	200 (10)			187	93.5	300 (15)			286	95.3
$CuSO_4$	280 (14)	192	68.5	192	68.5	280 (14)			140	50.0	120 (6)	77	64.1	77	64.1
	280 (14)	190	67.8	190	67.8	320 (16)			180	56.2	160 (8)	104	65.0	104	65.0
Total	560 (28)	382	68.2	382	68.2	600 (30)			320	53.3	280 (14)	181	64.6	181	64.6
$MnSO_4$	280 (14)	226	80.7	186	66.4	280 (14)	194	69.2	168	60.0	200 (10)	168	84.0	142	71.0
	200 (10)	143	71.5	119	59.5	200 (10)	140	70.0	123	61.5	200 (10)	151	75.5	139	69.5
Total	480 (24)	369	76.8	305	63.5	480 (24)	334	69.5	291	60.0	400 (20)	319	79.7	281	70.2
$CoSO_4$	280 (14)	214	76.4	214	76.4	280 (14)	209	74.6	209	74.6	160 (8)	140	87.5	139	86.8
	280 (14)	218	77.8	218	77.8	240 (12)			178	74.1	200 (10)	162	81.0	160	80.0
						200 (10)			161	80.5					
Total	560 (28)	432	77.1	432	77.1	720 (36)			548	76.1	360 (18)	302	83.8	299	83.0
$NiSO_4$	280 (14)	208	74.2	184	65.7	260 (13)			157	60.3	140 (7)	92	65.7	88	62.8
	200 (10)	152	76.0	138	69.0	280 (14)	164	58.5	156	55.7	200 (10)	131	65.5	119	59.5
Total	480 (24)	360	75.0	322	67.0	540 (27)			313	57.9	340 (17)	223	65.5	207	60.8
$ZnSO_4$	280 (14)	162	57.8	154	55.0	240 (12)			142	59.1	140 (7)	88	62.8	81	57.8
	200 (10)	121	60.5	116	58.0	280 (14)			134	47.8	200 (10)	130	65.0	119	59.5
						160 (8)			91	56.8	200 (10)	141	70.5	123	61.5
Total	480 (24)	283	58.9	270	56.2	680 (34)			367	53.9	540 (27)	359	66.4	323	59.8
$CdSO_4$	280 (14)	196	70.0	112	40.0	280 (14)	212	75.5	100	35.7	140 (7)	126	90.0	126	90.0
	400 (20)	300	75.0	186	46.5	280 (14)	152	54.2	114	40.7	200 (10)	149	74.5	149	74.5
	200 (10)	144	72.0	91	45.5										
Total	880 (44)	640	72.7	389	44.2	560 (28)	364	65.0	214	38.2	340 (17)	275	80.8	275	80.8

adult flies which had grown in the culture media mentioned above, were transferred to the media for the emergence test. The larvae whose parents had spent their larval stages in the media supplemented with metallic salts were called Mn-, Co-, Ni-, Zn- and Cd-strains respectively, according to the metallic salts with which their parents had been trained. Numbers of culture bottles and those of larvae used in the tests are shown in Table 1. Emergence and pupation rates in the test media containing ED_{50} of metallic salts were measured.

The experimental results and those of statistical examinations of them are shown in Tables 1~3.

In the normal medium, emergence rate of each strain did not differ from that of the control strain (Cu-strain, $0.20>\alpha>0.10$; Mn-strain, $0.10>\alpha>0.05$; Co-strain, $0.20>\alpha>0.10$; Ni-strain, $0.50>\alpha>0.30$; Zn-strain, $0.70>\alpha>0.50$; Cd-strain, $0.50>\alpha>0.30$.

The Mn-strain had higher emergence rates in both $MnSO_4$ and $CoSO_4$ test media than the control strain and lower one in $NiSO_4$ test medium, but there was seen no more difference in the emergence rates among other test media than those between the Mn-strain and the control strain. The Co-strain showed a higher emergence rate only in $CoSO_4$ test medium and lower ones in $MnSO_4$ and $CdSO_4$ test media, when compared with the control strain. Emergence rates of the Ni-strain were higher than those of the control strain in $CuSO_4$, $MnSO_4$, $CoSO_4$ and $NiSO_4$ test media, but the result was reversal in $CdSO_4$ test medium. In the case of the Zn-strain, the emergence rate was higher in $CoSO_4$, $NiSO_4$ test media and lower in $CdSO_4$ test medium, as compared with the control strain. The Cd-strain showed a higher emergence rate in all test media.

The above-mentioned results will be summarized as follows :

1) The Mn-, Co-, Ni- and Cd-strains showed markedly higher resistance in each test medium containing the same metallic salts as used in the training, but on the contrary, the Zn-strain did not show resistance to $ZnSO_4$ test medium.

2) The Mn-strain had cross resistance to $CoSO_4$ test medium and collateral sensitivity to $NiSO_4$ test medium.

3) The Co-strain showed collateral sensitivity to both $MnSO_4$ and $CdSO_4$ media.

4) The Ni-strain exhibited cross resistance to all test media, except $CdSO_4$ medium to which it showed collateral sensitivity.

5) The Zn-strain had cross resistance to $CoSO_4$ and $NiSO_4$ test media and collateral sensitivity to $CdSO_4$ test medium.

6) The Cd-strain exhibited markedly cross resistance to all test media.

7) The Cu-strain showed cross resistance to $ZnSO_4$ test medium and collateral sensitivity to $CoSO_4$ test medium.

Table 2. Examinations of differences in emergence

Between strains	Control	$CuSO_4$	$MnSO_4$
Control : Cu	$0.20 > \alpha > 0.10$	$0.05 > \alpha > 0.02$*	$0.70 > \alpha > 0.50$
: Mn	$0.10 > \alpha > 0.05$	$0.90 > \alpha > 0.80$	$0.001 > \alpha$*
: Co	$0.20 > \alpha > 0.10$	$0.10 > \alpha > 0.05$	$0.001 > \alpha$*
: Ni	$0.50 > \alpha > 0.30$	$0.001 > \alpha$*	$0.01 > \alpha > 0.001$*
: Zn	$0.70 > \alpha > 0.50$	$0.50 > \alpha > 0.30$	$0.70 > \alpha > 0.50$
: Cd	$0.50 > \alpha > 0.30$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
Cu : Mn	$0.90 > \alpha > 0.80$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
: Co	$0.20 > \alpha > 0.10$	$0.95 > \alpha > 0.90$	$0.001 > \alpha$*
: Ni	$0.70 > \alpha > 0.50$	$0.20 > \alpha > 0.10$	$0.05 > \alpha > 0.02$*
: Zn	$\alpha > 0.99$	$0.01 > \alpha$*	$0.20 > \alpha > 0.10$
: Cd	$0.50 > \alpha > 0.30$	$0.90 > \alpha > 0.80$	$0.001 > \alpha$*
Mn : Co	$0.90 > \alpha > 0.80$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
: Ni	$0.70 > \alpha > 0.50$	$0.001 > \alpha$*	$0.10 > \alpha > 0.05$
: Zn		$0.20 > \alpha > 0.10$	$0.01 > \alpha > 0.001$*
: Cd	$0.70 > \alpha > 0.50$	$0.05 > \alpha > 0.02$*	$0.90 > \alpha > 0.80$
Co : Ni	$0.98 > \alpha > 0.95$	$0.10 > \alpha > 0.05$	$0.001 > \alpha$*
: Zn	$0.90 > \alpha > 0.80$	$0.001 > \alpha$*	$0.001 > \alpha$*
: Cd	$0.70 > \alpha > 0.50$	$0.70 > \alpha > 0.50$	$0.001 > \alpha$*
Ni : Zn	$0.70 > \alpha > 0.50$	$0.001 > \alpha$*	$0.50 > \alpha > 0.30$
: Cd	$\alpha > 0.99$	$0.50 > \alpha > 0.30$	$0.05 > \alpha > 0.02$*
Zn : Cd	$0.70 > \alpha > 0.50$	$0.01 > \alpha > 0.001$*	$0.01 > \alpha > 0.001$*

* significant

Table 3. Examinations of differences in emergence

Between media	Control	Cu	Mn
Control : $CuSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*	$0.001 > \alpha$*
: $MnSO_4$	$0.001 > \alpha$*	$0.001 < \alpha$*	$0.001 > \alpha$*
: $CoSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*	$0.001 > \alpha$*
: $NiSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*	$0.001 > \alpha$*
: $ZnSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*	$0.001 > \alpha$*
: $CdSO_4$	$0.001 > \alpha$*	$0.001 > \alpha$*	$0.001 > \alpha$*
$CuSO_4$: $MnSO_4$	$0.10 > \alpha > 0.05$	$0.02 > \alpha > 0.01$*	$0.001 > \alpha$*
: $CoSO_4$	$0.20 > \alpha > 0.10$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
: $NiSO_4$	$0.05 > \alpha > 0.02$*	$0.02 > \alpha > 0.01$*	$0.001 > \alpha$*
: $ZnSO_4$	$0.20 > \alpha > 0.10$	$0.80 > \alpha > 0.70$	$0.001 > \alpha$*
: $CdSO_4$	$0.80 > \alpha > 0.70$	$0.001 > \alpha$*	$0.20 > \alpha > 0.10$
$MnSO_4$: $CoSO_4$	$0.05 > \alpha > 0.02$*	$0.50 > \alpha > 0.30$	$0.01 > \alpha > 0.001$*
: $NiSO_4$	$0.20 > \alpha > 0.10$	$0.98 > \alpha > 0.95$	$0.001 > \alpha$*
: $ZnSO_4$	$0.50 > \alpha > 0.30$	$0.02 > \alpha > 0.01$*	$0.001 > \alpha$*
: $CdSO_4$	$0.70 > \alpha > 0.50$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
CoSO : $NiSO_4$	$0.001 > \alpha$*	$0.70 > \alpha > 0.50$	$0.001 > \alpha$*
: $ZnSO_4$	$0.001 > \alpha$*	$0.01 > \alpha > 0.001$*	$0.001 > \alpha$*
: $CdSO_4$	$0.01 > \alpha > 0.001$*	$0.20 > \alpha > 0.10$	$0.01 > \alpha > 0.001$*
$NiSO_4$: $ZnSO_4$	$0.70 > \alpha > 0.50$	$0.05 > \alpha > 0.02$*	$0.01 > \alpha > 0.001$*
: $CdSO_4$	$0.50 > \alpha > 0.30$	$0.05 > \alpha > 0.02$*	$0.001 > \alpha$*
$ZnSO_4$: $CdSO_4$	$0.80 > \alpha > 0.70$	$0.001 > \alpha$*	$0.05 > \alpha > 0.02$*

* significant

rates among strains shown in Table 1 (χ^2-test).

Medium CoSO$_4$	NiSO$_4$	ZnSO$_4$	CdSO$_4$
0. 05 > α >0. 02*	0. 10 > α >0. 05	0. 001> α *	0. 30 > α >0. 20
0. 001> α *	0. 02 > α >0. 01*	0. 20 > α >0. 10	0. 90 > α >0. 80
0. 001> α *	0. 30 > α >0. 20	0. 90 > α >0. 80	0. 001> α *
0. 001> α *	0. 001> α *	0. 70 > α >0. 50	0. 02 > α >0. 01*
0. 001> α *	0. 05 > α >0. 02*	0. 50 > α >0. 30	0. 001> α *
0. 001> α *	0. 01 > α >0. 001*	0. 01 > α >0. 001*	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 50 > α >0. 30
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 01 > α >0. 001*	0. 05 > α >0. 02*	0. 20 > α >0. 10
0. 001> α *	0. 70 > α >0. 50	0. 01 > α >0. 001*	0. 001> α *
0. 001> α *	0. 30 > α >0. 20	0. 30 > α >0. 20	0. 001> α *
0. 10 > α >0. 05	0. 10 > α >0. 05	0. 50 > α >0. 30	0. 001> α *
0. 95 > α >0. 90	0. 001> α *	0. 01 > α >0. 001*	0. 0 1> α >0. 001*
0. 98 > α >0. 95	0. 001> α *	0. 01 > α >0. 001*	0. 001> α *
0. 10 > α >0. 05	0. 001> α *	0. 001> α *	0. 001> α *
0. 20 > α >0. 10	0. 001> α *	0. 05 > α >0. 02*	0. 001> α *
0. 30 > α >0. 20	0. 001> α *	0. 10 > α >0. 05	0. 001> α *
0. 01 > α >0. 001*	0. 001> α *	0. 001> α *	0. 001> α *
0. 70 > α >0. 50	0. 01 > α >0. 001*	0. 50 > α >0. 30	0. 05 > α >0. 02*
0. 001> α *	0. 10 > α >0. 05	0. 30 > α >0. 20	0. 001> α *
0. 05 > α >0. 02*	0. 50 > α >0. 30	0. 10 > α >0. 05	0. 001> α *

rates among media shown in Table 1 (χ^2-test).

Strain Co	Ni	Zn	Cd
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 20 > α >0. 10	0. 02 > α >0. 01*	0. 20 > α >0. 10
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 80 > α >0. 70	0. 20 > α >0. 10	0. 50 > α >0. 30
0. 001> α *	0. 001> α *	0. 90 > α >0. 80	0. 30 > α >0. 20
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 20 > α >0. 10	0. 50 > α >0. 30	0. 01 > α >0. 001*
0. 001> α *	0. 05 > α >0. 02*	0. 05 > α >0. 02*	0. 01 > α >0. 001*
0. 001> α *	0. 001> α *	0. 001> α *	0. 01 > α >0. 001*
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 70 > α >0. 50
0. 10 > α >0. 05	0. 001> α *	0. 20 > α >0. 10	0. 90 > α >0. 80
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *
0. 001> α *	0. 001> α *	0. 001> α *	0. 001> α *

In the next place, the resistance of each strain in each test medium will be described comparatively.

1) In $CuSO_4$ test medium

Strains more resistant than the control were the Cu-, Ni- and Cd-strains. The other strains did not differ from the control strain.

2) In $MnSO_4$ test medium

Strains more resistant than the control strain were the Mn-, Ni- and Cd-strains. The Co-strain was less resistant than the control strain.

3) In $CoSO_4$ test medium

All the strains tested except the Cu-strain which showed lower resistance exhibited higher resistance than the control strain.

4) In $NiSO_4$ test medium

Strains more resistant than the control strain were the Ni-, Zn- and Cd-strains. Only the Mn-strain was less resistant than the control strain.

5) In $ZnSO_4$ test medium

Strains more resistant than the control strain were the Cu- and Cd-strains.

6) In $CdSO_4$ test medium

Only the Cd-strain was more resistant than the control. The Co-, Ni- and Zn-strains were less resistant than the control.

Table 4. Developmental rates of each strain on the media containing various

Strain		Cu-strain		Mn-strain		Co-strain	
Average periods from hatching to / Test medium		Pupation (days)	Emergence (days)	Pupation (days)	Emergence (days)	Pupation (days)	Emergence (days)
$CuSO_4$		7.20	12.16	8.38	12.11	8.00	11.26
	s. d	(0.73)	(0.42)	(1.41)	(2.06)	(1.34)	(0.61)
MnSO		5.37	8.22	4.84	8.41	8.35	11.80
	s. d	(0.61)	(0.56)	(0.53)	(0.83)	(0.47)	(1.04)
$CoSO_4$		9.97	14.37	7.36	11.42	9.05	12.86
	s. d	(0.99)	(1.85)	(0.85)	(1.22)	(0.95)	(1.27)
$NiSO_4$		4.87	9.01	5.33	9.43	6.95	10.38
	s. d	(0.55)	(0.13)	(0.86)	(0.55)	(0.83)	(0.67)
$ZnSO_4$		7.63	11.83	7.67	11.98	10.82	15.40
	s. d	(1.14)	(2.40)	(1.67)	(1.34)	(2.35)	(1.78)
$CdSO_4$		9.21	13.37	6.92	10.93	14.14	17.00
	s. d	(0.88)	(0.78)	(0.72)	(0.39)	(2.03)	(1.24)

c. *Developmental rate*

The developmental rate of each strain on media supplemented with metallic salts was investigated in order to see one aspect of change in resistance. The same method as used previously by the present author (1959 a, b) was used. The results are shown in Table 4 and Figs. 1~2 and its statistical examinations are shown in Tables 5 and 6.

From the results shown in Tables 4~6 and Figs. 1~2, we can see the following things.

1) In $MnSO_4$ test medium

The Mn-strain (4.84 days) was significantly shorter in the length of the larval period than the control strain (5.33 days) but there was no significant difference in the length of the period up to emergence between the two (Mn-strain, 8.41 days ; control strain, 8.18 days).

2) In $CoSO_4$ test medium

The Co-strain was significantly shorter in the lengths of the larval periods (control strain 9.81 days ; Co-strain, 9.05 days) and the period up to emergence (control strain, 13.97 days ; Co-strain, 12.86 days) than the control.

3) In $NiSO_4$ test medium

There was no difference in the length of the larval period between the control

kinds of metallic salts. Figures in parentheses show standard deviations (s. d).

Ni-strain		Zn-strain		Cd-strain		Control	
Pupation (days)	Emergence (days)	Pupation (days)	Emergence (days)	Pupation (days)	Emergence (days)	Pupation (days)	Emergence (days)
6.26	10.58	7.16	11.16	7.64	12.38	8.19	13.33
(1.26)	(0.89)	(1.25)	(1.42)	(2.59)	(0.91)	(0.63)	(3.04)
5.61	8.00	5.71	9.03	5.83	9.15	5.33	8.18
(2.16)	(0.24)	(0.46)	(0.00)	(1.13)	(0.42)	(0.54)	(0.48)
7.34	10.17	8.97	11.67	9.51	14.58	9.81	13.97
(1.70)	(1.63)	(1.49)	(1.90)	(1.34)	(1.12)	(1.12)	(1.52)
5.84	8.45	6.77	10.58	5.92	9.29	5.55	9.09
(1.81)	(0.75)	(0.86)	(0.61)	(0.83)	(0.24)	(0.68)	(0.68)
6.60	10.33	7.88	10.83	8.90	12.33	7.40	10.45
(1.65)	(1.30)	(1.27)	(1.43)	(1.59)	(1.32)	(0.99)	(1.51)
7.76	11.35	7.81	11.60	8.60	12.26	9.54	14.78
(0.92)	(0.93)	(0.81)	(1.03)	(1.18)	(1.04)	(0.97)	(1.05)

strain (5.55 days) and the Ni-strain (5.84 days), but the Ni-strain (8.45 days) was significantly shorther in the length of the period up to emergence than the control (9.09 days).

4) In $ZnSO_4$ test medium

The Zn-strain was significantly longer in the length of the larval period than the control strain (Zn-strain, 7.88 days ; control strain, 7.40 days). No difference was found in the length of the period up to emergence between the two (Zn-strain, 10.83 days ; control strain, 10.45 days).

5) In $CdSO_4$ test medium

The Cd-strain was significantly faster in the developmental rate than the control strain (larval period : Cd-strain, 8.60 days ; control strain, 9.54 days ; period up to emergence : Cd-strain, 12.26 days ; control strain, 14.78 days).

The above-mentioned results will be summarized as follows :

When the normal flies were cultured in media containing sublethal doses of $CuSO_4$, $CoSO_4$ and $CdSO_4$ respectively during the larval stage, the flies of the next generation developed faster than the normal control flies. A shortening in the length of the larval stage was remarkable with the Mn-strain in $MnSO_4$ test medium and a shortening in the period up to emergence was observed with the Ni-strain in $NiSO_4$ test medium. On the contrary, in the case of the Zn-strain, the larval stage became longer than the normal strain in $ZnSO_4$ test medium. It is interesting that the Zn-strain which showed less resistance to $ZnSO_4$ than the control, when comparison was made with emergence rate, developed slower than the control in $ZnSO_4$ test medium.

Next, the comparisons of developmental rates of each strain in a given medium were made statistically from the results described in Tables 4~6. Main results obtained are as follows.

1) In $CuSO_4$ test medium

The strains which did not differ in developmental rate from the control were the Mn- and Co-strains. The Cu-, Ni- and Zn-strains were faster than the control, but the Cd-strain showed shorter larval period than the control, while it did not differ from the control in the period up to emergence.

2) In $MnSO_4$ test medium

It must be noticeable that only the Mn-strain was shorter in the larval period than the control strain, while all the strains tested except the Cu-strain which did not differ from the control strain showed longer larval period than the control. As for the period up to emergence, the Cu- and Mn- strains did not differ significantly from the control strain, the Ni-strain was shorter and the Co-, Zn- and Cd-strains were longer than the control strain significantly.

Table 5. Examinations of differences in developmental rates in each medium among strains (t-test).

Examinations of differences among average periods from hatching to / Between strains	Medium: $CuSO_4$ Pupation	$CuSO_4$ Emergence	$MnSO_4$ Pupation	$MnSO_4$ Emergence	$CoSO_4$ Pupation	$CoSO_4$ Emergence	$NiSO_4$ Pupation	$NiSO_4$ Emergence	$ZnSO_4$ Pupation	$ZnSO_4$ Emergence	$CdSO_4$ Pupation	$CdSO_4$ Emergence
Control : Cu	$0.001>\alpha$*	$0.001>\alpha$*	$0.40>\alpha>0.30$	$0.60>\alpha>0.50$	$0.30>\alpha>0.20$	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*
: Mn	$0.20>\alpha>0.10$	$0.30>\alpha>0.20$	$0.02>\alpha>0.01$*	$0.40>\alpha>0.30$	$0.001>\alpha$*	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Ce	$0.40>\alpha>0.30$	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Ni	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Zn	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$\alpha>0.90$	$0.001>\alpha$*	$0.001>\alpha$*
: Cd	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
Cu : Mn	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.90>\alpha>0.80$	$0.60>\alpha>0.50$	$0.001>\alpha$*	$0.001>\alpha$*
: Co	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Ni	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Zn	$0.80>\alpha>0.70$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.001>\alpha$*
: Cd	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.70>\alpha>0.60$	$0.001>\alpha$*	$0.001>\alpha$*	$0.02>\alpha>0.01$*	$0.001>\alpha$*	$0.001>\alpha$*
Mn : Co	$0.20>\alpha>0.10$	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Ni	$0.40>\alpha>0.30$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$\alpha>0.90$	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Zn	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*
: Cd	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*
Co : Ni	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Zn	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.70>\alpha>0.60$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Cd	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
Ni : Zn	$0.001>\alpha$*	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.70>\alpha>0.60$	$0.20>\alpha>0.10$
: Cd	$0.001>\alpha$*	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.001>\alpha$*	$0.001>\alpha$*	$0.40>\alpha>0.30$	$0.001>\alpha$*	$0.001>\alpha$*
Zn : Cd	$0.02>\alpha>0.01$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*

* significant

Table 6. Examinations of differences in developmental rates of each strain among media (t-test).

Examinations of differences among average periods from hatching to / Between media	Strain Control		Cu		Mn		Co		Ni		Zn		Cd	
	Pupation	Emergence	Pupation	Emergence	Pupation	Emergence	Pupation	Emergence	Pupation	Emergence	Pupation	Emergence	Pupation	Emergence
Control : $CuSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
: $MnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
: $CoSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
: $NiSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
: $ZnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
: $CdSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*										
$CuSO_4$: $MnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.01 > α >0.001*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*
: $CoSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.01 > α >0.001*	0.001> α*	0.001> α*	0.001> α*	0.05 > α >0.02*	0.001> α*	α > 0.90	0.001> α*	0.001> α*
: $NiSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.10 > α >0.05	0.001> α*	0.05 > α >0.02*	0.001> α*	0.001> α*	0.001> α*
: $ZnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.02 > α >0.01*	0.01 > α >0.001*	0.70 > α >0.60	0.001> α*	0.001> α*	0.20 > α >0.10	0.20 > α >0.10	0.02 > α >0.01*	0.01 > α >0.001*	0.001> α*	0.80 > α >0.70
: $CdSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	α > 0.90	0.001> α*	0.50 > α >0.40
$MnSO_4$: $CoSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.70 > α >0.60	0.001> α*	0.001> α*	0.001> α*
: $NiSO_4$	0.01 > α >0.001*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.30 > α >0.20	0.001> α*	0.001> α*	0.001> α*	0.60 > α >0.50	0.01 > α >0.001*
: $ZnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> ν*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*
: $CdSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*
$CoSO_4$: $NiSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0·001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*
: $ZnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.10 > α >0.05	0.01 > α >0.001*	0.001> α*	0.001> α*	0.01 > α >0.001*	0.50 > α >0.40	0.001> α*	0.01 > α >0.001*	0.001> α*	0.001> α*
: $CdSO_4$	0.05 > α >0.02*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.05 > α >0.02*	0.001> α*	0.001> α*	0.90 > α >0.80	0.001> α*	0.001> α*
$NiSO_4$: $ZnSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.01 > α >0.001*	0.001> α*	0.001> α*	0.20 > α >0.10	0.001> α*	0.001> α*
: $CdSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*
$ZnSO_4$: $CdSO_4$	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.001> α*	0.01 > α >0.001*	0.001> α*	0.001> α*	0.70 > α >0.60	0.01 > α >0.001*	0.20 > α >0.10	0.70 > α >0.60

* Significant

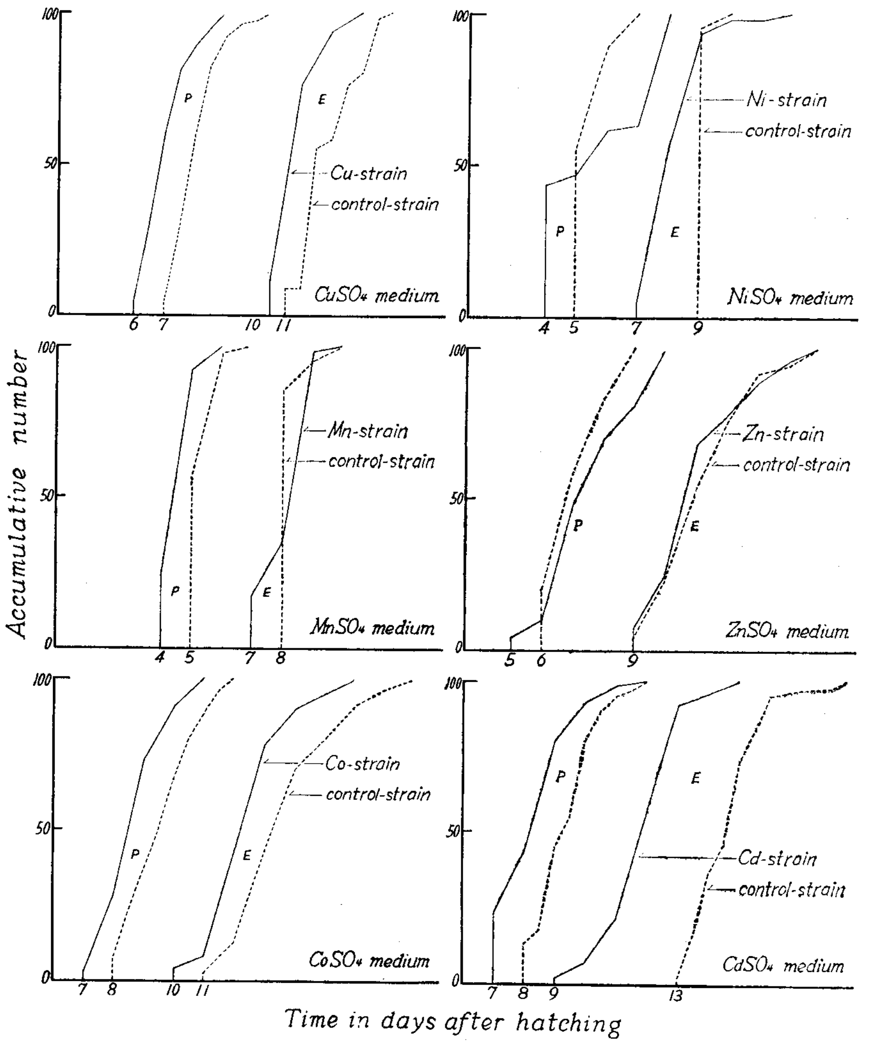
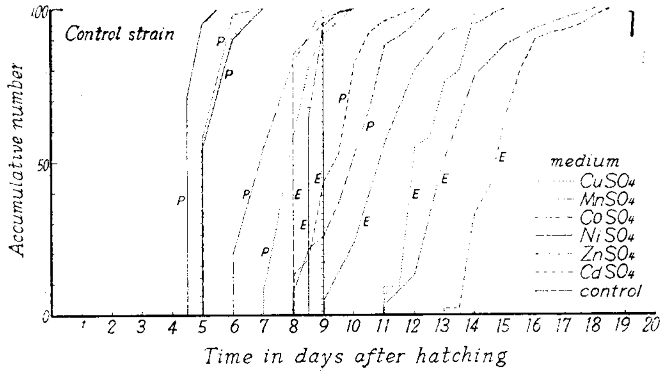
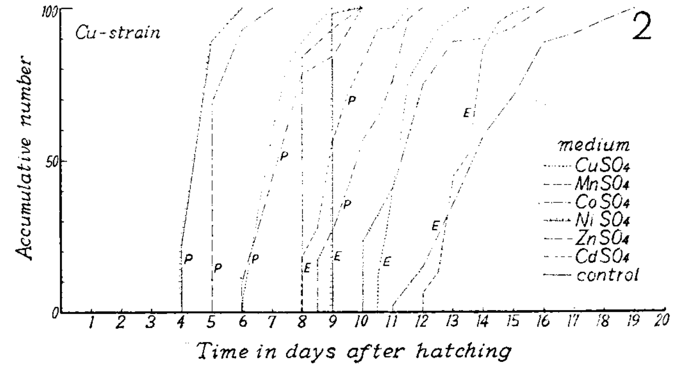


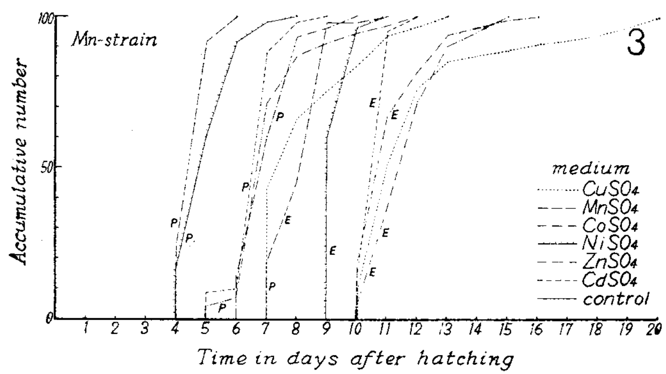
Fig. 1. Developmental rate of each variant strain in the medium containing the same metallic salt as used for the training.

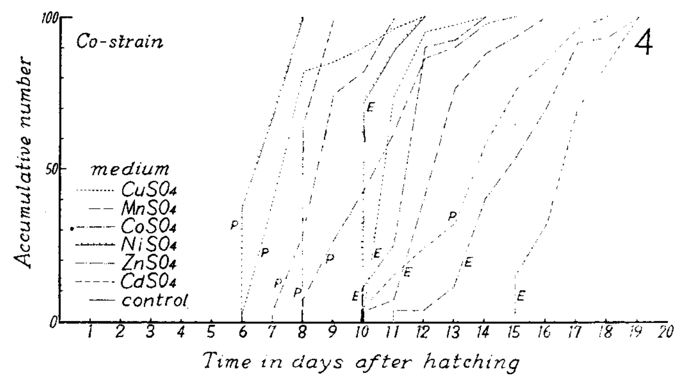
P : accumulative number of pupae.

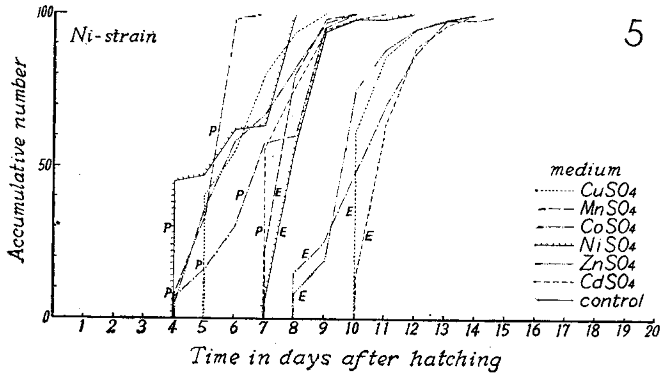
E : accumulative number of adults.

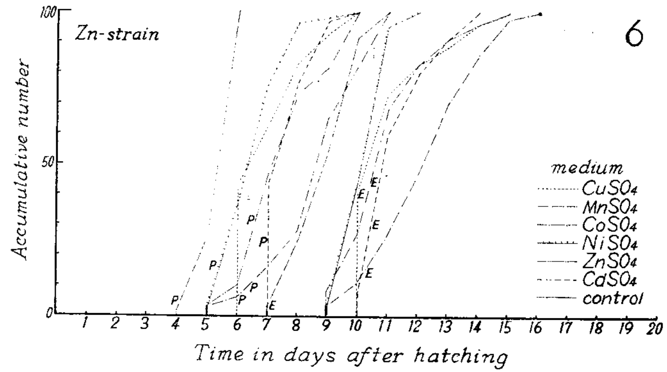

Control strain
1
Accumulative number
Time in days after hatching
medium
CuSO4
MnSO4
CoSO4
NiSO4
ZnSO4
CdSO4
control


Cu-strain
2
Accumulative number
Time in days after hatching
medium
CuSO4
MnSO4
CoSO4
NiSO4
ZnSO4
CdSO4
control


Mn-strain
3
Accumulative number
Time in days after hatching
medium
CuSO4
MnSO4
CoSO4
NiSO4
ZnSO4
CdSO4
control


Co-strain
4
Accumulative number
Time in days after hatching
medium
CuSO4
MnSO4
CoSO4
NiSO4
ZnSO4
CdSO4
control





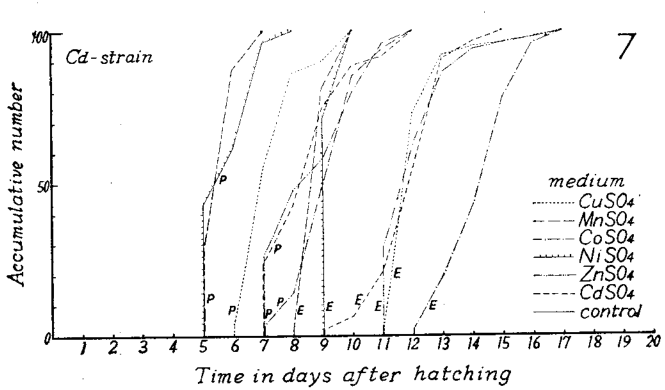


Fig. 2. Results of the developmental test in media containing various kinds of metallic salts.

1. Developmental rates of the control strain.
2. Developmental rates of the Cu-strain.
3. Developmental rates of the Mn-strain.
4. Developmental rates of the Co-strain.
5. Developmental rates of the Ni-strain.
6. Developmental rates of the Zn-strain.
7. Developmental rates of the Cd-strain.

3) In $CoSO_4$ test medium

Only the Cu-strain did not differ from the control strain. The Mn-, Co-, Ni- and Zn-strains showed significantly faster developmental rate than the control. In the case of Cd-strain, larval period became shorter but the period up to emergence became longer than the control.

4) In $NiSO_4$ test medium

The Cu-strain was significantly faster in the developmental rate than the control strain, but, on the contrary, the Co-, Zn- and Cd- strains were highly significantly slower. There was no difference in the length of the larval period between the Mn-strain and the control strain, but the Mn-strain had highly significantly longer period up to emergence than the control strain. In the case of Ni-strain, the larval period became longer but the period up to emergence became highly significantly shorter than the control strain.

5) In $ZnSO_4$ test medium

The Ni-strain was accelerated its developmental rate, but both Co- and Cd-strains were retarded than the control strain. In the cases of Cu- and Mn- strains, only the period up to emergence became longer than the control. It is interesting that the Zn-strain showed longer larval period than the control strain, while there was no difference in the length of the period up to emergence between the two.

6) In $CdSO_4$ test medium

All the strains tested except the Co-strain which became significantly slower, showed highly significantly faster developmental rate than the control strain.

Judging from the results mentioned above, it can be said that each strain obtained by the training in a culture medium containing sublethal dose of each salt is faster in the developmental rate than the control strain in the culture medium containing ED_{50} of the same salt as that used in the training, except the case of $ZnSO_4$. When comparisons are made among the strains on the same medium, the strain established by the training in that medium containing sublethal dose of the metallic salt is not always fastest on the medium containing ED_{50} of the same metallic salt as that used in the training.

d. *Comparative studies in the oviposition number and the hatching rate*

The experimental methods used in this test were the same ones as these used in the second report (Yanagishima and Suzuki 1959 b). Ten flies, 5 males and 5 females, were put into a oviposition tube, immediately after the emergence. Five such tubes were prepared. Oviposition numbers were counted during 15 days after emergence as follows and at the same time, the hatching rate was determined. A slide glass with the normal culture medium on it was inserted into the oviposition tube and it was exchanged with a new one every day, and the number of eggs laid on it was counted. After counting the number of eggs, the larvae which had already hatched out were removed from the slide glass. The slide glass was then maintained at 25°C under a moist condition for 24 hours to know the number of hatched larvae.

The oviposition number and the hatching rate are shown in Tables 7 and 8. The Mn- and Ni-strains showed significantly greater oviposition number than the control strain, whereas no difference was observed in the oviposition number between the control and the other strains.

The Zn-strain did not differ in the hatching rate from the control strain, but the Cu- and Ni-strains were higher and the Mn-, Co- and Cd- strains were lower than the control strain, each highly significantly. After all, only the Ni-strain was greater in the oviposition number and the hatching rate. In some cases only the hatching rate became higher accompanied with no change in the oviposition number, and in other cases, the latter became greater, without any changes in the former. Judging from these results, it can be said that all the strains tested, but for the Zn-strain, must have been given some effects in their genital organs by being reared in the media supplemented with metallic salts.

Table 7. Average numbers of eggs laid by a female of each strain during 15 days after emergence.

No. of tubes*	Strain						
	Control	Cu	Mn	Co	Ni	Zn	Cd
1	216. 8	242. 6	242. 2	198. 2	277. 4	218. 6	211. 4
2	241. 5	296. 3	313. 4	236. 6	296. 9	231. 6	214. 6
3	209. 4	202. 4	275. 7	247. 8	268. 2	225. 4	141. 1
4	272. 2	230. 5	325. 1	145. 2	237. 6	244. 6	229. 2
5	245. 2	256. 4	368. 2	250. 4	300. 6	265. 6	241. 8
Average	237. 0	245. 6	304. 9	215. 6	276. 1	249. 7	207. 6

* Each tube contains 5♀×5♂

Examinations of differences between strains (t-test).

Control strain	: Cu-strain	$0.70 > \alpha > 0.60$
	: Mn	$0.05 > \alpha > 0.02$*
	: Co	$0.40 > \alpha > 0.30$
	: Ni	$0.05 > \alpha > 0.02$*
	: Zn	$0.60 > \alpha > 0.50$
	: Cd	$0.30 > \alpha > 0.20$
Cu-strain	: Mn-strain	$0.10 > \alpha > 0.05$
	: Co	$0.30 > \alpha > 0.20$
	: Ni	$0.20 > \alpha > 0.10$
	: Zn	$0.90 > \alpha > 0.80$
	: Cd	$0.20 > \alpha > 0.10$
Mn-strain	: Co-strain	$0.02 > \alpha > 0.01$*
	: Ni	$0.40 > \alpha > 0.30$
	: Zn	$0.10 > \alpha > 0.05$
	: Cd	$0.01 > \alpha > 0.001$*
Co-strain	: Ni-strain	$0.05 > \alpha > 0.02$*
	: Zn	$0.30 > \alpha > 0.20$
	: Cd	$0.80 > \alpha > 0.70$
Ni-strain	: Zn-strain	$0.20 > \alpha > 0.10$
	: Cd	$0.02 > \alpha > 0.01$*
Zn-strain	: Cd-strain	$0.20 > \alpha > 0.10$

* significant

Table 8. Hatching rate of each strain on the control medium during 15 days after emergence.

Strain		Control	Cu	Mn	Co	Ni	Zn	Cd
Sum of 5 tubes (each tube contain 5♀×5♂)	Total number of eggs	4200	4862	4911	4163	4338	5100	4005
	No. of larvae	3596	4323	4011	3449	3807	4317	3354
	Hatching rate %	85.61	88.91	81.67	82.84	87.75	84.64	83.74

Examinations of differences in hatching rate between strains (χ^2-test).

Control strain	: Cu-strain	$0.001 > \alpha$*	Mn-strain	: Co-strain	$0.20 > \alpha > 0.10$
	: Mn	$0.001 > \alpha$*		: Ni	$0.001 > \alpha$*
	: Co	$0.001 > \alpha$*		: Zn	$0.001 > \alpha$*
	: Ni	$0.01 > \alpha > 0.001$*		: Cd	$0.02 > \alpha > 0.01$*
	: Zn	$0.20 > \alpha > 0.10$	Co-strain	: Ni-strain	$0.001 > \alpha$*
	: Cd	$0.02 > \alpha > 0.01$*		: Zn	$0.05 > \alpha > 0.02$*
	: Mn	$0.001 > \alpha$*		: Cd	$0.30 > \alpha > 0.20$
Cu-strain	: Co-strain	$0.001 > \alpha$*	Ni-strain	: Zn-strain	$0.001 > \alpha$*
	: Ni	$0.10 > \alpha > 0.05$		: Cd	$0.001 > \alpha$*
	: Zn	$0.001 > \alpha$*	Zn-strain	: Cd-strain	$0.30 > \alpha > 0.20$
	: Cd	$0.001 > \alpha$*			

* significant

e. *Comparison of the longevity of each strain*

In the next place, the author examined the longevity of flies of each strain, rearing them on a sugar solution containing the same kind of metallic salts as that used in the training of each strain. The same methods used in the previous test were employed (YANAGISHIMA 1961 a). The same test bottle as designed by OHSAWA and TSUKUDA (1956) was used throughout this test. The test solution was 0.1 M sucrose solution containing ED_{50} of various kinds of metallic salts.

A simple 0.1 M sucrose solution was used as the control. Ten male flies or 10 female flies were put into a test bottle and the number of survivors was counted every day.

Mean longevity in days and the survival rate of each strain on the salt-supplemented solution are shown in Fig. 3. 1–6 and Tables 9~13, and those on the simple solution, in Fig. 4. 1–3 and Table 14. From the results obtained, the following things can be said.

On the test solutions, containing each metallic salt by which the parents of the tested flies had been trained, the Cu-, Co-, Ni- and Zn- strains died sooner than the control strain regardless of sex, while in the case of the Cd-strain, female flies died sooner than the control flies, but male flies did not differ from the control strain. It is interesting that the Mn-strain could live longer than the control on the test solution supplemented with 15 mM of $MnSO_4$ regardless of sex.

On the simple sucrose solution, both the Cu-strain and the Mn-strain could live longer than the control regardless of sex, but the Ni-strain died sooner. In the

Table 9. Results of the longevity test performed with the control and Mn-strains in 0.1 M sucrose solution containing 15 mM $MnSO_4$.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of life	♀ Mean length of life (days)	♀ Standard deviation
Control	60	5.70	1.55	80	8.12	1.12
Mn-strain	50	7.18	1.09	50	9.30	0.93

Examinations of differences (t-test).

♂ $0.001 > \alpha$* ♀ $0.001 > \alpha$* * significant

Table 10. Results of the longevity test performed with the control and Co-strains in 0.1 M sucrose solution containing 0.5 mM $CoSO_4$.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of flies	♀ Mean length of life (days)	♀ Standary deviation
Control	60	18.46	1.38	40	18.98	1.82
Co-strain	40	16.90	1.10	40	15.30	2.94

Examinations of differences (t-test).

♂ $0.001 > \alpha$* ♀ $0.001 > \alpha$* * significant

Table 11. Results of the longevity test performed with the control and Ni-strains in 0.1 M sucrose solution containing 5.0 mM $NiSO_4$.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of flies	♀ Mean length of life (days)	♀ Ssandard deviation
Control	50	6.06	0.23	50	5.82	0.88
Ni-strain	60	2.00		60	2.00	

Examinations of differences (t-test).

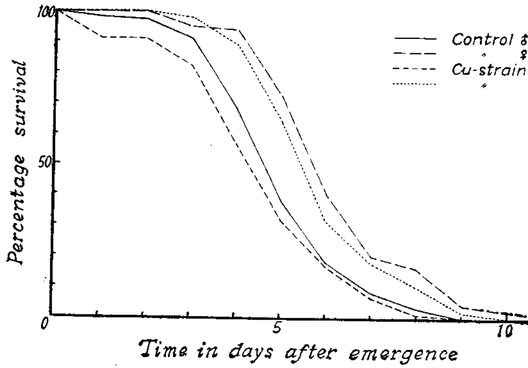
♂ $0.001 > \alpha$* ♀ $0.001 > \alpha$* * significant

Table 12. Results of the longevity test performed with the control and Zn-strains in 0.1 M sucrose solution containing 20 mM $ZnSO_4$.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of flies	♀ Mean length of life (days)	♀ Standard deviation
Control	60	4.26	0.44	40	4.80	0.51
Zn-strain	50	3.26	0.52	50	4.04	0.27

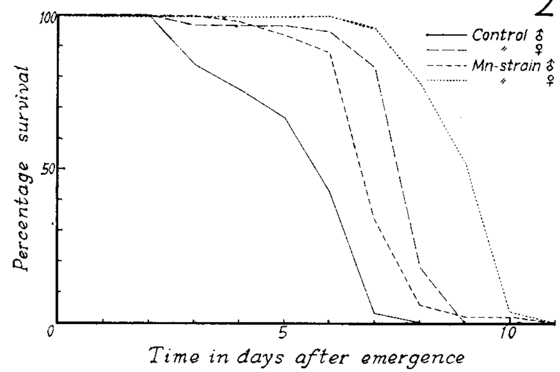
Examinations of differences (t-test).

♂ $0.001 > \alpha$* ♀ $0.001 > \alpha$* * significant



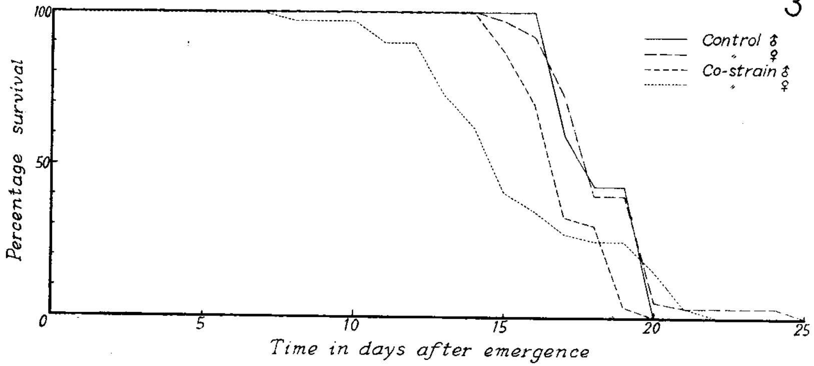
Control ♂
〃 ♀
Cu-strain ♂
〃 ♀
Percentage survival
100
50
0
5
10
Time in days after emergence

1



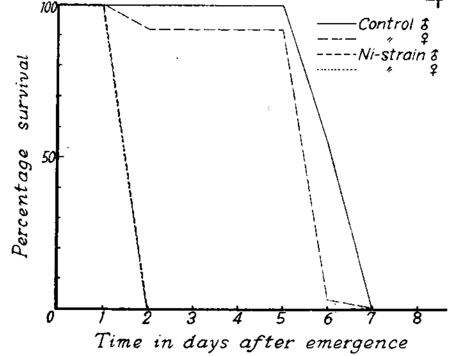
Control ♂
〃 ♀
Mn-strain ♂
〃 ♀
Percentage survival
100
50
0
5
10
Time in days after emergence

2



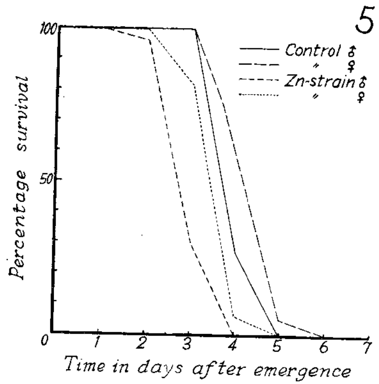
Control ♂
〃 ♀
Co-strain ♂
〃 ♀
Percentage survival
100
50
0
5
10
15
20
25
Time in days after emergence

3



Control ♂
〃 ♀
Ni-strain ♂
〃 ♀
Percentage survival
100
50
0
1
2
3
4
5
6
7
8
Time in days after emergence

4



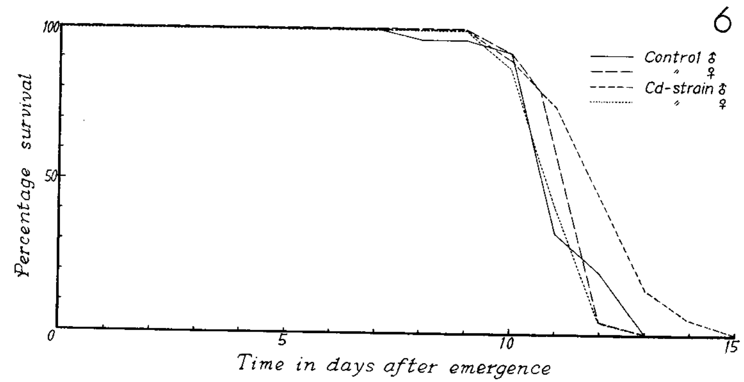


Fig. 3. Results of the longevity test with 0.1 M sucrose solution containing various metallic salts.

1. Survival curves of the control and Cu-strains on the solution containing 4 mM of $CuSO_4$.
2. Survival curves of the control and Mn-stsains on the solution containing 15 mM $MnSO_4$.
3. Survival curves of the control and Co-strains on the solutions containing 0.5 mM $CoSO_4$.
4. Survival curves of the control and Ni-strains on the solution containing 5.0 mM $NiSO_4$.
5. Survival curves of the control and Zn-strains on the solution containing 20 mM $ZnSO_4$.
6. Survival curves of the control and Cd-strains on the solution containing 0.07 mM $CdSO_4$.

Table 13. Results of the longevity test performed with the control and Cd-strains in 0.1 M sucrose solution containing 0.07 mM $CdSO_4$.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of flies	♀ Mean length of life (days)	♀ Standard deviation
Control	60	11.63	1.02	60	11.76	0.56
Cd-strain	40	11.80	0.98	50	11.34	0.70

Examinations of differences (t-test).

♂ $0.50 > \alpha > 0.40$ ♀ $0.001 > \alpha$* * significant

Table 14. Results of the longevity test performed with all the variant strains in simple 0.1 M sucrose solution.

Strain	♂ Number of flies	♂ Mean length of life (days)	♂ Standard deviation	♀ Number of flies	♀ Mean length of life (days)	♀ Standerd deviation
Control	60	24.98	3.87	60	23.70	3.80
Cu	70	26.27	2.76	70	26.22	7.71
Mn	50	27.60	3.52	50	26.14	2.81
Co	40	26.75	5.33	40	25.40	4.19
Ni	40	19.60	7.99	40	20.42	5.66
Zn	50	26.28	6.05	50	24.88	4.17
Cd	40	27.62	6.38	40	24.90	3.72

Examinations of differences in longevity (t-test).

		♂	♀
Control-strain	: Cu-strain	$0.05 > \alpha > 0.02$*	$0.01 > \alpha > 0.001$*
	: Mn	$0.001 > \alpha$*	$0.001 > \alpha$*
	: Co	$0.10 > \alpha > 0.05$	$0.05 > \alpha > 0.02$*
	: Ni	$0.001 > \alpha$*	$0.01 > \alpha > 0.001$*
	: Zn	$0.20 > \alpha > 0.10$	$0.20 > \alpha > 0.10$
	: Cd	$0.02 > \alpha > 0.01$*	$0.20 > \alpha > 0.10$
Cu-strain	: Mn-strain	$0.05 > \alpha > 0.02$*	$0.90 > \alpha > 0.80$
	: Co	$0.60 > \alpha > 0.50$	$0.20 > \alpha > 0.10$
	: Ni	$0.001 > \alpha$*	$0.001 > \alpha$*
	: Zn	$\alpha > 0.90$	$0.05 > \alpha > 0.02$*
	: Cd	$0.20 > \alpha > 0.10$	$0.05 > \alpha > 0.02$*
Mn-strain	: Co-strain	$0.40 > \alpha > 0.30$	$0.40 > \alpha > 0.30$
	: Ni	$0.001 > \alpha$*	$0.001 > \alpha$*
	: Zn	$0.20 > \alpha > 0.10$	$0.10 > \alpha > 0.05$
	: Cd	$\alpha > 0.90$*	$0.10 > \alpha > 0.05$
Co-strain	: Ni-strain	$0.001 > \alpha$*	$0.001 > \alpha$*
	: Zn	$0.70 > \alpha > 0.60$	$0.60 > \alpha > 0.50$
	: Cd	$0.60 > \alpha > 0.50$	$0.60 > \alpha > 0.50$
Ni-strain	: Zn-strain	$0.001 > \alpha$*	$0.001 > \alpha$*
	: Cd	$0.001 > \alpha$*	$0.001 > \alpha$*
Zn-strain	: Cd-strain	$0.40 > \alpha > 0.30$	$\alpha > 0.90$

* significant

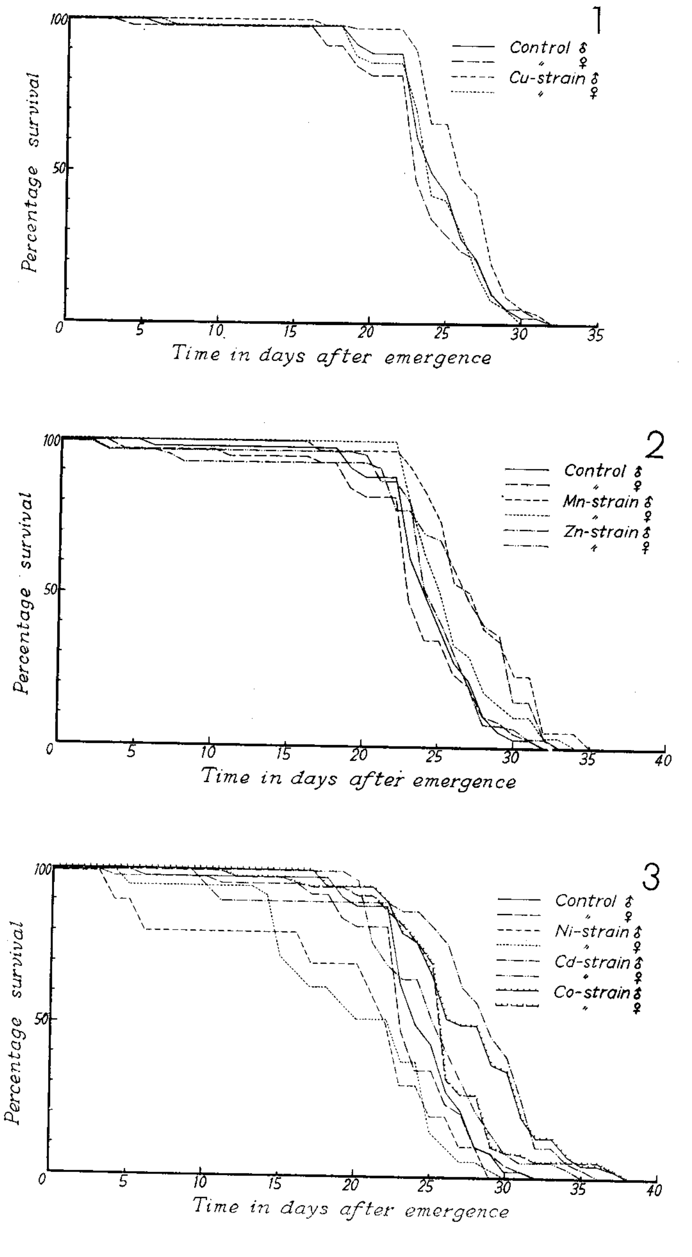


Fig. 4. Results of the longevity test with 0.1 M of sucrose solution.

1. Survival curves of the control and Cu-strains.
2. Survival curves of the control, Zn- and Mn-strains.
3. Survival curves of the control, Ni-, Cd- and Co-strains.

case of the Co-strain, male flies did not differ from the control one but female flies lived longer than the control. In the Cd-strain, male flies showed a tendency to live longer than the control strain and in the Zn-strain, no difference in the longevity from the control strain was observed regardless of sex.

The above-mentioned results suggest that the flies whose parents had been spent in salt-supplemented media during larval stages showed markedly changed characters in physiological activities.

f. *Enzyme inhibitor test*

In order to add further knowledges about differences in physiological activities among the strains mentioned above, the effects of some enzyme inhibitors different in their physiological actions on emergence rates were studied.

Enzyme inhibitors used were 2, 4-dinitrophenol, As_2O_3, NaF and *p*-chloromerc-uribenzoate. Both the concentrations of the inhibitors and the numbers of culture

Table 16. Examinations of differences in emergence rates among strains (χ^2-test).

Between strains	Inhibitor			
	NaF	2. 4-Dinitrophenol	As_2O_3	P. C. M. B.
Control : Cu	$0.20>\alpha>0.10$	$0.70>\alpha>0.50$	$0.01>\alpha>0.001$*	$0.99>\alpha>0.98$
: Mn	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.001>\alpha>$*	$0.90>\alpha>0.80$
: Co	$0.30>\alpha>0.20$	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$
: Ni	$0.30>\alpha>0.20$	$0.20>\alpha>0.10$	$0.20>\alpha>0.10$	$0.95>\alpha>0.90$
: Zn	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.70>\alpha>0.50$
: Cd	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.02>\alpha>0.01$*	$0.80>\alpha>0.70$
Cu : Mn	$0.02>\alpha>0.01$*	$0.20>\alpha>0.10$	$0.30>\alpha>0.20$	$0.70>\alpha>0.50$
: Co	$0.30>\alpha>0.20$	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$
: Ni	$0.50>\alpha>0.30$	$0.20>\alpha>0.10$	$0.10>\alpha>0.05$	$0.70>\alpha>0.50$
: Zn	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$
: Cd	$0.20>\alpha>0.10$	$0.01>\alpha>0.001$*	$0.95>\alpha>0.90$	$\alpha>0.99$
Mn : Co	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.90>\alpha>0.80$	$0.30>\alpha>0.20$
: Ni	$0.05>\alpha>0.02$*	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$\alpha>0.99$
: Zn	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.02>\alpha>0.01$*	$0.95>\alpha>0.90$
: Cd	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.80>\alpha>0.70$
Co : Ni	$0.30>\alpha>0.20$	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.90>\alpha>0.80$
: Zn	$0.50>\alpha<0.30$	$0.05>\alpha>0.02$*	$0.10>\alpha>0.05$	$0.90>\alpha>0.80$
: Cd	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.50>\alpha>0.30$	$0.30>\alpha>0.20$
Ni : Zn	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.95>\alpha>0.90$
: Cd	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$
Zn : Cd	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$

* significant

Table 15. Emergence rates of various strains on the media containing various kinds of enzyme inhibitors. Figures in parentheses show numbers of bottles.

Strain / Inhibitor	m Mol	Control No. of larvae	Control Pupated No.	%	Control Emerged No.	%	Cu-strain No. of larvae	Cu-strain Pupated No.	%	Cu-strain Emerged No.	%	Mn-strain No. of larvae	Mn-strain Pupated No.	%	Mn-strain Emerged No.	%	Co-strain No. of larvae	Co-strain Pupated No.	%	Co-strain Emerged No.	%
NaF	2	140 (7)			99	70.1	140 (7)			86	61.4	140 (7)			108	77.1	140 (7)			66	47.1
		140 (7)			94	67.1	200 (10)			116	58.0	140 (7)			112	80.0	140 (7)			78	55.4
		140 (7)			92	65.7															
	Total	420 (21)			285	67.9	340 (17)			202	59.4	280 (14)			220	78.6	280 (14)			144	51.4
	3	200 (10)			62	31.0	140 (7)			50	35.7	140 (7)			54	38.6	140 (7)			29	20.7
		140 (7)			57	40.7	140 (7)			38	27.1	140 (7)			57	40.7	140 (7)			40	28.6
		140 (7)			48	34.3															
	Total	480 (24)			167	34.8	280 (14)			88	31.4	280 (14)			111	39.6	280 (14)			69	24.6
	4	140 (7)			27	19.3	140 (7)			22	15.7	140 (7)			39	27.9	140 (7)			16	11.4
		140 (7)			30	21.4	140 (7)			11	7.9	140 (7)			37	26.4	140 (7)			9	6.4
		140 (7)			22	15.7	140 (7)			10	7.1										
	Total	420 (21)			79	18.8	420 (21)			43	10.2	280 (14)			76	27.1	280 (14)			25	8.9
2, 4-dinitrophenol	0.1	140 (7)			127	90.7	140 (7)			121	86.4	140 (7)			117	83.6	140 (7)			135	96.4
		140 (7)			125	89.3	140 (7)			107	76.4	140 (7)			112	80.0	140 (7)			126	90.0
		120 (6)			109	90.8															
	Total	400 (20)			361	90.3	280 (14)			228	81.4	280 (14)			229	81.8	280 (14)			261	93.2
	0.2	140 (7)			68	48.6	140 (7)			57	40.7	140 (7)			49	35.0	140 (7)			107	76.4
		140 (7)			64	45.7	140 (7)			53	37.9	140 (7)			47	33.6	140 (7)			91	65.0
		140 (7)			56	40.0															
	Total	420 (21)			188	44.8	280 (14)			110	39.3	280 (14)			96	34.3	280 (14)			198	70.0
	0.3	140 (7)			45	32.1	140 (7)			38	20.0	140 (7)			39	27.9	140 (7)			72	51.4
		140 (7)			40	28.6	140 (7)			21	22.1	140 (7)			41	29.3	140 (7)			57	40.7
		140 (7)			33	23.6															
	Total	420 (21)			118	28.1	280 (14)			59	21.1	280 (14)			80	28.6	280 (14)			129	46.1

Strain / Inhibitor	m Mol	Ni-strain No. of larvae	Ni-strain Pupated No.	%	Ni-strain Emerged No.	%	Zn-strain No. of larvae	Zn-strain Pupated No.	%	Zn-strain Emerged No.	%	Cd-strain No. of larvae	Cd-strain Pupated No.	%	Cd-strain Emerged No.	%
NaF	2	140 (7)			83	59.3	140 (7)			58	41.4	140 (7)			79	56.4
		140 (7)			78	55.7	140 (7)			84	60.0	140 (7)			77	55.0
	Total	280 (14)			161	57.5	280 (14)			142	50.7	280 (14)			156	55.7
	3	140 (7)			51	36.4	140 (7)			49	35.0	140 (7)			31	22.1
		140 (7)			53	37.9	140 (7)			52	37.1	140 (7)			29	20.7
							100 (5)			35	35.0					
	Total	280 (14)			104	37.1	380 (19)			136	35.8	280 (14)			60	21.4
	4	140 (7)			19	13.6	140 (7)			15	10.7	140 (7)			17	12.1
		140 (7)			20	14.3	140 (7)			12	8.6	140 (7)			18	12.9
							100 (5)			13	13.0					
	Total	280 (14)			39	13.9	380 (19)			40	10.5	280 (14)			35	12.5
2, 4-dinitrophenol	0.1	140 (7)			109	77.9	140 (7)			124	88.6	140 (7)			117	83.8
		140 (7)			111	79.3	140 (7)			119	85.0	140 (7)			120	85.7
	Total	280 (14)			220	78.6	280 (14)			243	86.8	280 (14)			237	84.6
	0.2	140 (7)			69	47.3	140 (7)			102	72.9	140 (7)			88	62.9
		140 (7)			74	52.9	140 (7)			90	64.3	140 (7)			93	66.4
	Total	280 (14)			143	51.1	280 (14)			192	68.6	280 (14)			181	64.6
	0.3	140 (7)			30	21.4	140 (7)			88	62.9	140 (7)			33	23.6
		140 (7)			32	22.9	140 (7)			85	60.7	140 (7)			32	22.9
	Total	280 (14)			62	22.1	280 (14)			173	61.8	280 (14)			65	23.2

Strain		Control					Cu-strain					Mn-strain				
Inhibitor	m Mol	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%
As_2O_3	0.5	140 (7)	100	71.4	88	62.9	140 (7)	93	66.4	72	51.4	140 (7)	90	64.3	48	34.3
		140 (7)	106	75.7	87	62.1	140 (7)	101	72.1	82	58.6	140 (7)	82	58.6	49	35.0
		140 (7)	102	72.9	98	70.0										
	Total	420 (21)	308	73.3	273	65.0	280 (14)	194	69.3	154	55.0	280 (14)	172	61.4	97	34.6
	0.6	140 (7)	87	62.1	50	35.7	200 (10)	132	66.0	38	19.0	140 (7)	46	32.9	8	5.7
		140 (7)	78	55.7	38	27.1	140 (7)	81	57.9	20	14.3	140 (7)	42	30.0	11	7.9
	Total	280 (14)	165	58.9	88	31.4	340 (17)	213	62.6	58	17.1	280 (14)	88	31.4	19	6.8
	0.75	140 (7)	21	15.0	8	5.7	140 (7)	7	5.0	—	—	140 (7)	1	0.7	—	—
		140 (7)	18	12.9	3	2.1	140 (7)	12	8.6	—	—	140 (7)	—	—	—	—
		140 (7)	27	19.3	4	2.9	140 (7)	2	1.4	1	0.7					
	Total	420 (21)	66	15.7	15	3.6	420 (21)	21	5.0	1	0.2	280 (14)	1	0.4	—	—
P. C. M. B.	0.015	140 (7)			131	93.5	140 (7)			114	81.4	140 (7)			122	87.1
		200 (10)			180	90.0	140 (7)			131	93.6	140 (7)			130	92.9
		140 (7)			131	93.5										
	Total	480 (24)			442	92.1	280 (14)			245	87.5	280 (14)			252	90.0
	0.020	140 (7)			125	89.3	140 (7)			121	87.4	160 (8)			131	81.9
		200 (10)			176	88.0	160 (8)			144	90.0	160 (8)			127	79.4
		140 (7)			113	80.7										
	Total	480 (24)			414	86.3	300 (15)			265	88.3	320 (16)			258	80.6
	0.025	140 (7)			114	81.4	140 (7)			113	80.7	140 (7)			108	77.1
		200 (10)			168	84.0	140 (7)			110	78.6	140 (7)			100	71.4
		120 (6)			96	80.0										
	Total	460 (23)			378	82.2	280 (14)			223	79.6	280 (14)			208	74.3

Strain		Co-strain					Ni-strain				
Inhibitor	m Mol	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%
As_2O_3	0.5	200 (10)	104	52.0	71	35.5	140 (7)	81	57.9	53	37.9
		140 (7)	64	45.7	46	32.9	140 (7)	79	56.4	54	38.6
	Total	340 (17)	168	49.4	117	34.4	280 (14)	160	57.1	107	38.2
	0.6	200 (10)	53	26.5	18	9.0	140 (7)	63	45.0	31	22.1
		140 (7)	31	22.1	10	7.1	140 (7)	70	50.0	29	20.7
	Total	340 (17)	84	24.7	28	8.2	280 (14)	133	47.5	60	21.4
	0.75	200 (10)	5	2.5	—	—	140 (7)	6	4.3	1	0.7
		200 (10)	—	—	—	—	140 (7)	3	2.1	—	—
		140 (7)	1	0.7	—	—					
	Total	540 (27)	6	1.1	—	—	280 (14)	9	3.2	1	0.4
P. C. M. B.	0.015	140 (7)			126	90.0	140 (7)			124	88.6
		140 (7)			117	83.6	140 (7)			127	90.7
	Total	280 (14)			243	86.8	280 (14)			251	89.6
	0.020	140 (7)			102	72.9	140 (7)			115	82.1
		140 (7)			100	71.4	140 (7)			110	78.6
	Total	280 (14)			202	72.1	280 (14)			225	80.4
	0.025	140 (7)			97	69.3	140 (7)			109	77.9
		140 (7)			98	70.0	140 (7)			101	72.1
	Total	280 (14)			195	69.6	280 (14)			210	75.0

Strain		Zn-strain					Cd-strain				
Inhibitor	m Mol	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Pupated No.	%	Emerged No.	%
As_2O_3	0.5	140 (7)	80	57.1	50	35.7	160 (8)	59	36.9	29	18.1
		140 (7)	67	47.9	43	30.7	160 (8)	57	35.6	29	18.1
	Total	280 (14)	147	52.5	93	33.2	320 (16)	116	36.3	58	18.1
	0.6	140 (7)	54	38.6	23	16.4	160 (8)	36	22.5	5	3.1
		140 (7)	48	34.3	20	14.3	160 (8)	37	23.1	5	3.1
	Total	280 (14)	102	36.4	43	15.4	320 (16)	73	22.8	10	3.1
	0.75	140 (7)	5	3.6	1	0.7	160 (8)	26	16.3	1	0.6
		140 (7)	9	6.4	—	—	160 (8)	20	12.5	—	—
	Total	280 (14)	14	5.0	1	0.4	320 (16)	46	14.4	1	0.3
P. C. M. B.	0.015	140 (7)			118	84.3	200 (10)			174	87.0
		140 (7)			127	90.7	140 (7)			116	82.9
	Total	280 (14)			245	87.5	340 (17)			290	85.3
	0.020	140 (7)			107	76.4	200 (10)			166	83.0
		120 (6)			92	76.7	140 (7)			131	93.6
	Total	260 (13)			199	76.5	340 (17)			297	87.4
	0.025	140 (7)			96	68.6	160 (8)			126	78.8
		140 (7)			97	69.3	140 (7)			110	78.6
	Total	280 (14)			193	68.9	300 (15)			236	78.7

Table 17. Examinations of differences in emergence rates among strains, according to concentrations of enzyme inhibitors (χ^2-test).

Inhibitor / m Mol / Between strains	NaF			2, 4-dinitrophenol			As_2O_3			P. C. M. B.		
	2	3	4	0.1	0.2	0.3	0.5	0.6	0.75	0.015	0.020	0.025
Control : Cu	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.20>\alpha>0.10$	$0.05>\alpha>0.02$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$
: Mn	$0.01>\alpha>0.001$*	$0.30>\alpha>0.20$	$0.02>\alpha>0.01$*	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.98>\alpha>0.95$	$0.001>\alpha$*	$0.001>\alpha$*	—	$0.50>\alpha>0.30$	$0.05>\alpha>0.02$*	$0.02>\alpha>0.01$*
: Co	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	—	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
: Ni	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.02>\alpha>0.01$*	$0.50>\alpha>0.30$	$0.05>\alpha>0.02$*	$0.05>\alpha>0.02$
: Zn	$0.001>\alpha$*	$0.90>\alpha>0.80$	$0.01>\alpha>0.001$*	$0.20>\alpha>0.10$	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.02>\alpha>0.01$*	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*
: Cd	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.05>\alpha>0.02$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*	$0.95>\alpha>0.90$	$0.30>\alpha>0.20$
Cu : Mn	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.01>\alpha>0.001$*	$\alpha>0.99$	$0.30>\alpha>0.20$	$0.05>\alpha>0.02$*	$0.001>\alpha$*	$0.001>\alpha$*	—	$0.50>\alpha>0.30$	$0.02>\alpha>0.01$*	$0.20>\alpha>0.10$
: Co	$0.10>\alpha>0.05$	$0.10>\alpha>0.05$	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	—	$0.90>\alpha>0.80$	$0.001>\alpha$*	$0.01>\alpha>0.001$*
: Ni	$0.70>\alpha>0.50$	$0.20>\alpha>0.10$	$0.20>\alpha>0.10$	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.90>\alpha>0.80$	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.70>\alpha>0.50$	$0.70>\alpha>0.50$	$0.02>\alpha>0.01$*	$0.30>\alpha>0.20$
: Zn	$0.05>\alpha>0.02$*	$0.30>\alpha>0.20$	$0.99>\alpha>0.98$	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.70>\alpha>0.50$	$0.90>\alpha>0.80$	$0.001>\alpha$*	$0.01>\alpha>0.001$*
: Cd	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.001>\alpha$*	—	$0.50>\alpha>0.30$	$0.80>\alpha>0.70$	$0.90>\alpha>0.80$
Mn : Co	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.99>\alpha>0.98$	$0.70>\alpha>0.50$	—	$0.30>\alpha>0.20$	$0.02>\alpha>0.01$*	$0.30>\alpha>0.20$
: Ni	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.50>\alpha>0.30$	$0.001>\alpha$*	—	$\alpha>0.99$	$0.99>\alpha>0.98$	$0.95>\alpha>0.90$
: Zn	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.01>\alpha>0.001$*	—	$0.50>\alpha>0.30$	$0.10>\alpha>0.05$	$0.20>\alpha>0.10$
: Cd	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.10>\alpha>0.05$	—	$0.20>\alpha>0.10$	$0.05>\alpha>0.02$*	$0.30>\alpha>0.20$
Co : Ni	$0.20>\alpha>0.10$	$0.01>\alpha>0.001$*	$0.10>\alpha>0.05$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*	—	$0.50>\alpha>0.30$	$0.05>\alpha>0.02$*	$0.20>\alpha>0.10$
: Zn	$0.80>\alpha>0.70$	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$	$0.02>\alpha>0.01$*	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.90>\alpha>0.80$	$0.01>\alpha>0.001$*	—	$0.90>\alpha>0.80$	$0.30>\alpha>0.20$	$0.95>\alpha>0.90$
: Cd	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.30>\alpha>0.20$	$0.05>\alpha>0.02$*	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*	—	$0.70>\alpha>0.50$	$0.001>\alpha$*	$0.02>\alpha>0.01$*
Ni : Zn	$0.20>\alpha>0.10$	$0.80>\alpha>0.70$	$0.30>\alpha>0.20$	$0.02>\alpha>0.01$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.30>\alpha>0.20$	$0.10>\alpha>0.05$	$\alpha>0.99$	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$	$0.20>\alpha>0.10$
: Cd	$0.80>\alpha>0.70$	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.10>\alpha>0.05$	$0.01>\alpha>0.001$*	$0.90>\alpha>0.80$	$0.001>\alpha$*	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.20>\alpha>0.10$	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$
Zn : Cd	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*

* significant

bottles used are shown in Table 15. Fundamental methods used in this test are described in the previous report (YANAGISHIMA 1961 a).

In Table 15, the results of this test and in Tables 16 and 17, their statistical examinations, are shown respectively.

The Cu–, Ni–, Zn– and Cd– strains were all more sensitive to all the enzyme inhibitors tested than the control strain ; in other words, there was no specificity in reaction to enzyme inhibitors. The Mn–strain was more sensitive to all the enzyme inhibitors except NaF, and the Co–strain was more resistant to 2, 4–dinitrophenol but more sensitive to the other enzyme inhibitors, than the control strain.

It can be said from these results that raising in sensitivity of flies to various kinds of enzyme inhibitors is observed when their parents have been trained with metallic salts and this phenomenon may probably indicate that raising in activity of metabolism in general is taken place with the treatment in the sublethal metallic salts.

II. Continuance of Acquired Characters

In the 5 th report, the present author (1961 c), has demonstrated that the copper resistance acquired by training larvae in a medium containing sublethal dose of copper, can be transmitted from generation to generation through sexual reproductions during culture in the normal medium. Furthermore, it has been demonstrated here that some metallic salts other than $CuSO_4$ can also produce resistant strains, just as $CuSO_4$ did. It is of great interest to know whether these resistant strains behave like the copper resistant strain in hereditary patterns. Some experiments performed to know the hereditary characters of these resistant strains will be described in the following.

Resistant strains used in this test were obtained by rearing larvae of the normal strain in each culture medium containing sublethal dose of each metallic salt. The adult flies which had emerged were transferred to oviposition tubes for laying eggs. The larvae hatched out from these eggs were transferred to the normal medium to grow up to adult flies (F_1 generation). These flies of F_1 generation were made to deposit eggs on the normal medium and the larvae hatched (F_2 generation) were transferred to the test media containing ED_{50} of metallic salts to measure emergence rates. In this case, the flies subjected to the test media were denoted as the Cu–, Mn–, Co–, Zn–, Ni and Cd–strains, according to the metal with which the grand parents had been trained. The experimental results are shown in Table 18. The examinations of results were performed referring to this table together with the results described in Table 1, which shows the emergence rates of flies in the ED_{50} test medium, whose parents had grown up in the sublethal training medium. The results may be summarized as follows :

Table 18. Emergence rates of various variant strains in the
For the method to make variant strains see text.

Strain	Control			Cu-strain			Mn-strain		
Test medium	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%
$CuSO_4$	100(5)	53	53.0	140(7)	94	67.1	140(7)	70	50.0
	100(5)	56	56.0	140(7)	98	70.0	140(7)	93	66.4
Total	200(10)	109	54.5	280(14)	192	68.5	280(14)	163	58.2
$MnSO_4$	100(5)	53	53.0	100(5)	57	57.0	160(8)	78	48.8
	100(5)	61	61.0	140(7)	77	55.0	140(7)	67	47.9
Total	200(10)	114	57.0	240(12)	134	55.8	300(15)	145	48.3
$CoSO_4$	100(5)	62	62.0	100(5)	54	54.0	160(8)	118	73.8
	100(5)	67	67.0	140(7)	86	61.4	140(7)	97	69.2
Total	200(10)	129	64.5	240(12)	140	58.3	300(15)	215	71.6
$NiSO_4$	100(5)	52	52.0	100(5)	58	58.0	160(8)	77	48.1
	100(5)	55	55.0	140(7)	69	49.3	140(7)	64	45.7
Total	200(10)	107	53.5	240(12)	127	52.9	300(15)	141	47.0
$ZnSO_4$	100(5)	46	46.0	100(5)	49	49.0	150(8)	51	31.9
	100(5)	55	55.0	140(7)	79	56.4	140(7)	43	30.7
Total	200(10)	101	50.5	240(12)	128	53.5	300(15)	94	31.3
$CdSO_4$	100(5)	54	54.0	100(5)	37	37.0	160(8)	95	59.4
	100(5)	43	43.0	140(7)	56	40.6	140(7)	85	60.7
Total	200(10)	97	48.5	240(12)	93	38.7	300(15)	180	60.0

a. *Examinations of the results shown in Table 18*

The results of the statistical examinations are shown in Tables 19~20. We can see the following facts from the results.

1) When the cu-strain was cultured in the normal medium for one generation, the acquired copper resistance did not change, but the cross resistance to $NiSO_4$ and $ZnSO_4$ media became so weak that no difference could be found from the control strain, and the collateral sensitivity to $CdSO_4$ and $CoSO_4$ became insignificant statistically, though the emergence rate was lower than the control strain.

2) The Mn-strain could keep the cross resistance to $CoSO_4$ and $CdSO_4$ media and the collateral sensitivity to $NiSO_4$ and $ZnSO_4$ media, and it showed a lower emergence rate in $MnSO_4$ medium than the control though the difference was statistically insignificant.

3) The Co-strain could keep the cross resistance to $CuSO_4$, the collateral sensitivity to $CdSO_4$, $NiSO_4$ and $MnSO_4$; at the latter two cases, however, the degrees were insignificant statistically. It is of interest that $CoSO_4$ resistance of the Co-strain became statistically insignificant, though the emergence rate itself was higher than

test media containing ED_{50} doses of various metallic salts.
Figures in parentheses show numbers of bottles.

Co-strain			Ni-strain			Zn-strain			Cd-strain		
No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%
100(5)	69	69.0	100(5)	54	54.0	100(5)	64	64.0	200(10)	135	67.5
140(7)	89	63.6	140(7)	83	59.2	140(7)	83	59.2	140(7)	97	69.2
240(12)	158	65.8	240(12)	137	57.0	240(12)	147	61.2	340(17)	232	68.2
140(7)	72	51.4	100(5)	48	48.0	100(5)	59	59.0	140(7)	68	48.6
140(7)	70	50.0	140(7)	67	47.8	140(7)	77	55.0	140(7)	70	50.0
280(14)	142	50.7	240(12)	115	47.9	240(12)	136	56.7	280(14)	138	49.2
160(8)	117	73.1	140(7)	111	79.3	140(7)	106	75.7	160(8)	104	65.0
140(7)	98	70.0	140(7)	97	69.2	140(7)	98	70.0	140(7)	85	60.7
300(15)	215	71.6	280(14)	208	74.2	280(14)	204	72.8	300(15)	189	63.0
100(5)	46	46.0	100(5)	68	68.0	100(5)	58	58.0	160(8)	97	60.6
140(7)	68	48.6	140(7)	74	52.8	140(7)	71	50.7	140(7)	88	62.9
240(12)	114	47.5	240(12)	142	59.1	240(12)	129	53.7	300(15)	185	61.6
140(7)	77	55.0	120(6)	70	58.3	140(7)	72	51.4	160(8)	96	60.0
140(7)	69	49.3	140(7)	68	48.6	140(7)	69	49.3	140(7)	81	57.8
280(14)	146	52.1	260(13)	138	53.0	280(14)	141	50.3	300(15)	177	59.0
100(5)	10	10.0	100(5)	13	13.0	120(6)	35	29.2	140(7)	12	8.5
140(7)	16	11.4	140(7)	16	11.4	140(7)	43	30.0	140(7)	10	7.1
240(12)	26	10.8	240(12)	29	12.0	260(13)	78	30.0	280(14)	22	7.8

that of the control strain.

4) The Ni-strain could keep the cross resistance to $CoSO_4$ and the collateral sensitivity to $CdSO_4$, but it did not show significant resistance to $NiSO_4$.

5) The Zn-strain could keep the cross resistance to $CoSO_4$ (though the difference was statistically insignificant) and the collateral sensitivity to $CdSO_4$. No difference was seen in the emergence rate in $ZnSO_4$ test medium between the control and the Zn-strains.

6) The Cd-strain could keep the cross resistance to $CuSO_4$, $NiSO_4$ and $ZnSO_4$, and the collateral sensitivity to $MnSO_4$. It showed a lower emergence rate in $CdSO_4$ medium than the control. The difference was statistically significant in both $CuSO_4$ and $CdSO_4$ test media.

b. *Comparison of the results shown in Table 18 with those in Table 1*

Table 18 was obtained with the flies whose grand parents had been subjected to the sublethal doses of various metallic salts but parents were cultured by the normal medium. On the other hand, the results shown in Table 1 were obtained by using the flies which had grown up in the medium containing sublethal doses

Table 19. Examinations of differences in emergence rates of various strains

Between media	Control	Cu	Mn
Control : $CuSO_4$			
: $MnSO_4$			
: $CoSO_4$			
: $NiSO_4$			
: $ZnSO_4$			
: $CdSO_4$			
Cu : $MnSO_4$	$0.70>\alpha>0.50$	$0.01>\alpha>0.001$*	$0.05>\alpha>0.02$*
: $CoSO_4$	$0.10>\alpha>0.05$	$0.02>\alpha>0.01$*	$0.001>\alpha$*
: $NiSO_4$	$0.90>\alpha>0.80$	$0.001>\alpha$*	$0.01>\alpha>0.001$*
: $ZnSO_4$	$0.50>\alpha>0.30$	$0.001>\alpha$*	$0.001>\alpha$*
: $CdSO_4$	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.80>\alpha>0.70$
Mn : $CoSO_4$	$0.20>\alpha>0.10$	$0.70>\alpha>0.50$	$0.001>\alpha$*
: $NiSO_4$	$0.70>\alpha>0.50$	$0.10>\alpha>0.05$	$0.90>\alpha>0.80$
: $ZnSO_4$	$0.30>\alpha>0.20$	$0.70>\alpha>0.50$	$0.001>\alpha$*
: $CdSO_4$	$0.20>\alpha>0.10$	$0.01>\alpha$*	$0.001>\alpha>0.001$*
Co : $NiSO_4$	$0.30>\alpha>0.20$	$0.30>\alpha>0.20$	$0.001>\alpha$*
: $ZnSO_4$	$0.01>\alpha>0.001$*	$0.50>\alpha>0.30$	$0.001>\alpha$*
: $CdSO_4$	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*
Ni : $ZnSO_4$	$0.70>\alpha>0.50$	equal	$0.001>\alpha$*
: $CdSO_4$	$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.01>\alpha>0.001$*
Zn : $CdSO_4$	$0.80>\alpha>0.70$	$0.01>\alpha>0.001$*	$0.001>\alpha$*

* significant

Table 20. Examinations of differences in emergence rates on various media

Between strains	$CuSO_4$	$MnSO_4$	$CoSO_4$
Control : Cu	$0.01>\alpha>0.001$*	$0.90>\alpha>0.80$	$0.30>\alpha>0.20$
: Mn	$0.50>\alpha>0.30$	$0.10>\alpha>0.05$	$0.20>\alpha>0.10$
: Co	$0.05>\alpha>0.02$*	$0.30>\alpha>0.20$	$0.20>\alpha>0.10$
: Ni	$0.70>\alpha>0.50$	$0.10>\alpha>0.05$	$0.05>\alpha>0.02$*
: Zn	$0.20>\alpha>0.10$	$0.98>\alpha>0.95$	$0.10>\alpha>0.05$
: Cd	$0.01>\alpha>0.001$*	$0.20>\alpha>0.10$	$0.90>\alpha>0.80$
Cu : Mn	$0.02>\alpha>0.01$*	$0.10>\alpha>0.05$	$0.01>\alpha>0.001$*
: Co	$0.70>\alpha>0.50$	$0.30>\alpha>0.20$	$0.01>\alpha>0.001$*
: Ni	$0.01>\alpha>0.001$*	$0.10>\alpha>0.05$	$0.001>\alpha$*
: Zn	$0.20>\alpha>0.10$	$0.95>\alpha>0.90$	$0.001>\alpha$*
: Cd	$\alpha>0.99$	$0.20>\alpha>0.10$	$0.50>\alpha>0.30$
Mn : Co	$0.10>\alpha>0.05$	$0.70>\alpha>0.50$	equal
: Ni	$0.90>\alpha>0.80$	$\alpha>0.99$	$0.70>\alpha>0.50$
: Zn	$0.70>\alpha>0.50$	$0.10>\alpha>0.05$	$0.90>\alpha>0.80$
: Cd	$0.02>\alpha>0.01$*	$0.90>\alpha>0.80$	$0.05>\alpha>0.02$*
Co : Ni	$0.10>\alpha>0.05$	$0.70>\alpha>0.50$	$0.50>\alpha>0.30$
: Zn	$0.50>\alpha>0.30$	$0.30>\alpha>0.20$	$0.90>\alpha>0.80$
: Cd	$0.70>\alpha>0.50$	$\alpha=0.80$	$0.05>\alpha>0.02$*
Ni : Zn	$0.50>\alpha>0.30$	$0.10>\alpha>0.05$	$0.80>\alpha>0.70$
: Cd	$0.01>\alpha>0.001$*	$0.90>\alpha>0.80$	$0.01>\alpha>0.001$*
Zn : Cd	$0.10>\alpha>0.05$	$0.20>\alpha>0.10$	$0.02>\alpha>0.01$*

* gignificans

among media different in metallic salt supplements (χ^2-test).

Strain	Co	Ni	Zn	Cd
	$0.001>\alpha$*	$0.10>\alpha>0.05$	$0.50>\alpha>0.30$	$0.001>\alpha$*
	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.01>\alpha>0.001$*	$0.70>\alpha>0.50$
	$0.001>\alpha$*	$0.80>\alpha>0.70$	$0.20>\alpha>0.10$	$0.10>\alpha>0.05$
	$0.01>\alpha>0.001$*	$0.50>\alpha>0.30$	$0.02>\alpha>0.01$*	$0.05>\alpha>0.02$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.01>\alpha>0.001$*
	$0.70>\alpha>0.50$	$0.02>\alpha>0.01$*	$0.70>\alpha>0.50$	$0.01>\alpha>0.001$*
	$0.95>\alpha>0.90$	$0.30>\alpha>0.20$	$0.20>\alpha>0.10$	$0.05>\alpha>0.02$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.95>\alpha>0.90$
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.50>\alpha>0.30$
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.50>\alpha>0.30$	$0.30>\alpha>0.20$	$0.50>\alpha>0.30$	$0.70>\alpha>0.50$
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*

among various strains (χ^2-test).

Medium	$NiSO_4$	$ZnSO_4$	$CdSO_4$
	$0.98>\alpha>0.95$	$0.70>\alpha>0.50$	$0.10>\alpha>0.05$
	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.02>\alpha>0.01$*
	$0.30>\alpha>0.20$	$0.50>\alpha>0.30$	$0.001>\alpha$*
	$0.30>\alpha>0.20$	$0.70>\alpha>0.50$	$0.001>\alpha$*
	$0.98>\alpha>0.95$	$0.98>\alpha>0.95$	$0.001>\alpha$*
	$0.10>\alpha>0.05$	$0.10>\alpha>0.05$	$0.001>\alpha$*
	$0.30>\alpha>0.20$	$0.001>\alpha$*	$0.001>\alpha$*
	$0.30>\alpha>0.20$	$0.90>\alpha>0.80$	$0.001>\alpha$*
	$0.20>\alpha>0.10$	$0.98>\alpha>0.95$	$0.001>\alpha$*
	$0.95>\alpha>0.90$	$0.70>\alpha>0.50$	$0.05>\alpha>0.02$*
	$0.10>\alpha>0.05$	$0.30>\alpha>0.20$	$0.001>\alpha$*
	$0.98>\alpha>0.95$	$0.001>\alpha$*	$0.001>\alpha$*
	$0.01>\alpha>0.001$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.20>\alpha>0.10$	$0.001>\alpha$*	$0.001>\alpha$*
	$0.001>\alpha$*	$0.001>\alpha$*	$0.001>\alpha$*
	$0.02>\alpha>0.01$*	$0.90>\alpha>0.80$	$0.80>\alpha>0.70$
	$0.01>\alpha>0.001$*	$0.80>\alpha>0.70$	$0.001>\alpha$*
	$0.01>\alpha>0.001$*	$0.20>\alpha>0.10$	$0.50>\alpha>0.30$
	$0.50>\alpha>0.30$	$0.70>\alpha>0.50$	$0.001>\alpha$*
	$0.70>\alpha>0.50$	$0.20>\alpha>0.10$	$0.20>\alpha>0.10$
	$0.10>\alpha>0.05$	$0.05>\alpha>0.02$*	$0.001>\alpha$*

Table 21. Examinations of differences in emergence rates on test media with sublethal training media during larval stage (Table 1) and

medium \ strain	Control	Cu	Mn
$CuSO_4$	0.70 > α > 0.50	0.30 > α > 0.20	0.80 > α > 0.70
$MnSO_4$	0.70 > α > 0.50	0.90 > α > 0.80	0.001 > α *
$CoSO_4$	0.70 > α > 0.50	0.50 > α > 0.30	0.10 > α > 0.05
$NiSO_4$	0.50 > α > 0.30	0.50 > α > 0.30	0.05 > α > 0.02*
$ZnSO_4$	0.95 > α > 0.90	0.02 > α > 0.01*	0.001 > α *
$CdSO_4$	0.50 > α > 0.30	0.02 > α > 0.01*	0.05 > α > 0.02*

* significant

of various metallic salts. The comparison between these two results is made in Table 21, the brief conclusion of which is as follows.

1) Cu-strain

The cross resistance to $ZnSO_4$ and collateral sensitivity to $CdSO_4$ decreased markedly when the parent flies were returned to the normal medium, but the resistance to other metallic salts did not change.

2) Mn-strain

The resistance to $CuSO_4$ and $CoSO_4$ did not change but that to $MnSO_4$ and $ZnSO_4$ decreased significantly, while that to $NiSO_4$ and $CdSO_4$ increased markedly.

3) Co-strain

The resistance to $MnSO_4$ increased highly significantly and the resistance to the other metallic salts did not change.

4) Ni-strain

The resistance to $CoSO_4$ and $ZnSO_4$ slightly changed but that to the other metallic salts decreased significantly.

5) Zn-strain

The resistance to $CuSO_4$ increased but that to $CdSO_4$ decreased, while that to the other metallic salts did not change.

6) Cd-strain

The resistance to $MnSO_4$, $CoSO_4$ and $CdSO_4$ decreased highly significantly, while that to the other metallic salts remained unchanged.

These results will be summarized as follows :

The resistance acquired by culturing larvae in media containing sublethal doses of metallic salts could be kept after having been returned to the normal medium for one generation in the cases of $CuSO_4$ and $CoSO_4$, but decreased significantly in the cases of the other metallic salts. The cross resistance and the collateral sensitivity exhibited by the above mentioned strains trained in media supplemented seen between the variant strains whose parents had been treated those whose grand parents had been treated (Table 18).

Co	Ni	Zn	Cd
$0.50>\alpha>0.30$	$0.01>\alpha>0.001$*	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$
$0.001>\alpha$*	$0.001>\alpha$*	$0.50>\alpha>0.30$	$0.001>\alpha$*
$0.70>\alpha>0.50$	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.001>\alpha$*
$0.70>\alpha>0.50$	$0.05>\alpha>0.02$*	$0.50>\alpha>0.30$	$\alpha=0.70$
$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.50>\alpha>0.30$	$0.20>\alpha>0.10$
$0.98>\alpha>0.95$	$0.001>\alpha$*	$0.05>\alpha>0.02$*	$0.001>\alpha$*

with sublethal doses of metallic salts, also more or less changed, after a culture of a generation in the normal medium.

At last, in order to know more clearly the hereditary pattern of the acquired resistance, an *Index of the acquired resistance* was devised, which was calculated as follows. Taking the emergence rate (shown in Table 1) of the control strain in a definite test medium as 1.00, the *relative values* of emergence rates of various strains in this test medium were calculated. These relative values are the indices of the acquired resistance of each strain, which show at the same time the degrees of cross resistance or collateral sensitivity. The results are shown in Table 22.

Table 22. Indices of acquired resistance which were calculated as relative values of emergence rates of various strains on a certain test medium, when the emergence rate of the control strain on each test medium was estimated as 1.00. The original values of emergence rate are seen in Table 1.

Test medium \ Strain	Control	Cu	Mn	Co	Ni	Zn	Cd
Control	1.00	0.92	0.96	0.96	0.97	0.96	0.98
$CuSO_4$	1.00	1.12	0.99	1.09	1.19	0.93	1.12
$MnSO_4$	1.00	1.02	1.26	0.54	1.15	1.10	1.27
$CoSO_4$	1.00	0.87	1.25	1.18	1.29	1.22	1.33
$NiSO_4$	1.00	1.13	0.80	0.89	1.35	1.16	1.22
$ZnSO_4$	1.00	1.22	0.90	0.95	1.08	1.04	1.15
$CdSO_4$	1.00	0.92	0.99	0.21	0.83	0.72	1.52

	media
Cu-strain	Zn > Ni > Cu > Mn > Control = Cd > Co
Mn-strain	Mn > Co > Cu = Cd > Control > Zn > Ni
Co-strain	Co > Cu > Control > Zu > Ni > Mn > Cd
Ni-strain	Ni > Co > Cu > Mn > Zn > Control > Cd
Zn-strain	Co > Ni > Mn > Zn > Control > Cu > Cd
Cd-strain	Cd > Co > Mn > Ni > Zn > Cu > Control

When the data of Table 18 are used, the results will become as Table 23.

From Table 22 we can see that : 1) When each strain is tested in the test medium containing the same kind of metal as it had been trained, the resistance is higher than the control. The resistance of the Zn-strain, however, does not change from the control. 2) Four strains (Mn, Co, Ni and Cd), except Cu and Zn, are the most resistant among the various strains when they are tested on the same media as they had been trained respectively (see the under column of the table).

Table 23. Indices of acquired resistance of the variants, whose parents were cultured in the normal medium and grand parents were cultured with media containing sublethal metallic salts. The original values of emergence rate are seen in Table 18.

Test medium \ Strain	Control	Cu	Mn	Co	Ni	Zn	Cd
$CuSO_4$	1.00	1.25	1.06	1.20	1.04	1.12	1.25
$MnSO_4$	1.00	0.97	0.84	0.88	0.84	0.99	0.86
$CoSO_4$	1.00	0.90	1.10	1.10	1.15	1.12	0.97
$NiSO_4$	1.00	0.98	0.87	0.88	1.10	1.00	1.15
$ZnSO_4$	1.00	1.05	0.61	1.03	1.04	0.99	1.16
$CdSO_4$	1.00	0.79	1.23	0.22	0.24	0.61	0.16

	media
Cu-strain	Cu>Zu>Control>Ni>Mn>Co>Cd
Mn-strain	Cd>Co>Cu>Control>Ni>Mn>Zn
Co-strain	Cu>Co>Zn>Control>Mn=Ni>Cd
Ni-strain	Co>Ni>Zn=Cu>Control>Mn>Cd
Zn-strain	Cu=Co>Ni=Control>Mn=Zn>Cd
Cd-strain	Cu>Zn>Ni>Control>Co>Mn>Cd

Then, from Table 23 we can see that : 1) The indices of resistance of Mn- and Cd-strains, especially of the latter, are fairly decreased, when they are tested in the media containing the same kind of metals their grand parents had been trained. This seems to indicate the appearance of bad effect of the training of the grand parents. 2) The value of the Zn-strain is nearly 1, and when considered together with the data of Table 22, it can be said that the effect of pretraining in Zn medium is negligible. 3) On the other hand, the acquired resistance of Cu-strain is most stable ; those of the Co- and Ni-strains rank next. 4) Generally, the degree of index of each strain obtained on the same medium as it had been trained is lower than that shown in Table 22, except the Cu-strain, and this seems to show the acquired resistance of most strains is rather temporary, though that of the Cu-strain is stable enough as has been explained in the previous reports in detail.

Discussion

In the previous reports (YANAGISHIMA and SUZUKI 1959 a, b, YANAGISHIMA 1961 a, b, c) the author has demonstrated that copper induces a resistance to copper under conditions where no or, if any, little selection is possible, and this resistance can be transmitted through sexual reproductions and maintained more than several generations in the normal medium. It is important to determine whether the copper resistance is specific phenomenon to copper, in order to know the mechanisms of resistance and origin of resistance. In the present report, some experiments designed along this line were described.

It is clear from the results mentioned in this paper that not only copper but also other bivalent metals can induce resistant variations, but each variation caused by each metal is markedly specific. Among the metals treated, cobalt and copper show the same tendency to cause relatively stable resistance which can be kept through sexual reproductions for more than one generation on the normal culture medium, but the resistant variant caused by copper is not at all the same as one caused by cobalt.

Some other metals cause more or less unstable resistances which are apt to decrease when resistant variants are cultured on the normal medium. No correlation between the chemical nature of metals and the biological action mentioned above is found. As each variant caused by each metal is highly specific, and the variation can take place under the condition where no or, if any, little selection against the normal flies is possible, there is no inevitable necessity to explain the phenomenon described in this paper by random mutation followed by selections. As for the possible mechanisms supposed, the present author has already discussed in the previous paper, concentrating her attention on nucleo-cytoplasmic relations (1961 c).

Summary

When a strain of *Drosophila melanogaster* Oregon RS strain is cultured in a culture medium containing sublethal dose (0.5 mM) of $CuSO_4$, the flies of the next generation show an obvious resistance to a culture medium containing ED_{50} (4 mM) of $CuSO_4$, and such characters as developmental rate and ecological and physiological activities also change, associated with the acquisition of the copper resistance (YANAGISHIMA and SUZUKI 1959 a, b, YANAGISHIMA 1961 a, b, c). The changed characters mentioned above are transmissive through sexual reproduction even on the normal culture medium.

In order to make clear the mechanisms through which the resistance is acquired, it is necessary to know whether such a phenomenon as mentioned above is caused only by copper with high specificity or not. Comparative studies on the induction of resistant variations have been performed, using such bivalent metallic salts as $MnSO_4$, $CoSO_4$, $NiSO_4$ $ZnSO_4$ and $CdSO_4$. The larvae whose parents spent their larval stages in culture media containing sublethal doses of $MnSO_4$, $CoSO_4$, $NiSO_4$, $ZnSO_4$ and $CdSO_4$ are called Mn–, Co–, Ni–, Zn– and Cd– strains respectively. Main experimental results obtained are as follows :

1) The Mn–, Co–, Ni– and Cd– strains are obviously resistant to the test media containing ED_{50} of the same metallic salts with which the parent flies were trained, while the Zn–strain does not show resistance to $ZnSO_4$ test medium.

2) The Mn–, Co–, Ni–, Cd– and Cu–strains show highly significantly faster developmental rates than the control strain in the test medium containing ED_{50} of the same metallic salt as used in the training, but only the Zn–strain does not show such a tendency.

3) Some changes in oviposition numbers and hatching rates are observed when the trained strains are tested on the normal medium. The Ni–strain is greater in both the oviposition number and the hatching rate. The Mn– and Ni– strains show significantly greater oviposition numbers than the control. The Mn–, Co– and Cd–strains are lower in the hatching rate, while the Cu–strain is higher.

4) Significant differences are observed in the longevity on both simple sucrose solution and sucrose solutions supplemented with the metallic salts used for the training, among the variant strains induced with the metallic salts.

5) Sensitivity to various kinds of enzyme inhibitors different in their physiological actions was tested with each variant fly. All the variants produced by the training with the metallic salts except the Mn– and Co–strains are more sensitive to all the enzyme inhibitors tested than the control strain.

6) The Cu– and Co– strains can keep the resistance to copper and cobalt respectively, even after being reared for a generation in the normal medium, while in the other variants than the above mentioned two strains, the acquired resistance becomes weaker, when cultured on the normal medium. The cross resistance and collateral sensitivity observed in each variant are also kept more or less, even after it is cultured for a generation in the normal medium.

7) The *Index of acquired resistance* was calculated with each variant to compare one another. From the index, it can be seen that the resistance exhibited by each variant has high specificity.

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