

Study of the Effects of Potassium Chloride and Calcium Chloride on the Development of *Drosophila melanogaster*

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염화칼륨과 염화칼슘의 초파리 발생에 미치는 영향에 대하여

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적 요

초파리(*Drosophila melanogaster*)의 발생에 미치는 염화칼륨(KCl)과 염화칼슘(CaCl_2)의 단독 영향 및 상호 작용을 검토하기 위하여 6가지 농도의 KCl이나 CaCl_2 처리 미디어에 5계통의 초파리를 사육하여 우화율을 검토한 결과는 다음과 같다.

1. 우화율은 초파리 계통간에는 유의적인 차이를 볼 수 없으나, KCl이나 CaCl_2 의 농도간에는 매우 유의적인 차이가 있으며, 그 농도가 커짐에 따라 우화율이 낮아진다.

2. 우화율에 미치는 CaCl_2 의 억제적 효과는 KCl에 비하여 현저하게 크며, 이것은 초파리의 KCl에 대한 저항성이 CaCl_2 에 대한 것 보다 크다는 것을 뜻한다.

3. KCl이나 CaCl_2 미디어에 소량의 CaCl_2 이나 KCl를 첨가하면 KCl이나 CaCl_2 의 우화율에 대한 억제적 효과의 길항작용을 보이고 있으나, 좀더 다량을 가하면 억제적 효과가 더욱 촉진된다.

4. 따라서 초파리의 발생에 대한 K^+ 와 Ca^{++} 의 억제적 효과에 있어서 상호 작용이 관여하고 있음을 알 수 있다.

INTRODUCTION

Many investigations of the effects of some chemicals on the development of the drosophilid flies have been carried out. The effects of the some salts (e. g., Na,

Ca) contained in the food media on the development have been discussed by Rubinstein *et al.* (1936), Kalmus (1943), King (1953) and Loeb (1965). According to Miyoshi (1959), the toxic effect of the agent upon developing flies was found to depend on the positive Na^+ ion, not on the negative Cl^- ion and both K^+ and Ca^{++} ions produced a similar effect. Miyoshi (1961) also obtained a result of his experiment that the resistibility to sodium chloride varied in different strains of *D. melanogaster*. Miyoshi & Nakamura (1968) examined the effects of sodium chloride upon the embryonic development and found that eggs were quite insensitive to sodium chloride in the medium unless they were dechlorinated but the mortality of embryo was markedly high when they were subjected to the NaCl medium after being dechlorinated by sodium hypochlorite. It was found by Kang *et al.* (1970), Chung & Kang (1971), Kang (1971), Kang & Chung (1971) that the emergence rates of *D. melanogaster* (resistibility to NaCl) was not significantly different among the strains but strikingly different among concentrations of NaCl, the emergence rate decreased as the concentration of NaCl increased. The effects of NaCl on the hatching rates (Chung & Kang, 1972) and the emergence rates (Chung, 1972) of *D. melanogaster* when pretreated sodium hypochlorite were studied and they found that the hatching rates and the emergence rates were heterogeneous among concentrations of NaCl when pretreated NaOCl and decreased as concentration of NaCl increased. Kang (1972) reported that the emergence rates of *D. melanogaster* were significantly different among concentrations of NaCl or KCl but not among strains and the resistibility to KCl was rather strong compared to the resistibility to NaCl. Chung & Kang (1973) designed an experiment in which the emergence rates of *D. melanogaster* were examined when it was raised in the medium of various concentrations of NaCl and CaCl_2 in order to see if any effect of the food medium on the development of the six strains of the flies and obtained the results that the effect of CaCl_2 on the emergence rates was found to be greater than that of NaCl.

The present investigation is attempted to see if KCl and CaCl_2 affect the development of *D. melanogaster* and to examine the interaction of KCl and CaCl_2 on the emergence rates.

MATERIALS AND METHODS

The three wild strains (Oregon-R, Kwangju, and Choon-chun) and the two SD strains (SD^{NH}-2 and R-1) of *Drosophila melanogaster* were used in the present experiment. The genetic constitutions of the SD strains were described in the preceding paper (Kang *et al.*, 1970). Oregon-R is the standard laboratory wild stock. The Kwangju and the Choon-chun strains have been kept in the constant

temperature room for a few years since originally captured from Kwangju and Choonchun area in Korea, respectively. The food formula used in the present experiment is presented in the preceding paper (Kang *et al.*, 1970). The present experiment was performed at $25 \pm 1^\circ\text{C}$, and the KCl medium was prepared by adding KCl at a concentration of 0.0M (as the control), 0.1M, 0.3M, 0.5M, 0.8M, and 1.0M to the standard medium and the CaCl₂ medium was made by adding CaCl₂ at a concentration of 0.0M, 0.1M, 0.2M, 0.3M, 0.5M, and 0.8M to the standard medium.

Eggs from populations of five pairs of three to five day old flies of each strain were transferred to the food medium of KCl or CaCl₂ seven to nine hours after being laid and the number of emerging flies were counted and the emergence rate was calculated for each vial and averaged for each concentration of KCl and CaCl₂ in each strain.

In order to see the interaction of KCl and CaCl₂, several combined media were prepared by adding 0.05M, 0.1M, and 0.2M of CaCl₂ (or KCl) to each of six kinds of the KCl (or CaCl₂) media.

RESULTS

Six vials (3×10cm), each of which contained 50 eggs were prepared for each concentration of KCl or CaCl₂ in each strain of the flies.

The emergence rates of the five strains of *D. melanogaster*, when various concentrations of KCl were treated, are presented in Table 1 and Figure 1 (number in parenthesis of each table in the text indicates the number of flies emerged). The emergence rate, when KCl was treated is not significantly different among strains (analysis of variance indicates: $F=2.04$, $n_1=4$, $n_2=20$, $p>0.05$) but strikingly different among concentrations of KCl (analysis of variance indicates:

Table 1. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing various concentrations of KCl

Strain	No. of eggs tested	Concentration of KCl					
		0.0M	0.1M	0.3M	0.5M	0.8M	1.0M
SD ^{NH} -2	300	87.7 (263)	86.7 (260)	82.3 (247)	70.7 (212)	50.3 (151)	31.7 (95)
R-1	300	90.3 (271)	41.7 (275)	84.7 (254)	71.3 (214)	54.7 (164)	33.3 (100)
Oregon-R	300	86.7 (260)	84.3 (253)	80.7 (242)	69.7 (209)	52.3 (157)	37.3 (112)
Kwangju	300	89.3 (268)	85.7 (257)	83.7 (251)	73.3 (220)	53.3 (160)	32.7 (98)
Choonchun	300	87.0 (261)	88.7 (266)	84.3 (253)	68.3 (205)	50.7 (152)	35.3 (106)

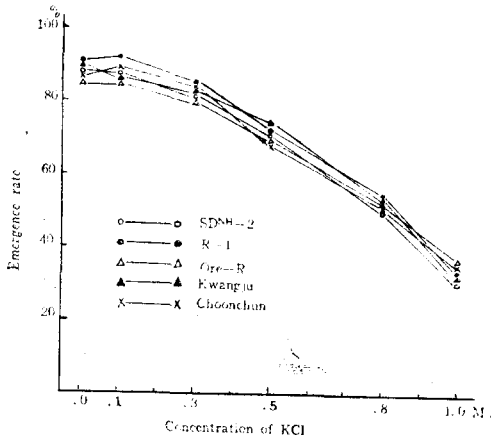


Fig. 1. Graph showing emergence rates listed in Table 1.

and Figure 2. The emergence rate, as seen in the table and the figure, is not significantly different among strains but markedly different among concentrations of CaCl_2 . The analysis of variance also shows no significant difference among strains: $F=2.75$, $n_1=4$, $n_2=16$, $p>0.05$, and significantly different among concentrations of CaCl_2 : $F=39.68$, $n_1=4$, $n_2=16$, $p<0.01$. As seen in Table 2 and Figure 2, the emergence rate is almost homogeneous from the concentration of CaCl_2 , 0.0M to 0.1 M but the rate decreases to about 58–67% at 0.2M and then drops continuously to about 20–27% at 0.3M. The rate decreases even more markedly at 0.5M (about 1%). No flies emerged from the medium of 0.8M of CaCl_2 . This implies that the five strains of *D. melanogaster* in the present experiment are considerably resistible to the CaCl_2 from a concentration of 0.0M to

$F=673.51$, $n_1=5$, $n_2=20$, $p<0.01$), as illustrated in Table 1 and Figure 1. The emergence rate is found to be almost constant from 0.0M to 0.1M of KCl but the rate decreases to about 81–85% at 0.3M, about 68–71% at 0.5M, and then drops markedly to about 50–55% at 0.8 M and to 32–37% at 1.0M of KCl. This suggests that the five strains of *D. melanogaster* in the present experiment is resistible from a concentration of 0.0M to 0.5M but susceptible from 0.5M or higher concentrations of KCl.

The single effect of CaCl_2 on the emergence rates is illustrated in Table 2,

Table 2. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing various concentrations of CaCl_2

Strain	No. of eggs tested	Concentration of CaCl_2					
		0.0M	0.1M	0.3M	0.3M	0.5M	0.8M
SDNH-2	300	87.7 (263)	86.0 (258)	67.3 (202)	24.3 (73)	1.7 (5)	— (0)
R-1	300	90.3 (271)	88.3 (265)	64.7 (194)	26.3 (79)	1.3 (4)	— (0)
Oregon-R	300	86.7 (260)	84.3 (253)	61.3 (184)	27.0 (81)	2.3 (7)	— (0)
Kwangju	300	89.3 (268)	80.7 (242)	57.7 (173)	18.3 (55)	— (0)	— (0)
Choonchun	300	87.0 (261)	81.7 (245)	60.7 (182)	20.3 (61)	0.7 (2)	— (0)

0.1M but susceptible from 0.2M or higher concentrations fo CaCl₂. If the comparison of affecting pattern on the emergence rates between KCl and CaCl₂ is made, the considerable difference is found in the present experiment. As clearly manifested in Figure 1 and 2, the emergence rate, when CaCl₂ is treated, decreases more markedly than when KCl is treated. No flies emerged from the medium containing 0.8 M of CaCl₂, whereas quite a few flies emerged from the medium of 0.8M and 1.0M of KCl. This is the remarkable contrast between the affecting pattern of KCl and CaCl₂, on the emergence rates and this implies that the resistibility of CaCl₂ on the emergence rates and that the resistibility of Drosophila to KCl is greater than to CaCl₂.

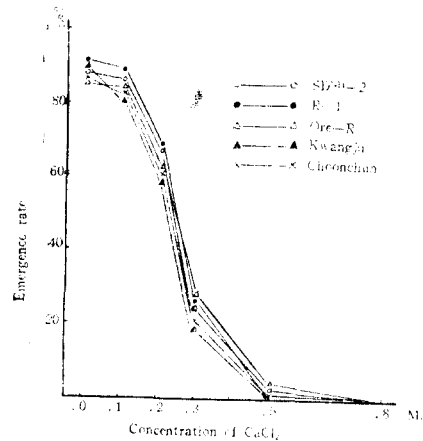


Fig. 2. Graph showing emergence rates listed in Table 2.

The interaction of KCl and CaCl₂ on the emergence rates is examined by preparing the combined media and the results are presented in Tables 3—8 and Figures 3—8. The emergence rates obtained from the media containing each concentration of KCl +0.05M, 0.1M, or 0.2M of CaCl₂ are illustrated in Tables 3—5 and Figures 3—5. As seen in Table 3 and Figure 3, no change is seen in the emergence rates when 0.05M of CaCl₂ is added to each concentration of KCl. Analysis of variance indicates that the emergence rate is not significantly different among strains of the flies (F=2.89, n₁=4, n₂=16, p>0.05) but significantly heterogeneous among concentrations of KCl (F=175.95, n₁=4, n₂=16, p<0.01). However, the affecting

Table 3. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.05M of CaCl₂ plus various concentrations of KCl

Strain	No. of eggs tested	Control	Conc. of KCl & 0.05M CaCl ₂			
			0.1M	0.3M	0.5M	0.8M
SD#-2	300	87.7	86.3 (259)	84.7 (254)	73.7 (221)	59.3 (178)
R-1	300	90.3	88.7 (266)	85.3 (256)	72.3 (217)	57.7 (173)
Oregon-R	300	86.7	85.3 (256)	83.7 (251)	74.7 (224)	52.7 (158)
Kwangju	300	89.3	87.0 (261)	83.0 (249)	76.3 (229)	56.3 (169)
Choonchun	300	87.0	86.7 (260)	85.3 (256)	70.7 (212)	54.3 (163)

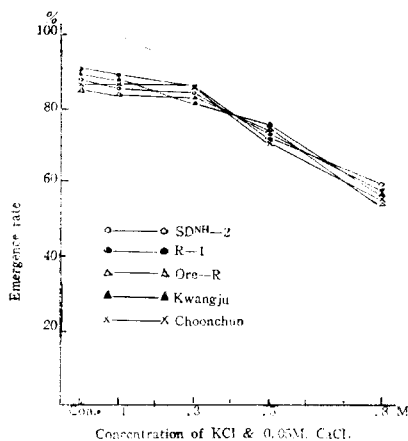


Fig. 3. Graph showing emergence rates listed in Table 3.

This decrease is much more remarkable than in Figure 1, which is the case of no addition of CaCl₂ to the KCl medium, especially it decreases 14–21% at 0.8M of KCl. This implies that CaCl₂ may enhance inhibitory effect of KCl on the emergence rates.

The results of the experiment undertaken by using food media containing 0.2M of CaCl₂ plus various concentrations of KCl are shown in Table 5 and Figure 5.

As illustrated in the table and the figure, the emergence rate is also significantly different among concentrations of KCl ($F=675.81$, $n_1=3$, $n_2=12$, $p<0.01$) but not different among strains ($F=2.87$, $n_1=4$, $n_2=12$, $p>0.05$).

As seen in the graph, the reducing rate of the emergence rates is much greater, as the concentration of KCl increases, than in the case of no addition of CaCl₂. The emergence rate begins to decrease from 0.1M (62–73%) and 40% at 0.3M,

pattern of KCl on the emergence rates is influenced by the addition of CaCl₂ as its concentration is increased.

The emergence rates obtained from the medium containing each concentration of KCl +0.1M of CaCl₂ are presented in Table 4 and Figure 4.

As shown in Table 4 and Figure 4, the emergence rates are not significantly different among strains ($F=1.98$, $n_1=4$, $n_2=16$, $p>0.05$) but significantly different among concentrations of KCl ($F=452.48$, $n_1=4$, $n_2=16$, $p<0.01$).

The emergence rate decreases as the concentration of KCl increases and this

Table 4. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.1M of CaCl₂ plus various concentrations of KCl

Strain	No. of eggs tested	Control	Conc. of KCl & 0.1M CaCl ₂			
			0.1M	0.3M	0.5M	0.8M
SDNH-2	300	87.7	88.3 (265)	82.7 (248)	70.3 (211)	21.3 (64)
R-1	300	90.3	86.7 (260)	84.3 (253)	67.7 (203)	15.0 (45)
Oregon-R	300	86.7	84.0 (252)	78.3 (235)	62.3 (187)	14.3 (43)
Kwangju	300	89.3	82.7 (248)	79.3 (238)	63.7 (191)	18.7 (56)
Choonchun	300	87.0	85.3 (256)	79.7 (239)	64.7 (194)	16.7 (50)

Table 5. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.2M of CaCl₂ plus various concentrations of KCl

Strain	No. of eggs tested	Control	Conc. of KCl & 0.2M CaCl ₂			
			0.1M	0.3M	0.5M	0.8M
SDNH-2	300	87.7	62.3 (187)	40.7 (122)	21.3 (64)	— (0)
R-1	300	90.3	72.3 (217)	44.7 (134)	23.3 (70)	— (0)
Oregon-R	300	86.7	73.7 (221)	46.7 (140)	21.3 (64)	— (0)
Kwangju	300	89.3	71.3 (213)	43.3 (130)	18.7 (56)	— (0)
Choonchun	300	87.0	68.3 (205)	42.0 (126)	14.3 (43)	— (0)

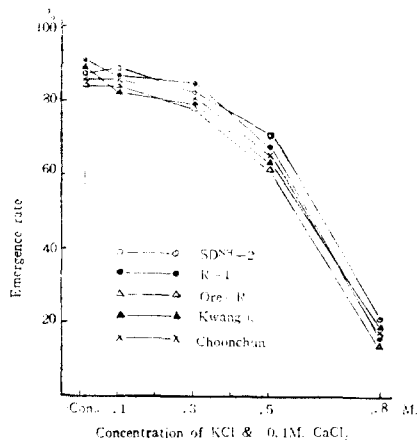


Fig. 4. Graph showing emergence rates listed in Table 4.

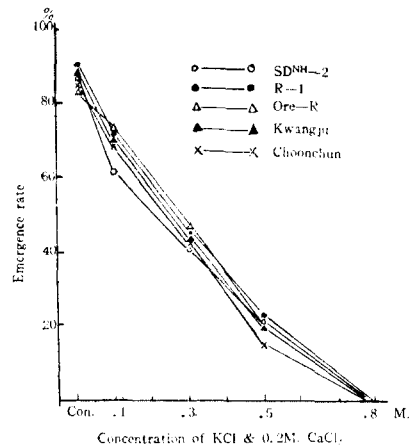


Fig. 5. Graph showing emergence rates listed in Table 5.

about 20% at 0.5M. No flies emerged from the medium containing 0.8M of KCl plus 0.2M of CaCl₂. Here is also the conspicuous enhancement of inhibitory effect of KCl by the addition of CaCl₂, that is, the addition of CaCl₂ to the KCl medium results in weak resistibility of the flies to KCl.

In order to see any change in the inhibitory effects of CaCl₂ on the emergence rates of flies if any concentration of KCl is added to the CaCl₂ medium, several combined media (0.05M, 0.01M or 0.2M of KCl plus 0.1M, 0.2M, 0.3M, or 0.5M, of CaCl₂) are prepared and the emergence rates obtained from the media are presented in Tables 6–8 and Figures 6–8.

The emergence rate obtained from the media of 0.05M of KCl plus various concentrations of CaCl₂ is not significantly different among strains of the flies (analysis of variance: $F=0.13$, $n_1=4$, $n_2=12$, $p>0.05$), but strikingly different

Table 6. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.05M of KCl plus various concentrations of CaCl₂

Strain	No. of eggs tested	Control	Conc. of CaCl ₂ & 0.05M KCl			
			0.1M	0.2M	0.3M	0.5M
SD ^{NH} -2	300	87.7	86.7 (260)	71.3 (214)	38.7 (116)	— (0)
R-1	300	90.3	88.3 (267)	67.7 (203)	37.3 (112)	— (0)
Oregon-R	300	86.7	83.3 (250)	70.7 (212)	45.3 (136)	— (0)
Kwangju	300	89.3	80.7 (242)	73.3 (220)	36.7 (110)	— (0)
Choonchun	300	87.0	82.7 (248)	66.7 (200)	44.7 (134)	— (0)

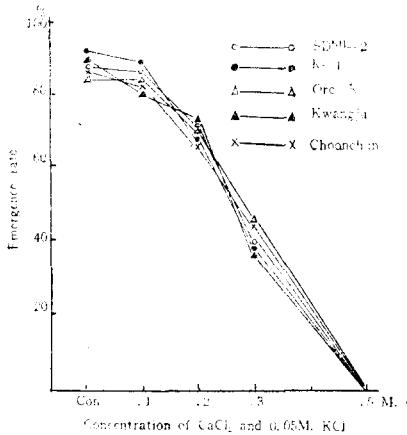


Fig. 6. Graph showing emergence rates listed in Table 6.

among concentrations of CaCl₂ ($F=200.81$, $n_1=3$, $n_2=12$, $p \ll 0.01$), as seen in Table 6 and Figure 6.

If the comparison of the graph in Figure 6 and in Figure 2, it is found that the emergence rate in the addition of 0.05M of KCl to 0.3M of CaCl₂ (38—45%) is greater than in no addition of KCl to 0.3M of CaCl₂ (20—27%). This implies that the inhibitory effect of CaCl₂ on the emergence rates of the flies is counteracted by the addition of KCl.

The emergence rates obtained from the media containing 0.1M of KCl plus various concentrations of CaCl₂ are shown in Table

Table 7. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.1M of KCl plus various concentrations of CaCl₂

Strain	No. of eggs tested	Control	Conc. of CaCl & 0.1M. KCl			
			0.1M	0.2M	0.3M	0.5M
SD ^{NH} -2	300	87.7	85.3 (256)	60.7 (182)	23.3 (70)	— (0)
R-1	300	90.3	85.7 (257)	63.3 (190)	24.7 (74)	— (0)
Oregon-R	300	86.7	82.3 (247)	65.7 (197)	30.3 (91)	— (0)
Kwangju	300	89.3	81.7 (245)	61.3 (184)	25.7 (77)	— (0)
Choonchun	300	87.0	83.7 (251)	59.7 (179)	22.7 (68)	— (0)

7 and Figure 7. As in other cases, there is a remarkable difference in the emergence rates among concentrations of CaCl₂ (analysis of variance also indicates $F=879.78$, $n_1=3$, $n_2=12$, $p<0.01$) but not a significant difference among the five strains of the flies ($F=1.29$, $n_1=4$, $n_2=12$, $p>0.05$). The graph of Figure 7 is similar to that in Figure 2 with the exception that no flies emerged from the medium containing 0.1M of KCl plus various concentrations of CaCl₂ (a few flies emerged from the single CaCl₂ medium). This suggests that the addition of 0.1M of CaCl₂ medium neither enhance nor suppress the inhibitory effect of CaCl₂ on emergence rates of flies.

The emergence rates obtained from the media containing 0.2M of KCl plus various concentrations of CaCl₂ are listed in Table 8 and illustrated by graphs in Figure 8. As seen in the table and the figure, no difference of emergence rates is

Table 8. Emergence rates(%) of the five strains of *D. melanogaster* in the media containing 0.2M of KCl plus various concentrations of CaCl₂

Strain	No. of eggs tested	Control	Conc. of CaCl ₂ & 0.2M KCl			
			0.1M	0.2M	0.3M	0.5M
SD ^{NH} -2	300	87.7	73.7 (221)	38.7 (116)	2.7 (8)	— (0)
R-1	300	90.3	77.3 (232)	39.7 (119)	0.3 (1)	— (0)
Oregon-R	300	86.7	70.3 (211)	36.7 (110)	3.7 (11)	— (0)
Kwangju	300	89.3	72.7 (218)	40.3 (121)	1.3 (4)	— (0)
Choonchun	300	87.0	69.7 (209)	42.3 (127)	1.7 (5)	— (0)

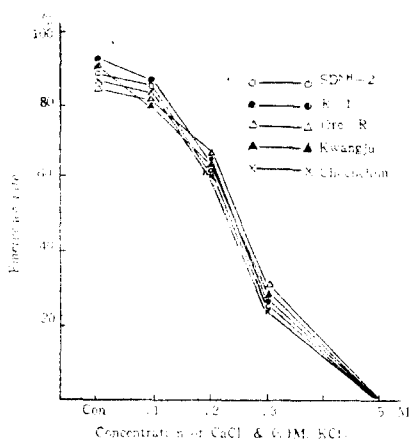


Fig. 7. Graph showing emergence rates listed in Table 7.

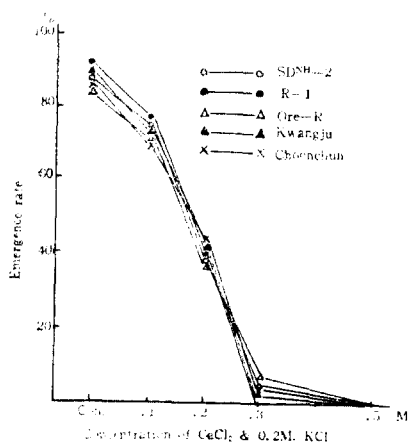


Fig. 8. Graph showing emergence rates listed in Table 8.

observed among the five strains of the flies ($F=0.74$, $n_1=4$, $n_2=12$, $p>0.05$) but a significant difference appears among concentrations of CaCl_2 ($F=154.44$, $n_1=3$, $n_2=12$, $p\leq 0.01$). As illustrated in Figure 8, the addition of 0.2M of KCl to the CaCl_2 medium enhances strongly the inhibitory effect of CaCl_2 on the emergence rates of the drosophilid flies.

In short, CaCl_2 influences the effect of KCl on the emergence rates of the flies much more conspicuously than KCl does, and this becomes manifested as the concentration of KCl or CaCl_2 increases.

DISCUSSION

The present experiment is undertaken to see the single effect and the interaction of KCl and CaCl_2 on the emergence rates of *D. melanogaster* and the results show that the emergence rate is not significantly different among strains of the flies but conspicuously heterogeneous among concentrations of KCl or CaCl_2 . This is agreed with the results of the previous papers in which the effects of NaCl on the emergence rates of the drosophilid flies were examined (Kang *et al.* 1970, Kang & Chung 1971, Kang 1971, 1972, Chung & Kang 1971, 1973). Thus it is clear that both of KCl and CaCl_2 have an inhibitory effect on the emergence rates of drosophilid flies as NaCl does.

It is also found from the present experiment that the inhibitory effect of CaCl_2 on the emergence rates is greater than that of KCl. This may be due to the strong inhibitory action of CaCl_2 on the hatchability of the flies. Kang (1972) compared the effect of NaCl to KCl on the emergence rates of *D. melanogaster* and obtained a result that the inhibitory effect of NaCl on the emergence rates was greater than those of KCl, implying that the resistibility of the flies to KCl was greater than to NaCl. Chung & Kang (1973) indicated that the inhibitory effect of CaCl_2 on the emergence rates was greater than that of NaCl; in other words, the resistibility of the flies to NaCl was greater than to CaCl_2 . Thus, the order of resistibility of *D. melanogaster*, as Chung & Kang (1973) pointed out, might be $\text{KCl} > \text{CaCl}_2$.

The examination of the interaction of KCl and CaCl_2 on the emergence rates results in the finding that the addition of very small amount of CaCl_2 or KCl (0.05M) to the KCl or CaCl_2 media counteracts the inhibitory effect of KCl or CaCl_2 . The addition of a little greater amount of CaCl_2 or KCl (0.1M and 0.2M) to the KCl or CaCl_2 media, on the other hand, enhance the inhibitory effect of KCl or CaCl_2 . This implies that there may be some antagonism between ions (K^+ , Ca^{++}) and moreover some special concentration ratio may be involved in this antagonism. Miyoshi (1959) found that the toxic action of high concentration of

NaCl was counteracted by the addition of a small amount of either K⁺ or Ca⁺⁺ ions. Thus the result of the present experiment may be interpreted by Miyoshi's finding.

SUMMARY

In order to see the single effect and interaction of potassium chloride (KCl) and calcium chloride (CaCl₂) on the development of *Drosophila melanogaster*, the emergence rates of the five strains of the flies were examined from the media containing 0.0M, 0.05M, 0.1M, 0.2M of CaCl₂ or KCl plus various concentrations of KCl or CaCl₂, and the results are presented as follows:

1. The emergence rate of *D. melanogaster* is not significantly different among strains but strikingly different among concentrations of KCl or CaCl₂; the emergence rate decreases as concentration of KCl or CaCl₂ increases.

2. The inhibitory effect of CaCl₂ on the emergence rates is greater than that of KCl, implying that the resistibility of the flies to KCl is greater than to CaCl₂.

3. The addition of small amount of CaCl₂ or KCl to KCl or CaCl₂ media counteracts the inhibitory effect of KCl or CaCl₂, but the addition of a little greater amounts of CaCl₂ or KCl to the KCl or CaCl₂ media enhance the inhibitory effect of the salts.

4. Thus, there may be some interaction of K⁺ and Ca⁺⁺ ions in the inhibitory effect on the development (emergence rates) of *Drosophila melanogaster*.

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