Studies on the Mode of the Toxic Action of Silver Nitrate upon Mosquito Larvae[1), 2)]

By

Kenji Suzuki

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(With 5 Text-figures)

By absorbing water and various salts through the anal gills, mosquito larvae have the ability constantly to regulate osmotic pressure in the body to the most appropriate state for the maintenance of their existence. This is known to be a characteristic function common to the anal gills of aquatic dipterous larvae (Krogh, 1939). In his previous work (Suzuki, 1959), the present writer observed the fact that heavy metal salts are vastly different from alkali metal salts in the mode of action upon mosquito larvae; the former is absorbed in a fairly dilute concentration and finally, kills the larvae within a comparatively short time. Moreover, it was ascertained that amongst 11 heavy metal salts used, silver nitrate exhibits the most violent toxic action to mosquito larvae. The present study was undertaken for the purpose of clarifying the mode of action of silver nitrate upon mosquito larvae.

Material and method: Fourth instar larvae of *Culex pipiens pallens* Coquillett, reared in the laboratory, were employed in all the experiments. Toxicity of silver nitrate was estimated according to the procedure described in detail in the previous paper (Suzuki, 1959). Twenty larvae were transferred to the solution in which was dissolved a given amount of silver nitrate; glass distilled water was used as a solvent through the experiments. Inspection was made at suitable interval (within 30 minutes) within a day. Larvae exhibiting no response to pricking with a needle were regarded as dead; the number was recorded together with the time of inspection. The relationship of cumulative mortality of the larvae for the logarithm of death time in minutes is expressed by the equation, $T=K\times 10^{np}$ or $\log T=\log K+np$, where T is time in minutes; P is cumulative mortality and K, n, are constants. Thus, the time of 50% mortality (TD_{50}) was easily calculated from the equation. In the course of experiment, food was not given to the larvae. All the experiments were conducted with water at temperatures ranging from 17°C to 22°C.

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1) Contribution No. 488 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) This work was in part supported by a Grant-in-Aid for Scientific Research of Hokkaido Prefecture.

Results

Experiment 1: Each 20 larvae were soaked in 10^{-2}%, 10^{-3}%, 10^{-4}%, 10^{-5}% and 10^{-6}% solutions of silver nitrate. As shown in Fig. 1, a rectilinear relation exists between log TD_{50} of the larvae and log concentration of silver nitrate within the range from 10^{-2}% to 10^{-5}%. The relation may be written as the equation, $TC^n=K$ or $\log T+n\log C=\log K$. By the computation of regression, $\log T+0.456788\log C=0.365244$ was obtained as the equation corresponding to the case. But $\log TD_{50}$ values of the larvae transferred to 10^{-6}% and to glass distilled water (control) were each remarkably larger than those of the larvae bred at the concentrations above 10^{-5}%; namely, the mosquito larvae lived in 10^{-6}% solution and in distilled water longer than in the solutions over 10^{-5}%. Furthermore, the difference of TD_{50} was statistically insignificant between 10^{-6}% solution and distilled water. It was thus surmised that 10^{-6}% of silver nitrate has scarcely any toxic action.

Experiment 2: The larvae were removed to 100 ml solutions of 10^{-2}%, 10^{-3}%, and 10^{-4}% $AgNO_3$, to each solution 1 ml of 1% reduced glutathion having been added.

Experiment 3: Immediately after the amputation of anal gills, the larvae were brought into 10^{-2}%, 10^{-3}% and 10^{-4}% solutions of silver nitrate. The results of Experiments 2 and 3 also are illustrated in Fig. 1. As seen theirin, the equation $TC^n=K$ could not be applied to the results of these two experiments. However, values of TD_{50} at each concentration in Exps. 2 and 3 were always greater than TD_{50} at the corresponding concentrations of Exp. 1, and among three experiments just referred to, TD_{50} values in Exp. 3 were the largest and those of TD_{50} in Exp. 2 were next to them. In addition, the differences of TD_{50} at the mutually corresponding concentartions among these three experiments were proved to be significant by the statistical test. Therefore, it was presumed from the facts mentioned above that the larvae of which the anal gills have been amputated exceedingly prolong the duration of survival, and that reduced glutathion restrains the toxic action of silver nitrate.

Experiment 4: In order to investigate the protective action of reduced glutathion, three series of experiments were designed.

a) 100 ml of 10^{-3}% silver nitrate solutions, each solution plus 2 ml, 5 ml and 10 ml of 0.01% reduced glutathion; 100 ml of 10^{-3}% silver nitrate solution without adding reduced glutathion.

b) 100 ml of 10^{-4}% silver nitrate solutions, each plus 2 ml, 5 ml and 10 ml of 0.01% reduced glutathion; 100 ml of 10^{-4}% silver nitrate solution without adding reduced glutathion.

c) 100 ml of solutions, each containing 2 ml, 5 ml and 10 ml of 0.01% reduced glutathion; 100 ml of glass distilled water.

Twenty larvae were placed in each solution. Fig. 2 shows the results of the experiments. In Exp. a, no protective effect of reduced glutathion upon

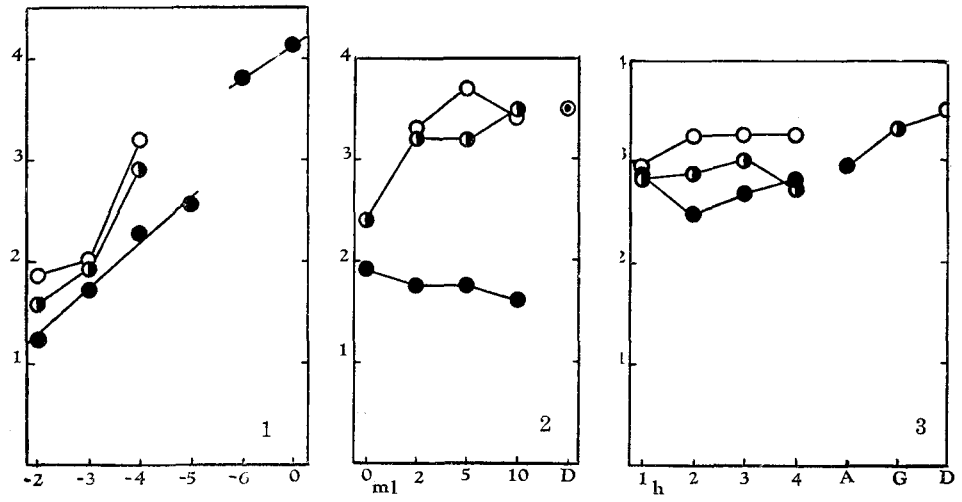

Fig. 1. Fig. 2. Fig. 3.

Fig. 1. The relation between log time in minutes required for arriving at 50% mortality (TD_{50}) and log concentration of silver nitrate. Ordinate, log TD_{50}; abscissa, log concentration (%). In the following figures also, ordinate expresses log TD_{50}. Open, semi-open and solid circles show each the results of Exps. 3, 2 and 1.

Fig. 2. The relation of log TD_{50} for the amounts of reduced glutathion added to the silevr solution and distilled water. Abscissa, amounts of reduced glutathion; solid, semi-open and open circles show each the results of Exps. 4 a, b and c; a double circle expresses log TD_{50} of larvae reared in distilled water.

Fig. 3. The relation of log TD_{50} for the duration of time of immersing larvae previously in reduced glutathion and silver nitrate solutions. Abscissa, duration of time; open, semi-open and solid circles show each the results of Exps. 5 f, e and d. Circles over abscissa A, G and D indicate log TD_{50} of larvae reared in 10^{-4}% silver nitrate, 0.001% reduced glutathion and distilled water without receiving previous treatment respectively.

the mosquito larvae was found. On the other hand, in Exp. b, reduced glutathion evidenced a conspicuous protective effect. In Exp. a, the amount of reduced glutathion was probably not so plentiful as to protect the larvae from the toxic action of silver nitrate. From the results of Exp. c, it was clarified that the difference of TD_{50} in each concentration of reduced glutathion from TD_{50} in distilled water is statistically insignificant; accordingly, it may be stated that reduced glutathion is not poisonous to the mosquito larvae in the concentrations used here.

Experiment 5 : For further examination of the protective action of reduced glutathion, four experiments were conducted.

d) After immersion in 10^{-4}% silver nitrate solution for 1, 2, 3 and 4 hours,

each 20 larvae were transferred to 0.001% reduced glutathion.

e) In reverse of Exp. d, after immersion in 0.001% reduced glutathion for 1, 2, 3 and 4 hours, the larvae were removed to 10^{-4}% silver nitrate.

f) After having been placed previously in 10^{-4}% silver nitrate solution for 1, 2, 3 and 4 hours, the larvae were transferred to glass distilled water.

g) For the purpose of judging the effect of such treatment as described above, the larvae were reared in distilled water, 0.001% reduced glutathion and 10^{-4}% silver nitrate solutions respectively, without being subjected to any of the previously described treatments.

Fig. 3 indicates the results of Exps. d, e, f and g. The results of Exp. d show that TD_{50} of the larvae was not shortened in parallel with the duration of time of immersion in silver nitrate solution; by comparison with Exp. g, the values of TD_{50} in this case were always smaller than those of the larvae reared in reduced glutathion without having been subjected to the previous treatment of silver nitrate. In Exp. e, it was observed that TD_{50} values were not necessarily proportionate to the duration of time of the treatment with reduced glutathion. Comparison with Exp. g reveals the fact that larvae subjected to previous treatment with reduced glutathion never survive longer than larvae bred in silver nitrate solution alone. It may therefore be said that the protective effect of previous treatment of reduced glutathion upon the mosquito larvae is doubtful. According to the results of Exp. f, TD_{50} values of the larvae which were removed from silver nitrate solution to distilled water nearly accord with each other, irrespective of the duration of time of immersion in the silver solution. It was confirmed by statistical test that TD_{50} values in Exp. f are without exception larger than corresponding TD_{50} in Exps. d and e. Hence, the conclusion is reasonably arrived at that alternate immersions into both silver nitrate and reduced glutathion solutions are effective in quicking the death of larvae, and that removal within 4 hours from silver solution to distilled water results in a slow recovery of larvae from the poisoning of silver nitrate.

Experiment 6 : Three sets of experiments were conducted with the intention of inquiring into the toxic action of silver nitrate under the presence of sodium and potassium ions.

h) Mosquito larvae were reared in 100 ml of 0.1% sodium nitrate solutions containing respectively 1 ml, 5 ml and 10 ml of 10^{-4}% silver nitrate.

i) Larvae were reared in 100 ml of 0.1% potassium nitrate solutions containing respectively the same amounts of 10^{-4}% silver nitrate as in Exp. h.

j) As the control group, the larvae were reared in 0.1% $NaNO_3$, 0.1% KNO_3, 10^{-4}% $AgNO_3$ and distilled water respectively.

The results of these experiments are graphed in Fig. 4. According to the figure, the addition of small amount of silver nitrate to the sodium nitrate solution postpones the death of the larvae beyond its occurrence in the solution including only sodium nitrate, exclusive of one exception (the sodium solution

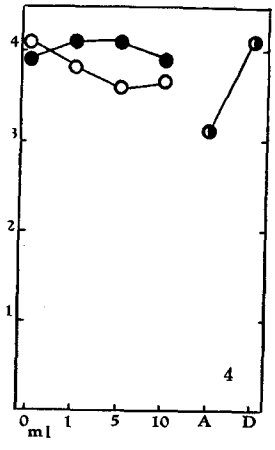

Fig. 4.

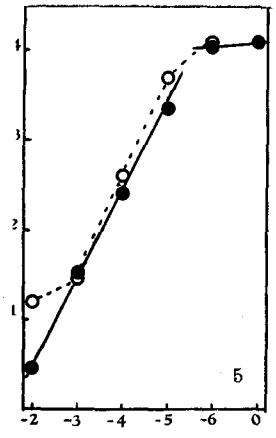

Fig. 5.

Fig. 4. The change of log TD_{50} when silver nitrate was added to sodium and potassium nitrate solutions. Abscissa, the annexed amounts of silver nitrate. Solid and open circles indicate the results of Exps. 6 h and i respectively: semi-open circles over abscissa A and D show each log TD_{50} in 10^{-4} % silver nitrate and in distilled water.

Fig. 5. The change of log TD_{50} of larvae bred under the artificial light condition and in darkness. Abscissa, log concentration of silver nitrate (%). Open and soild circles each express the results of Exps. 7 l and k.

plus 10 ml of 10^{-4} % silver nitrate). On the other hand, the addition of silver nitrate to potassium nitrate solution reduces the existence of larvae to a shorter period than in a single solution of silver nitrate. When 10^{-4} % silver nitrate was added, the larvae in the sodium solution outlived the larvae in the potassium solution; each value of TD_{50} in the sodium and potassium solutions lies nearly between the respective values of TD_{50} in distilled water and 10^{-4} % silver nitrate solution without any contents of potassium and sodium ions. From the results given just above, it may be easily supposed that the mode of action of silver nitrate differs respectively in the sodium and potassium solutions; the sodium ion is superior to the potassium ion in disturbing the toxic action of silver nitrate upon the mosquito larvae.

Experiment 7: Larvae were divided into two groups for the examination of the influence of light upon the toxic action of silver nitrate.

k) Twenty larvae were kept in each 10^{-2} %, 10^{-3} %, 10^{-4} %, 10^{-5} %, 10^{-6} % silver nitrate solutions and distilled water under the brightness of ca. 100 lux.

m) In darkness, the larvae were kept in the solutions of the same concentrations of silver nitrate as in Exp. k.

The results of these experiments are summarized in Figure 5. In Exp.

k, a rectilinear relation was recognized between log TD_{50} and log concentrations of silver nitrate, excepting the cases of 10^{-6}% solution and distilled water; the relation may be written as the equation, $\log T + 1.002314 \log C = -1.527106$. The values of TD_{50} in Exp. m were higher than those corresponding in Exp. k, with the exception of 10^{-3}% solution; the difference of mean values of TD_{50} between Exps. k and m was statistically clearly significant, that is to say, the death time of larvae is different according to whether light exists or not. Thus it may be concluded that the toxic effect of silver nitrate is somewhat reduced in darkness in comparison with that under light.

For observation of the influence of a sensitizer, erythrosine, upon the mode of action of silver nitrate, various experiments were separately carried out under the brightness of 100 lux.

Experiment 8: Each twenty larvae were bred in the following three solutions: 1. 100 ml of 10^{-4}% silver nitrate plus 1 ml of 0.1% erythrosine, 2. 100 ml of the same silver solution plus 10 ml of 0.1 % erythrosine, 3. the same silver solution without erythrosine.

Experiment 9: After immersion for 3 hours in 0.01% erythrosine and distilled water respectively, twenty larvae were removed to separate solutions of 10^{-4}% silver nitrate.

Experiment 10: Larvae were divided into four groups: the first two groups were each transferred to distilled water and 0.01% erythrosine after having been previously treated with 10^{-4}% silver nitrate solution for 30 minutes; the next two groups were each removed to 0.01% erythrosine and were left still in the original distilled water after having been soaked for 30 minutes in distilled water.

Table 1

	Treatment	No. of Trial	TD_{50} in minutes
Exp. 8	10^{-4}% $AgNO_3$ (100 ml)	2	653.09±60.30
	10^{-4}% $AgNO_3$ (100 ml) + 0.1% Erythrosine (1ml)	2	868.84±72.52
	10^{-4}% $AgNO_3$ (100ml) + 0.1% Erythrosine (10 ml)	1	72457×10^5
Exp. 9	0.01% Erythrosine $\xrightarrow{3\text{ hrs.}}$ 10^{-4}% $AgNO_3$	3	297.07±80.41
	Dist. water $\xrightarrow{3\text{ hrs.}}$ 10^{-4}% $AgNO_3$	3	510.12±75.02
Exp. 10	10^{-4}% $AgNO_3$ $\xrightarrow{30\text{ min.}}$ Dist. water	3	1223.48±100.30
	10^{-4}% $AgNO_3$ $\xrightarrow{30\text{ min.}}$ 0.01% Erythrosine	3	1777.92±210.52
	Dist. water $\xrightarrow{30\text{ min.}}$ 0.01% Erythrosine	3	1968.94±74.30
	Dist. water → Dist. water	3	2010.34±50.73

Table 1 presents the results of Experiments 8, 9 and 10. The results of Exp. 8 prove that the adding of erythrosine to the silver solution conspicuously lengthens TD_{50} values of the larvae according to the amount of addition; in other words, the toxicity of silver nitrate decreases under the presence of erythrosine. On the other hand, it was found in Exp. 9 that the previous treatment with erythrosine solution promotes remarkably the death of larvae by silver nitrate poisoning. It was surmised from the results of Exp. 10 that the larvae which were exposed to 10^{-4}% silver nitrate beforehand are capable of recovering from the poisoning to some extent as a result of the succeeding immersion in 0.01% erythrosine but never recover to completely non-poisonable state. Furthermore, the erythrosine solution itself could be inferred to be innocuous to the larvae in the concentrations used for the experiment from the fact that TD_{50} values nearly accorded with each other between the larvae which were removed from the erythrosine solution to distilled water and those which were left in distilled water through the whole course of the experiment.

Discussion

It was observed in this study that the relationship of log concentration of silver nitrate to log TD_{50} of larvae is expressed by a rectilinear line. This fact probably means that the death of larvae is quickened according to the amount of silver ions accumulated in the body proportionally to the concentration.

After the amputation of anal gills, the larvae lived considerably longer than the cases in which such treatment was not received (Fig. 1; Exps. 1 and 3). From this fact, it is presumed that the absorption of silver ions is in a marked degree disturbed by the amputation of the anal gills. The addition of reduced glutathion also weakened the toxicity of silver nitrate (Fig. 1; Exps. 1 and 2). In connection with the protective action of reduced glutathion coexisting in the solution of mercury salt, Corner and Rigler (1958) proposed the idea that the affinity of a thiol compound such as reduced glutathion for the mercury ion is greater than that of the body surface for the ion. So far as silver ions are concerned, the present writer also is of the opinion that before penetration into the body, a part of the silver ions may form complex ions with reduced glutathion innocuous to the larvae or may form insoluble salts difficult to absorb by an avidity stronger than that occurring between the surface of the anal gills and the silver ions; accordingly, the toxicity of silver nitrate is reduced. In this case, the essential condition probably is coexistence of both silver and glutathion ions in the external medium; for, alternately separate immersion in the solutions of both the salts mentioned above never postponed the death of larvae in comparison with the case of leaving the larvae in silver solution (Fig. 3; Exps. 5 e and f). The alternate immersion hastened the death of larvae more quickly than in the case of removal of larvae to distilled water after soaking into silver solution alone for 1 to 4 hours and than in the case of treatment with only reduced glutathion (Fig. 3; Exps. 5 d, e and f). Therefore, it is certain that in case either of previous or after-treatment, the separate immersion into reduced glutathion is practically not at all effective in allaying the toxicity of silver nitrate.

The time in which silver nitrate required to kill the larvae under the pre-

sence of sodium and potassium ions is much longer than in the solution with only the dissolved silver salt, and the mode of action of silver nitrate appears to differ with the cases of existence with either sodium or potassium ions; the coexistence of sodium ions fairly weakens the toxic action of silver nitrate to a greater extent than in the potassium solution (Fig. 4; Exps. 6 h and i). Treherne (1954) supposed the presence of a separate mechanism as to the accumulation of these two ions in the body, from the data showing that in the mosquito, *Aedes aegypti*, potassium ions did not compete with sodium for uptake. With respect to the relationship between the mechanism for the uptake of alkali metal ions and the decrease of toxicity of silver nitrate, the present writer has no satisfactory data; but the results of the experiments by Holm-Jensen (1948) seem to suggest a solution of the problem. According to him, the death of *Daphnia magna* in dilute sea water containing silver ions is attributable to the out-flow of sodium ions from the body resulting from the destruction of the mechanism of sodium intake accomplished by silver ions. If this view conforms to the case of the mosquito larvae, the following interpretation will be possible for the fact that when silver nitrate was added, TD_{50} values of the larvae in sodium solution were always delayed beyond the values in potassium solution. That is to say, in those cases, the flowing out of sodium ions from the body of larvae in the sodium solution was not so rapid as in the potassium solution; consequently, the death of larvae in the former was much longer than in the latter. Verification of the appropriateness of this interpretation remains for the future.

The toxic action of silver nitrate in the presence of light is inclined to be stronger than in darkness (Fig. 5 ; Exps. 7 k and m). This observation may suggest the existence of photosensitive substances in the cells of the anal gill, which promote exceedingly the cohesion with silver ions by means of light; the substances may be distributed on the body surface, too. The results of a series of experiments making use of a photosensitive substance, erythrosine, appear to support the above inference to some degree (Table 1). The larvae which were exposed to 0.01% erythrosine before removal to silver nitrate solution died more swiftly than the larvae not submitted to such a previous treatment of erythrosine (Exp. 9). The toxic action of erythrosine hardly comes into question at 0.01% concentration (Exp. 10). Therefore, the results of Exp. 9 probably mean that in light, the larvae rapidly produce deposits of erythrosine with the silver ions in the body by adsorption or absorption of erythrosine. The affinity of silver ions to erythrosine may be stronger than the avidity to combine with the cell contents because the silver solution dissolving erythrosine was proved to be more advantageous to the maintenance of life of the mosquito larvae than the solution including only silver nitrate (Exp. 8); furthermore, after-treatment with erythrosine was found to accelerate markedly the recovery of larvae from poisoning by silver nitrate (Exp. 10).

Finally, the writer wishes to express his hearty gratitude to Professor Tohru Uchida

for his helpful guidance and his kindness in reading through the manuscript.

Summary

1. Silver nitrate killed 50% of the larvae of the mosquito, *Culex pipiens pallens* within 20 minutes at 10^{-2}% concentration. Such a toxic action of the silver salt was proportioned to the concentration; the minimum concentration poisonous to the larvae was 10^{-5}%.

2. The toxicity of silver nitrate was weakened by the amputation of anal gills and the addition of reduced glutathion. In the case of alternate immersion of the larvae into reduced glutathion and silver nitrate solutions, the reduced glutathion could not alleviate the toxicity of the silver ions, notwithstanding previous or after-treatment. Thus, the coexistence of reduced glutathion and silver ions was surmised to be useful for the mitigation of the toxic action of silver nitrate.

3. The toxic action of silver nitrate present in each sodium nitrate and potassium nitrate solutions was not so strong as that of a simple solution of silver nitrate. The degree of alleviation of the toxicity seems to differ slightly between the sodium and the potassium solutions; the former was more effective than the latter in protection from the posioning.

4. The toxic action of silver nitrate in darkness is apt to occur later than in the artificial light. Concerning this, the following facts were found in the presence of light. In the alternate immersion into erythrosine and sliver nitrate solutions, previous treatment with erythrosine promoted the poisoning of the larvae by the silver salt: on the contrary, after-treatment with erythrosine restored the larvae from the poisoning to a tolerable degree; the addition of erythrosine to the silver solution was completely able to destroy the toxicity of the latter.

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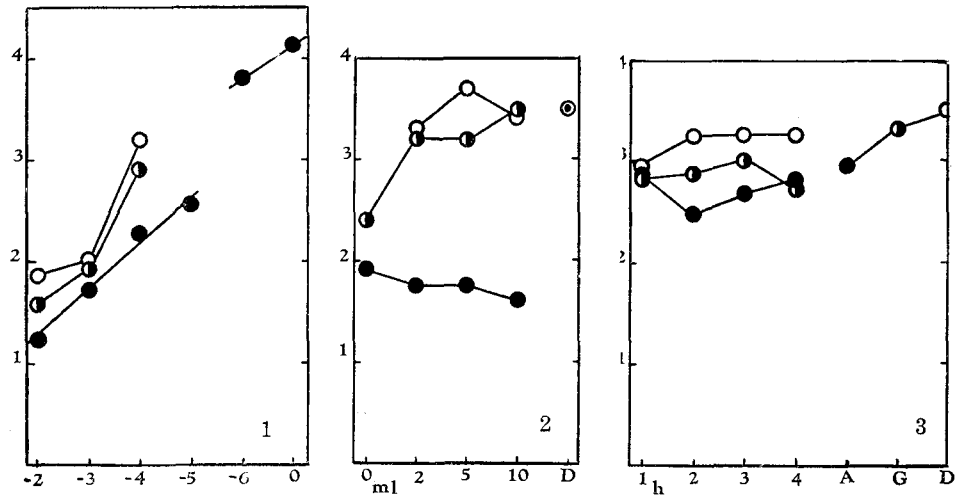

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f) After having been placed previously in 10^{-4}% silver nitrate solution for 1, 2, 3 and 4 hours, the larvae were transferred to glass distilled water.

g) For the purpose of judging the effect of such treatment as described above, the larvae were reared in distilled water, 0.001% reduced glutathion and 10^{-4}% silver nitrate solutions respectively, without being subjected to any of the previously described treatments.

Fig. 3 indicates the results of Exps. d, e, f and g. The results of Exp. d show that TD_{50} of the larvae was not shortened in parallel with the duration of time of immersion in silver nitrate solution; by comparison with Exp. g, the values of TD_{50} in this case were always smaller than those of the larvae reared in reduced glutathion without having been subjected to the previous treatment of silver nitrate. In Exp. e, it was observed that TD_{50} values were not necessarily proportionate to the duration of time of the treatment with reduced glutathion. Comparison with Exp. g reveals the fact that larvae subjected to previous treatment with reduced glutathion never survive longer than larvae bred in silver nitrate solution alone. It may therefore be said that the protective effect of previous treatment of reduced glutathion upon the mosquito larvae is doubtful. According to the results of Exp. f, TD_{50} values of the larvae which were removed from silver nitrate solution to distilled water nearly accord with each other, irrespective of the duration of time of immersion in the silver solution. It was confirmed by statistical test that TD_{50} values in Exp. f are without exception larger than corresponding TD_{50} in Exps. d and e. Hence, the conclusion is reasonably arrived at that alternate immersions into both silver nitrate and reduced glutathion solutions are effective in quicking the death of larvae, and that removal within 4 hours from silver solution to distilled water results in a slow recovery of larvae from the poisoning of silver nitrate.

Experiment 6 : Three sets of experiments were conducted with the intention of inquiring into the toxic action of silver nitrate under the presence of sodium and potassium ions.

h) Mosquito larvae were reared in 100 ml of 0.1% sodium nitrate solutions containing respectively 1 ml, 5 ml and 10 ml of 10^{-4}% silver nitrate.

i) Larvae were reared in 100 ml of 0.1% potassium nitrate solutions containing respectively the same amounts of 10^{-4}% silver nitrate as in Exp. h.

j) As the control group, the larvae were reared in 0.1% $NaNO_3$, 0.1% KNO_3, 10^{-4}% $AgNO_3$ and distilled water respectively.

The results of these experiments are graphed in Fig. 4. According to the figure, the addition of small amount of silver nitrate to the sodium nitrate solution postpones the death of the larvae beyond its occurrence in the solution including only sodium nitrate, exclusive of one exception (the sodium solution

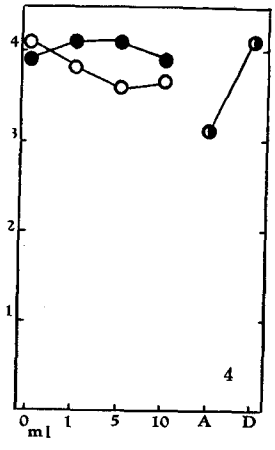

Fig. 4.

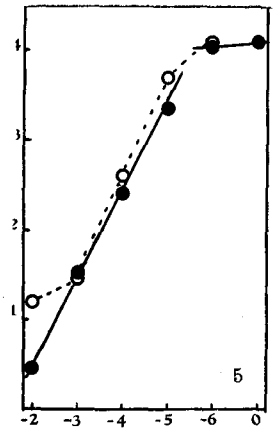

Fig. 5.

Fig. 4. The change of log TD_{50} when silver nitrate was added to sodium and potassium nitrate solutions. Abscissa, the annexed amounts of silver nitrate. Solid and open circles indicate the results of Exps. 6 h and i respectively: semi-open circles over abscissa A and D show each log TD_{50} in 10^{-4} % silver nitrate and in distilled water.

Fig. 5. The change of log TD_{50} of larvae bred under the artificial light condition and in darkness. Abscissa, log concentration of silver nitrate (%). Open and soild circles each express the results of Exps. 7 l and k.

plus 10 ml of 10^{-4} % silver nitrate). On the other hand, the addition of silver nitrate to potassium nitrate solution reduces the existence of larvae to a shorter period than in a single solution of silver nitrate. When 10^{-4} % silver nitrate was added, the larvae in the sodium solution outlived the larvae in the potassium solution; each value of TD_{50} in the sodium and potassium solutions lies nearly between the respective values of TD_{50} in distilled water and 10^{-4} % silver nitrate solution without any contents of potassium and sodium ions. From the results given just above, it may be easily supposed that the mode of action of silver nitrate differs respectively in the sodium and potassium solutions; the sodium ion is superior to the potassium ion in disturbing the toxic action of silver nitrate upon the mosquito larvae.

Experiment 7: Larvae were divided into two groups for the examination of the influence of light upon the toxic action of silver nitrate.

k) Twenty larvae were kept in each 10^{-2} %, 10^{-3} %, 10^{-4} %, 10^{-5} %, 10^{-6} % silver nitrate solutions and distilled water under the brightness of ca. 100 lux.

m) In darkness, the larvae were kept in the solutions of the same concentrations of silver nitrate as in Exp. k.

The results of these experiments are summarized in Figure 5. In Exp.

k, a rectilinear relation was recognized between log TD_{50} and log concentrations of silver nitrate, excepting the cases of 10^{-6}% solution and distilled water; the relation may be written as the equation, $\log T + 1.002314 \log C = -1.527106$. The values of TD_{50} in Exp. m were higher than those corresponding in Exp. k, with the exception of 10^{-3}% solution; the difference of mean values of TD_{50} between Exps. k and m was statistically clearly significant, that is to say, the death time of larvae is different according to whether light exists or not. Thus it may be concluded that the toxic effect of silver nitrate is somewhat reduced in darkness in comparison with that under light.

For observation of the influence of a sensitizer, erythrosine, upon the mode of action of silver nitrate, various experiments were separately carried out under the brightness of 100 lux.

Experiment 8: Each twenty larvae were bred in the following three solutions: 1. 100 ml of 10^{-4}% silver nitrate plus 1 ml of 0.1% erythrosine, 2. 100 ml of the same silver solution plus 10 ml of 0.1 % erythrosine, 3. the same silver solution without erythrosine.

Experiment 9: After immersion for 3 hours in 0.01% erythrosine and distilled water respectively, twenty larvae were removed to separate solutions of 10^{-4}% silver nitrate.

Experiment 10: Larvae were divided into four groups: the first two groups were each transferred to distilled water and 0.01% erythrosine after having been previously treated with 10^{-4}% silver nitrate solution for 30 minutes; the next two groups were each removed to 0.01% erythrosine and were left still in the original distilled water after having been soaked for 30 minutes in distilled water.

Table 1

	Treatment	No. of Trial	TD_{50} in minutes
Exp. 8	10^{-4}% $AgNO_3$ (100 ml)	2	653.09±60.30
	10^{-4}% $AgNO_3$ (100 ml) + 0.1% Erythrosine (1ml)	2	868.84±72.52
	10^{-4}% $AgNO_3$ (100ml) + 0.1% Erythrosine (10 ml)	1	72457×10^5
Exp. 9	0.01% Erythrosine $\xrightarrow{3\text{ hrs.}}$ 10^{-4}% $AgNO_3$	3	297.07±80.41
	Dist. water $\xrightarrow{3\text{ hrs.}}$ 10^{-4}% $AgNO_3$	3	510.12±75.02
Exp. 10	10^{-4}% $AgNO_3$ $\xrightarrow{30\text{ min.}}$ Dist. water	3	1223.48±100.30
	10^{-4}% $AgNO_3$ $\xrightarrow{30\text{ min.}}$ 0.01% Erythrosine	3	1777.92±210.52
	Dist. water $\xrightarrow{30\text{ min.}}$ 0.01% Erythrosine	3	1968.94±74.30
	Dist. water → Dist. water	3	2010.34±50.73

Table 1 presents the results of Experiments 8, 9 and 10. The results of Exp. 8 prove that the adding of erythrosine to the silver solution conspicuously lengthens TD_{50} values of the larvae according to the amount of addition; in other words, the toxicity of silver nitrate decreases under the presence of erythrosine. On the other hand, it was found in Exp. 9 that the previous treatment with erythrosine solution promotes remarkably the death of larvae by silver nitrate poisoning. It was surmised from the results of Exp. 10 that the larvae which were exposed to 10^{-4}% silver nitrate beforehand are capable of recovering from the poisoning to some extent as a result of the succeeding immersion in 0.01% erythrosine but never recover to completely non-poisonable state. Furthermore, the erythrosine solution itself could be inferred to be innocuous to the larvae in the concentrations used for the experiment from the fact that TD_{50} values nearly accorded with each other between the larvae which were removed from the erythrosine solution to distilled water and those which were left in distilled water through the whole course of the experiment.

Discussion

It was observed in this study that the relationship of log concentration of silver nitrate to log TD_{50} of larvae is expressed by a rectilinear line. This fact probably means that the death of larvae is quickened according to the amount of silver ions accumulated in the body proportionally to the concentration.

After the amputation of anal gills, the larvae lived considerably longer than the cases in which such treatment was not received (Fig. 1; Exps. 1 and 3). From this fact, it is presumed that the absorption of silver ions is in a marked degree disturbed by the amputation of the anal gills. The addition of reduced glutathion also weakened the toxicity of silver nitrate (Fig. 1; Exps. 1 and 2). In connection with the protective action of reduced glutathion coexisting in the solution of mercury salt, Corner and Rigler (1958) proposed the idea that the affinity of a thiol compound such as reduced glutathion for the mercury ion is greater than that of the body surface for the ion. So far as silver ions are concerned, the present writer also is of the opinion that before penetration into the body, a part of the silver ions may form complex ions with reduced glutathion innocuous to the larvae or may form insoluble salts difficult to absorb by an avidity stronger than that occurring between the surface of the anal gills and the silver ions; accordingly, the toxicity of silver nitrate is reduced. In this case, the essential condition probably is coexistence of both silver and glutathion ions in the external medium; for, alternately separate immersion in the solutions of both the salts mentioned above never postponed the death of larvae in comparison with the case of leaving the larvae in silver solution (Fig. 3; Exps. 5 e and f). The alternate immersion hastened the death of larvae more quickly than in the case of removal of larvae to distilled water after soaking into silver solution alone for 1 to 4 hours and than in the case of treatment with only reduced glutathion (Fig. 3; Exps. 5 d, e and f). Therefore, it is certain that in case either of previous or after-treatment, the separate immersion into reduced glutathion is practically not at all effective in allaying the toxicity of silver nitrate.

The time in which silver nitrate required to kill the larvae under the pre-

sence of sodium and potassium ions is much longer than in the solution with only the dissolved silver salt, and the mode of action of silver nitrate appears to differ with the cases of existence with either sodium or potassium ions; the coexistence of sodium ions fairly weakens the toxic action of silver nitrate to a greater extent than in the potassium solution (Fig. 4; Exps. 6 h and i). Treherne (1954) supposed the presence of a separate mechanism as to the accumulation of these two ions in the body, from the data showing that in the mosquito, *Aedes aegypti*, potassium ions did not compete with sodium for uptake. With respect to the relationship between the mechanism for the uptake of alkali metal ions and the decrease of toxicity of silver nitrate, the present writer has no satisfactory data; but the results of the experiments by Holm-Jensen (1948) seem to suggest a solution of the problem. According to him, the death of *Daphnia magna* in dilute sea water containing silver ions is attributable to the out-flow of sodium ions from the body resulting from the destruction of the mechanism of sodium intake accomplished by silver ions. If this view conforms to the case of the mosquito larvae, the following interpretation will be possible for the fact that when silver nitrate was added, TD_{50} values of the larvae in sodium solution were always delayed beyond the values in potassium solution. That is to say, in those cases, the flowing out of sodium ions from the body of larvae in the sodium solution was not so rapid as in the potassium solution; consequently, the death of larvae in the former was much longer than in the latter. Verification of the appropriateness of this interpretation remains for the future.

The toxic action of silver nitrate in the presence of light is inclined to be stronger than in darkness (Fig. 5 ; Exps. 7 k and m). This observation may suggest the existence of photosensitive substances in the cells of the anal gill, which promote exceedingly the cohesion with silver ions by means of light; the substances may be distributed on the body surface, too. The results of a series of experiments making use of a photosensitive substance, erythrosine, appear to support the above inference to some degree (Table 1). The larvae which were exposed to 0.01% erythrosine before removal to silver nitrate solution died more swiftly than the larvae not submitted to such a previous treatment of erythrosine (Exp. 9). The toxic action of erythrosine hardly comes into question at 0.01% concentration (Exp. 10). Therefore, the results of Exp. 9 probably mean that in light, the larvae rapidly produce deposits of erythrosine with the silver ions in the body by adsorption or absorption of erythrosine. The affinity of silver ions to erythrosine may be stronger than the avidity to combine with the cell contents because the silver solution dissolving erythrosine was proved to be more advantageous to the maintenance of life of the mosquito larvae than the solution including only silver nitrate (Exp. 8); furthermore, after-treatment with erythrosine was found to accelerate markedly the recovery of larvae from poisoning by silver nitrate (Exp. 10).

Finally, the writer wishes to express his hearty gratitude to Professor Tohru Uchida

for his helpful guidance and his kindness in reading through the manuscript.

Summary

1. Silver nitrate killed 50% of the larvae of the mosquito, *Culex pipiens pallens* within 20 minutes at 10^{-2}% concentration. Such a toxic action of the silver salt was proportioned to the concentration; the minimum concentration poisonous to the larvae was 10^{-5}%.

2. The toxicity of silver nitrate was weakened by the amputation of anal gills and the addition of reduced glutathion. In the case of alternate immersion of the larvae into reduced glutathion and silver nitrate solutions, the reduced glutathion could not alleviate the toxicity of the silver ions, notwithstanding previous or after-treatment. Thus, the coexistence of reduced glutathion and silver ions was surmised to be useful for the mitigation of the toxic action of silver nitrate.

3. The toxic action of silver nitrate present in each sodium nitrate and potassium nitrate solutions was not so strong as that of a simple solution of silver nitrate. The degree of alleviation of the toxicity seems to differ slightly between the sodium and the potassium solutions; the former was more effective than the latter in protection from the posioning.

4. The toxic action of silver nitrate in darkness is apt to occur later than in the artificial light. Concerning this, the following facts were found in the presence of light. In the alternate immersion into erythrosine and sliver nitrate solutions, previous treatment with erythrosine promoted the poisoning of the larvae by the silver salt: on the contrary, after-treatment with erythrosine restored the larvae from the poisoning to a tolerable degree; the addition of erythrosine to the silver solution was completely able to destroy the toxicity of the latter.

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