ON THE RESISTIBILITY OF *DROSOPHILA* TO SODIUM CHLORIDE.

II. DIFFERENCES IN THE RESISTIBILITY AT DIFFERENT DEVELOPMENTAL STAGES

YASUHIRO MIYOSHI¹⁾ AND KENJI NAKAMURA

Osaka College of Physical Education, Baba 311, Ibaraki city,
Osaka Prefecture 567

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It was reported that *bw* (Kyoto) of *D. melanogaster* exhibited the strong resistibility to high concentrations of sodium chloride in the culture medium, and this presented a striking contrast against low emergence rates of other strains in the same medium (Miyoshi 1961). By selecting through successive generations more than 50 with the NaCl medium, a more resistant strain was raised from this strain and was designated as *srb*. The results suggested that the resistibility of *bw* (Kyoto) strain to high concentrations of NaCl was a heritable character. In this paper, the present authors examined how NaCl of high concentrations in the medium affected the developmental process in both the resistant strains and the susceptible ones.

MATERIALS AND METHODS

Three resistant strains of *D. melanogaster*, MHW, *srb* and brown (*bw* (Kyoto)), and four susceptible strains, Oregon-R-S (abbreviated Ore-RS in the following), white (*w*), vermilion (*v*) and vestigial (*vg*) were used for materials. MHW has been obtained by intercrossing wild strains from three different localities of Japan (Minoo, Wakayama and Hiroshima), and by selecting the offsprings through successive generations with the NaCl medium. The resistibility of this strain is the highest among strains hitherto tested. The remaining strains have been reared for many years in the laboratory under constant cultural conditions using the regular koji (*Aspergillus oryzae* on moist rice) medium without an addition of NaCl.

The emergence rates²⁾ of these strains, both in the standard medium and in the NaCl medium were examined in the preliminary experiments, in which the resistibility of flies to NaCl was indicated by the proportion of emergence rate in the NaCl medium to that in the standard one. As illustrated in Table 1, there were striking differences among strains in the resistibility to NaCl. It was also noteworthy that the most resistant two strains MHW and *srb* exhibited higher emergence rate in the NaCl medium than in the

1) Deceased in January, 1963. The present series of works had been performed by the senior author in the Zoological Department of Kyoto University as the doctor thesis under the direction of the junior author. This paper is based on the manuscripts left by the senior author.

2) Throughout the text "emergence rate" does not mean number emerging per unit time but a simple percent emergence.

Table 1. Emergence rate and resistibilities in strains of *D. melanogaster* in the standard and 1.0M NaCl containing media.

Strain	No. of eggs tested		Emergence rate		Resistibility
	in standard medium	in NaCl medium	in standard medium	in NaCl medium	
MHW (R)	1000	1000	87.6 $\pm$ 1.63	95.6 $\pm$ 1.58	1.09 $\pm$ 0.012
srb (R)	1000	1000	79.8 $\pm$ 1.15	90.4 $\pm$ 0.86	1.13 $\pm$ 0.008
<i>bw</i> (Kyoto) (R)	1000	1000	71.6 $\pm$ 4.58	49.2 $\pm$ 5.28	0.69 $\pm$ 0.039
<i>w</i> (S)	800	800	86.0 $\pm$ 4.57	5.3 $\pm$ 0.50	0.06 $\pm$ 0.015
<i>v</i> (S)	1000	1000	91.8 $\pm$ 3.44	4.0 $\pm$ 2.04	0.04 $\pm$ 0.014
Ore-RS (S)	750	750	80.7 $\pm$ 4.46	3.1 $\pm$ 1.70	0.04 $\pm$ 0.012
<i>vg</i> (S)	750	750	88.6 $\pm$ 2.05	1.1 $\pm$ 0.73	0.01 $\pm$ 0.007

(R): Resistant to NaCl (S): Susceptible to NaCl

standard medium.

Before being subjected to the test, the strains have been reared for several generations in the semisynthetic standard medium instead of the regular koji medium to get rid of influences of the Na ions which might have been contained in the medium as a trace element and taken into the body. The methods of preparations of the standard medium and the NaCl medium which contained NaCl at a concentration of 1.0M were presented in the preceding paper. The resistibility was examined at each stage of development in separate experiments. Except in the embryonic stage, the flies used in each experiment were of two different classes or derivations, of which one had been laid and grown in the standard medium up to the stage to be tested and the other in the NaCl medium. Each group consisting of 50 individuals was put on a glass plate of 2.5 cm $\times$ 7.5 cm covered with 6 g of either the standard medium or the test medium. The plate was then introduced into a glass tube (12 cm $\times$ 3 cm), and kept at 25 $\pm$ 1°C.

RESULTS

Embryonic stage: To examine the effect of NaCl upon the embryonic development, three series of experiments were performed using the resistant strains, MHW and srb, and the susceptible strains, *v*, *vg* and Ore-RS.

In the first series of experiments, eggs laid by twenty pairs of 4-7 days old flies were collected within two hours after oviposition, and the number of hatched eggs was counted at the 27th hour after initiation of the experiment. The percentages of hatched eggs or the hatchabilities are illustrated in Table 2. In each strain there was little difference in the hatchability between two groups of eggs, one put on the NaCl medium and the other on the standard medium. Slight numerical differences in Table 2 were found to be insignificant by the chi-square test. The hatchabilities were also tested in the sterilized pure water, in Ephrussi & Beadle's physiological saline solution and in the sterilized water with NaCl at concentrations of 0.5 M, 1.0 M and 1.5 M, respectively. The results were found to be same with those in the standard medium.

Table 2. Hatchabilities and resistibilities in strains of *D. melanogaster* in the standard and 1.0M NaCl containing media.

Strain		No. of eggs tested		No. of hatched eggs		Percentage of hatched eggs		Resistibility
		in standard medium	in NaCl medium	in standard medium	in NaCl medium	in standard medium	in NaCl medium	
MHW	(R)	500	500	488	489	97.6±0.78	97.8±0.75	1.00±0.013
srb	(R)	500	500	483	487	96.6±0.85	97.4±1.03	1.01±0.017
<i>v</i>	(S)	500	500	491	485	98.2±0.63	97.0±1.13	0.99±0.010
Ore-RS	(S)	500	500	481	480	96.2±0.98	96.0±1.15	0.99±0.012
<i>vg</i>	(S)	500	500	459	449	91.8±1.38	89.8±1.46	0.98±0.022

(R): Resistant to NaCl (S): Susceptible to NaCl

In the second series of experiments, eggs were dechorionated by 1×10^{-3} percent NaOCl solution for about one minute, and embryos in the contraction stage were selected for the test. Five kinds of media were prepared as in the preceding experiment, and dechorionated eggs were immersed to a depth of about one cm in each medium. Eggs hatched between the 20th and the 26th hour of incubation, were counted and unhatched eggs were examined under the microscope at what stage they ceased to develop. As shown in Table 3, the results obtained in the present experiment exhibited a striking contrast against those in the first series. The hatchability of dechorionated eggs in

Table 3. Hatchabilities of dechorionated eggs in strains of *D. melanogaster* in Ephrussi & Beadle's physiological saline, in sterilized pure water and in the NaCl solutions of various concentrations. Each value in the table is based on 500 eggs of the contraction stage.

Strain		Percentage of hatched eggs in				
		physiological saline	pure water	0.5 M NaCl	1.0 M NaCl	1.5 M NaCl
MHW	(R)	95.0±1.48	80.2±2.32	60.4±2.42	48.0±2.42	23.8±2.87
srb	(R)	96.4±1.26	82.2±1.56	59.2±3.00	49.8±2.36	20.6±2.44
<i>v</i>	(S)	97.8±0.81	84.8±2.65	36.8±1.50	12.2±2.41	0.8±0.45
Ore-RS	(S)	95.2±1.44	79.4±1.71	40.4±1.22	15.8±2.58	0.8±0.26
<i>vg</i>	(S)	91.6±2.19	83.6±1.71	32.0±1.11	10.6±1.58	0.2±0.20

(R): Resistant to NaCl (S): Susceptible to NaCl

both the resistant and the susceptible strains decreased in the presence of NaCl in the medium. The percentages of unhatched eggs increased and the strain differences in the resistibility became clearer, as the concentration of NaCl increased. Embryos of both MHW and *srb*, being nearly equal with each other in the hatchability, were far more resistant than those of the other strains. Ore-RS remained at nearly the same level as *v* in the hatchability, and *vg* was most susceptible among these strains. The sterilized pure water exerted unfavourable effects upon embryos of all strains irrespective of differences in the resistibility to NaCl, and the hatchability in each strain was lowered by approximately 10-15 percent in this medium. Comparing these results with those obtained in the first series of experiments, it may be surmised that the embryos were

completely protected by the chorion from unfavourable effects of the test medium.

By examining the morphology of unhatched embryos, it was noticed that there was a marked difference in the stage critical to the embryonic development between the embryos in the NaCl containing media and those in the other ones. In the physiological saline and in the pure water, the unhatched embryos remained approximately at the stage of eruption of air into the trachea. On the other hand, the majority of the unhatched embryos in the NaCl media was at about the preblastokinetic stage, and none of unhatched egg developed beyond this stage up to the critical stage as in the other kinds of media. The time lapse between these two critical stages was nearly 10 hours under the ordinary conditions at 25°C. It should be noted that there was no distinction between the resistant strains and the susceptible ones with regard to the critical stage in each medium.

In addition to these series of experiments, dechorionated embryos which had grown in the standard medium up to the stage subsequent to the preblastokinetic stage were also subjected to the NaCl media. The embryos of all strains revealed very high hatchability of 90-95 percent irrespective of the strain differences in the resistibility. These results would suggest that NaCl of high concentrations affected the dechorionated embryos more seriously prior to the preblastokinetic stage than in the later stage.

The 1st instar larval stage: The effect of a high concentration of NaCl on the 1st instar larva was examined by means of the survival test in the medium containing 1.0M NaCl. Two series of experiments were performed using resistant MHW, *srb* and *bw* (Kyoto) strains and susceptible Ore-RS and *v* strains. In the 1st series, eggs were put either on the NaCl medium or on the standard medium. As in the preceding series of experiments, eggs with chorion were not affected by NaCl in the medium. The number of the 1st instar larvae was recorded at about the end of the 24th hour and unhatched eggs were discarded. Under the ordinary conditions the 1st instar stage ends nearly in 24 hours but there might occur some delay in the NaCl medium. Therefore, the number of the 2nd instar larvae molted was counted at the 24th-48th hour after hatching. Thus, the 1st instar larvae were subjected to the test through the whole length of the 1st instar stage from hatching to molting. In the second series, 8-10 hours aged larvae hatched on the standard medium were transferred to the test media and the number of molted larvae was counted.

The results obtained from these two series of experiments are presented in Table 4. The strain differences were clear in the percentage of survivors of the 1st instar larvae in the NaCl medium, and the differences were more conspicuous in the 1st series than in the 2nd series of experiments. In the 1st series, the percentage of survivors in each of the susceptible strains, Ore-RS and *v*, remained lower than six percent, while in *bw* (Kyoto) the survival rate was 60 percent and in *srb* and in MHW it exceeded 95 percent.

In the second series, the larvae were put on the test medium during later half of the 1st instar stage. Nearly half of the larvae survived in the susceptible strains and the survival rate increased in *bw* (Kyoto) by 20 percent, but it was unchanged in *srb* and in MHW as compared with that in the 1st series of experiment. These results would suggest that NaCl affected the larvae in the later half of the 1st instar development as

Table 4. Percent of survivals and resistibilities of the 1st instar larvae in strains of *D. melanogaster* in 1.0M NaCl containing medium.

Strain	Test medium	Time of transferring larvae to test medium	No. of 1st instar larvae tested	No. of 2nd instar larvae survived	Survival (%)	Resistibility
MHW (R)	NaCl	control in standard medium	287	264	92.0±1.71	
		before hatching	194	189	97.4±1.46	1.06
		8th-10th hour after hatching	188	181	96.3±1.31	1.05
srb (R)	NaCl	control in standard medium	340	305	89.7±2.49	
		before hatching	191	183	95.8±2.15	1.07
		8th-10th hour after hatching	235	227	96.6±0.52	1.08
bw (Kyoto) (R)	NaCl	control in standard medium	341	298	87.4±2.59	
		before hatching	190	114	60.0±7.00	0.69
		8th-10th hour after hatching	185	152	82.2±4.57	0.94
v (S)	NaCl	control in standard medium	475	452	95.2±0.75	
		before hatching	146	8	5.5±0.70	0.06
		8th-10th hour after hatching	339	173	51.0±1.99	0.54
Ore-RS (S)	NaCl	control in standard medium	462	441	95.5±0.85	
		before hatching	242	14	5.8±0.72	0.06
		8th-10th hour after hatching	240	113	47.1±3.72	0.49

(R): Resistant to NaCl (S): Susceptible to NaCl

well as in the earlier half.

The 2nd instar larval stage: In the examination of the effect of the NaCl medium on the 2nd instar larvae, a susceptible strain *w* was used in addition to the five strains used in the preceding series of experiments. The larvae to be tested were of two derivations in each strain, of which one passed the 1st instar stage in the NaCl medium and the other in the standard medium. Young larvae in the 2nd instar stage were collected within 3 hours after molting, thoroughly washed through three changes of Ringer solution and put either on the NaCl or the standard medium. The number of larvae accomplished the 2nd molting was counted at the 24th-48th hour.

As illustrated in Table 5, the 2nd instar larvae which had passed their 1st instar stage in the NaCl medium were hardly affected by the presence of NaCl in the medium. Thus the viability of all strains was 84-99 percent. On the other hand, the viability of the 2nd instar larvae which had passed the 1st instar stage in the standard medium and then passed their 2nd instar stage in the NaCl medium was high in the resistant strains but was low in the susceptible strains. However, the strain differences in the viability of these larvae were little as compared with those in the 1st instar stage.

These results indicated that the larval development in the 2nd instar stage was not affected so much by the NaCl medium, as far as the viability was concerned.

The 3rd instar larval stage: The lethal effect of the NaCl medium on the 3rd instar larvae was examined following the procedure of the preceding experiments. Strains

Table 5. Percent of survivals and resistibilities of the 2nd instar larvae in strains of *D. melanogaster* in 1.0M NaCl containing medium.

Strain		Medium, up to 1st molting	No. of 2nd instar larvae tested		No. of 3rd instar larvae survived		Survival (%)		Resisti- bility
			in standard medium	in NaCl medium	in standard medium	in NaCl medium	in standard medium	in NaCl medium	
MHW	(R)	standard	225	225	220	224	97.8±1.72	99.6±0.44	1.02
		NaCl	270	225	269	222	99.6±0.17	98.7±0.88	0.99
srb	(R)	standard	270	315	255	303	94.4±2.07	96.2±1.26	1.02
		NaCl	225	225	211	221	93.8±2.15	98.2±0.82	1.05
<i>bw</i> (Kyoto)	(R)	standard	360	315	334	270	92.8±1.19	85.7±2.42	0.92
		NaCl	315	315	301	302	95.6±1.39	95.9±0.95	1.00
<i>w</i>	(S)	standard	225	315	208	234	92.4±1.54	74.3±2.78	0.80
		NaCl	45	45	41	38	91.1	84.4	0.93
<i>v</i>	(S)	standard	315	315	310	247	98.4±0.79	78.4±4.38	0.80
		NaCl	45	45	41	42	91.1	93.3	1.02
Ore-RS	(S)	standard	225	270	212	200	94.2±1.51	74.1±3.95	0.79
		NaCl	45	45	40	40	88.9	88.9	1.00

(R): Resistant to NaCl (S): Susceptible to NaCl

Table 6. Percent of survivals and resistibilities of the 3rd instar larvae in strains of *D. melanogaster* in 1.0M NaCl containing medium.

Strain		Medium, up to 2nd molting	No. of 3rd instar larvae tested		No. of pupae		Survival (%)		Resisti- bility
			in standard medium	in NaCl medium	in standard medium	in NaCl medium	in standard medium	in NaCl medium	
MHW	(R)	standard	135	180	133	176	98.5±1.47	97.8±0.90	0.99
		NaCl	180	135	177	134	98.3±1.05	99.3±0.73	1.01
srb	(R)	standard	180	225	175	221	97.2±1.41	98.2±0.82	1.01
		NaCl	180	180	177	175	98.3±1.05	97.2±2.77	0.99
<i>v</i>	(S)	standard	180	180	175	148	97.2±1.05	82.2±3.96	0.85
		NaCl	90	90	83	81	92.2±3.35	90.0±1.10	0.98
Ore-RS	(S)	standard	180	225	173	189	96.1±1.90	84.0±3.10	0.87
		NaCl	135	135	127	128	94.1±3.23	94.8±1.97	1.01

(R): Resistant to NaCl (S): Susceptible to NaCl

used as materials were MHW, srb, *v* and Ore-RS. The 3rd instar larvae grown in the standard medium and those grown in the NaCl medium were transferred to the two kinds of the test media immediately after they had accomplished the 2nd molting. The puparia formed in each medium were counted at the early stage.

The results obtained are presented in Table 6. In each strain, more than 90 per cent of larvae, which had accomplished the 2nd molting in the NaCl medium, reached the pupal stage in both the NaCl and the standard media. In the 3rd instar larvae which had accomplished the 2nd molting in the standard medium, NaCl in the medium

fatally affected the pupation of the susceptible strains but not of the resistant strains. However, the effect of NaCl on the susceptible strains at the 3rd instar stage was far little as compared with those at the 1st and the 2nd instar stages.

In the foregoing experiments, the resistibility of each strain to the NaCl medium was tested only with regard to the viability. Other than such lethal effect, slowing down of the larval development was recognized in the NaCl medium. As shown in Table 7, the time needed from hatching to puparium formation in the standard medium

Table 7. Time lapses from hatching to white puparium formation in strains of *D. melanogaster* in the standard and 1.0M NaCl containing media. The range is given by 95 percent confidence limit.

Strain	Medium	No. of larvae tested	Time in hours		Significance of difference
			mean	range	
srb (R)	standard	156	96.3	93.0—99.6	—
	NaCl	155	96.5	92.5—100.5	
<i>bw</i> (Kyoto) (R)	standard	171	96.2	94.5—97.9	+
	NaCl	60	137.9	128.8—147.0	
Ore-RS (S)	standard	218	85.5	80.6—90.4	+
	NaCl	26	139.3	130.1—148.5	

(R): Resistant to NaCl (S): Susceptible to NaCl

was 85.5 hours in Ore-RS and 96 hours in both *srb* and *bw* (Kyoto). In the NaCl medium, however, it took 139 hours in Ore-RS and *bw* (Kyoto), while in *srb* it remained almost same as that in the standard medium.

At the end of the 3rd instar, larvae usually climbed up the wall of the culture vessel to pupate and seldom pupated on the surface of the culture medium. The larvae grown in the NaCl medium often pupated on the surface of the medium and the ratio of pupae on the wall decreased as the concentration of NaCl in the medium increased. There was no difference among the strains in the pupation behavior.

Pupal stage: Pupae do not take food. However, there would be possibilities that NaCl in the medium affects the life of pupae through the ionic action or/and the high osmotic pressure. The viability or the emergence rate of pupae in susceptible strains, *v* and Ore-RS, and in highly resistant strains, MHW and *srb*, was tested using two groups of pupae, one reared in the standard medium and the other survived the whole larval stage in the NaCl medium.

In both the resistant and the susceptible strains the emergence rate exceeded 96 percent (Table 8). The results would suggest that the pupal development in these strains was not affected fatally by NaCl in the medium.

However, so far as the duration of the pupal growth was concerned, there existed a remarkable difference between the two groups of pupae reared in the different media; it was especially so in the susceptible strain, Ore-RS. According to Bodenstein (1950) the duration of the pupal stage was 96 hours in *D. melanogaster* under the normal con-

Table 8. Percent of survivals and resistibilities of pupae in strains of *D. melanogaster* in 1.0M NaCl containing medium.

Strain		Medium, up to pupation	No. of pupae tested	No. of adults emerged	Survival (%)	Resistibility
MHW	(R)	standard	133	132	99.2±0.73	1.00
		NaCl	134	133	99.3±0.73	
srb	(R)	standard	175	172	98.3±1.14	1.02
		NaCl	175	175	100.0	
<i>v</i>	(S)	standard	175	175	100.0	0.96
		NaCl	81	78	96.3±1.30	
Ore-RS	(S)	standard	173	171	98.8±1.10	1.01
		NaCl	128	128	100.0	

(R): Resistant to NaCl (S): Susceptible to NaCl

Table 9. Duration of pupal stage in strains of *D. melanogaster* in the standard and 1.0M NaCl containing media.
The range is given by 95 percent confidence limit.

Strain		Medium, up to pupation	No. of pupae tested	Time in hours		Significance of difference
				mean	range	
srb	(R)	standard	45	116.5	113.3—119.7	—
		NaCl	50	117.5	111.2—123.8	
<i>bw</i> (Kyoto)	(R)	standard	50	115.8	112.5—119.1	+
		NaCl	36	135.1	124.5—145.7	
Ore-RS	(S)	standard	45	114.5	110.4—118.6	+
		NaCl	23	169.7	157.2—182.2	

(R): Resistant to NaCl (S): Susceptible to NaCl

ditions at 25°C. It is about 20 hours shorter than that in the present strains reared in the koji medium. As shown in Table 9, the pupal development in these strains tested took nearly 115 hours in the standard medium as long as in the koji medium and there was no significant differences among three strains tested. In the NaCl medium however, there was a noticeable delay in both Ore-RS and *bw* (Kyoto) but not in *srb*.

These strain differences might correspond to those in the larval susceptibility. It would be of some important meanings that the standard deviation in the duration of the pupal development in the NaCl medium was very small in *srb* as compared with that in other strains. This fact might suggest that the population of *srb* strain was more homogeneous than those in the other strains as far as the resistibility to NaCl was concerned.

Imaginal stage Effects of NaCl on the imago have been reported in the preceding paper (Miyoshi 1961).

DISCUSSION AND CONCLUSION

The strain differences of *D. melanogaster* in the resistibility to NaCl have been clearly shown in the preceding work, in which flies have been subjected to the selection pressure of the NaCl medium through their whole range of development. The present experiments reveal that there are differences in the sensitivity to NaCl at different developmental stages, and the strain differences are more distinct in early larval stages. The results are summarized in Figures 1 and 2. Figure 1 illustrates the cumulative percent of survivals of several strains through successive stages of development both in the standard medium and in the NaCl one. The values in the diagram are calculated from the percent of survivals in each stage obtained by separate experiment. In Figure 2, the resistibilities of four strains in the NaCl medium in each developmental stage are shown by the histograms.

Eggs of both the resistant and the susceptible strains are quite insensitive to NaCl in the medium unless the eggs are dechorionated. However, when they are subjected

Fig. 1. Graph showing the cumulative survivals (%) in strains of *D. melanogaster* in successive stages of development in both the medium containing 1.0M NaCl and the standard medium.

Fig. 2. Histogram showing resistibilities to NaCl in strains of *D. melanogaster* at each developmental stage. The resistibility is indicated at the proportion of the percent of survivals in the culture medium containing 1.0M NaCl to that in the standard medium.

to the NaCl medium after being dechorionated, the mortality of embryos is markedly high and the strain differences are more clearly demonstrated as the concentration of NaCl in the medium increases. By examining the morphology of the unhatched embryos, it becomes evident that the critical period of the susceptible embryos lies prior to the preblastokinetic stage. The haemolymph of *Drosophila* is believed to be almost isotonic with 0.2M NaCl. If it is true, any of the NaCl containing media used in the present experiments would yield a hypertonic environment to the flies. There will arise a question whether the death of the susceptible embryos in the NaCl media was induced by the high osmotic pressure of the environment or by the specific action of NaCl at high concentrations. In the subsequent paper, it will be elucidated that the effects of the media fatal to the developing flies may chiefly be attributed to the toxic action of the latter.

As stated above, the embryos are well protected by the chorion against the harmful effects of the external agents. The flies are more sensitive to high concentrations of NaCl in the medium at the larval stage than at the pupal stage. The mortality of larvae is highest at the 1st instar stage not only in the NaCl medium but in the standard medium. It is commonly believed that the sensitivity of *D. melanogaster* to insecticides or starvation (Bakker 1959) is highest at an early stage of the larval development. It should be noted that in the standard medium the emergence rate of the NaCl susceptible strains is higher than that of the resistant strains, it is also noticed that the most resistant strains, MHW and srb, show higher emergence rate in the NaCl medium than in the standard medium. These phenomena will be discussed in a separate work.

The development of the 2nd instar larvae is not affected very much by the NaCl medium, so far as the viability is concerned. It shows a striking contrast against the high susceptibility of the 1st instar larvae.

It is very interesting that even NaCl susceptible strains show high viability to high concentrations of NaCl at the 3rd instar 48-96 hours aged larvae. Data given by Beadle, Tatum and Clancy (1938) suggest that an organizational change takes place at about

70 hours after egg laying at 25°C in Ore-RS and *v;bw* strains. In the starvation experiments, Bakker (1959) states that in flies removed of the food at any time between the 42nd and the 48th hour of hatching the development is delayed, while remove of the normal food after 48 hours have passed does not affect the development to delay. On the other hand, a slight acceleration of development is observed in larvae deprived of food after the 2nd molt (Beadle, Tatum and Clancy 1938). In the present experiment, the time required for completing both the 1st and the 2nd instar larval stages is considerably prolonged in the 1.0 M NaCl medium. The time of the pupation in the larvae which had survived the whole larval stage in the NaCl medium is also delayed to some extent.

The flies are not fatally affected by the 1.0 M NaCl medium at the pupal stage, but the time for completion of the pupal stage is markedly prolonged. The strain differences in the prolongation of the development seem to correspond to those in the susceptibility to NaCl. As pupae do not feed, residual effects of the NaCl medium which had been taken during the larval stages would not be excluded from these results.

SUMMARY

(1) On the resistibility of *D. melanogaster* to sodium chloride, the present authors examined the resistibility to NaCl of high concentrations in the medium at the different developmental stages in both the resistant and the susceptible strains.

(2) Eggs of both the resistant and the susceptible strains are quite insensitive to NaCl in the medium unless they are dechorionated. However, when they are subjected to the NaCl medium after being dechorionated, the mortality of embryos is markedly high. The strain differences in the resistibility are more clearly demonstrated as the concentration of NaCl increases. By examining the morphology of the unhatched embryos, it becomes evident that the critical period of the susceptible embryos lies prior to the preblastokinetic stage.

(3) Larvae of *D. melanogaster* are more sensitive to high concentration of NaCl at the 1st instar larval stage than at succeeding stages. The strain differences in the resistibility are most clearly demonstrated at the 1st instar larval stage but do not correspond to the strain differences in the viability under the normal conditions.

(4) The viability of the 2nd instar larvae is less affected by the NaCl medium than that of the 1st instar larvae.

(5) In the 3rd instar larval stage, even NaCl susceptible strains are hardly affected by high concentrations of NaCl.

(6) Time required for completing the 1st and the 2nd instar larval stages is considerably prolonged in the NaCl medium. At the 3rd instar stage, the development of survivors is more or less delayed.

(7) Flies are not fatally affected by the NaCl medium at the pupal stage, but the time for completion of the pupal stage is markedly prolonged. The strain differences in the prolongation of the development also seem to correspond to those in the susceptibility to NaCl at the larval stage.

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