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Depigmentation of Planarian eye treated with salts of dithiocarbamide and related chemicals

by

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In our previous paper (Kido and Kishida, 1960), it was reported that the depigmentation of planarian eye occurred more rapidly in the solution of thiocarbamide mixed with iodine than in the solution of thiocarbamide alone. Thus, we assumed that an oxidation product derived from thiocarbamide may be more effective for causing depigmentation of the eye than thiocarbamide itself. Under the situation, Kishida (1960) carried out an extensive experiment. When the worms were treated with solutions containing thiocarbamide and each of some oxidizers other than iodine, the eye-pigment disappeared with the same speed as in the case with iodine. On the other hand, it has been known that an oxidizer reacts with thiocarbamide to produce a salt of dithiocarbamide (Bis [amino-imino-methyl] disulfid), if the mixed solution is kept in a favorable condition (Claus, 1885, McGowan, 1886, 1887, Storch 1890, Marshall, 1902). However, it has not yet been tested whether the salt may be effective for causing the depigmentation of planarian eye.

The present paper deals with this point. In addition, by testing the effect of some other chemicals related to thiocabamide and dithiocarbamide in relation to their chemical structures, it was hoped to learn the nature of the chemical reactions involved in the depigmentation of the eye.

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Material and Method

The material used was exclusively an asexual form of *Dugesia gonocephala* collected in Kohiraso, the vicinity of Kanazawa City. After starvation over a week, ten worms were reared together in a Petri-dish filled with 50 cc of the test solution at 20－22°C without giving any food. The test solution was not changed throughout the experiment.

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The chemicals used in the present experiment, dithiocarbamide dichloride ($C_2H_6N_4S_2 \cdot 2HCl$), dithiocarbamide dibromide ($C_2H_6N_4S_2 \cdot 2HBr$), dithiocarbamide diodide ($C_2H_6N_4S_2 \cdot 2HJ$), dithiocarbamide dioxalate ($C_2H_6N_4S_2 \cdot 2C_2H_2O_4$), dithiocarbamide dinitrate ($C_2H_6N_4S_2 \cdot 2HNO_3$), thiocarbamide nitrate ($CSN_2H_4 \cdot HNO_3$) and carbamide nitrate ($CON_2H_4 \cdot HNO_3$) were carefully prepared in our laboratory. But prior to the use each chemical was identified by examining their physico-chemical properties.

Experimental result

The degrees of the depigmentation of planarian eye treated with the test solutions were graded into six types, i. e., N, A, B, C, D and E, as were already described in our previous paper (Kido and Kishida, 1960).

Experiment 1. *Treatment with salts of dithiocarbamide*

Five salts of dithiocarbamide, a) –dichloride, b) –dibromide, c) –diodide, d) –dioxalate, e) –dinitrate, were respectively prepared as 10^{-4}M aqueous solution.

Table 1. Depigmentation of planarian eye treated with the salts of dithiocarbamide.

Salt of dithiocarbamide	Types of depigmented eye	Days after treatment			
		1	2	3	4
a)-dichloride	N	17.5*	0.0	0.0	0.0
	A	5.0	7.5	0.0	0.0
	B	10.0	5.0	0.0	0.0
	C	42.5	12.5	0.0	0.0
	D	15.0	25.0	2.5	0.0
	E	10.0	50.0	97.5	100.0
b)-dibromide	N	10.0	0.0	0.0	0.0
	A	17.5	7.5	0.0	0.0
	B	20.0	2.5	0.0	0.0
	C	37.5	12.5	7.5	0.0
	D	15.0	32.5	0.0	0.0
	E	0.0	45.0	92.5	100.0
c)-diiodide	N	5.0	0.0	0.0	0.0
	A	15.0	5.0	0.0	0.0
	B	7.5	0.0	0.0	0.0
	C	50.0	10.0	5.0	0.0
	D	17.5	7.5	2.5	0.0
	E	5.0	77.5	92.5	100.0
d)-dioxalate	N	17.5	0.0	0.0	0.0
	A	2.5	2.5	0.0	0.0
	B	2.5	0.0	0.0	0.0
	C	27.5	5.0	0.0	0.0
	D	35.0	7.5	2.5	0.0
	E	15.0	85.0	97.5	100.0
e)-dinitrate	N	10.0	0.0	0.0	0.0
	A	15.0	5.0	0.0	0.0
	B	5.0	2.5	0.0	0.0
	C	27.5	12.5	0.0	0.0
	D	40.0	20.0	2.5	0.0
	E	2.5	60.0	97.5	100.0

* Numbers indicate percentage.

As is shown in Table 1, on the first day after the treatment, all types of the depigmented eye from N to E were observed, but types C and D appeared in more than 50% of the whole in every experimental group. On the second day type N in which the depigmentation did not occur, was not found, but types D and E exceeded 70%. On the third day completely depigmented eye (type E) reached into 97.5 % in a), 92.5% in b), 92.5% in c), 97.5% in d) and 97.5% in e). In each experimental group from a) to e), the time required for the complete disappearance of eye-pigment in all the worms was four days. But when the worms were treated with 10^{-4}M solution of thiocarbamide, the depigmentation of the eye was not found at all even on the fourth day after the treatment.

From these facts it is revealed that the salts of dithiocarbamide mentioned above are approximately equal to each other in their depigmentating effect, and much more effective than thiocarbamide itself.

Experiment 2. *Treatment with chemicals related to thiocarbamide and to salts of dithiocarbamide*

a) *Treatment with thiocarbamide nitrate.* Since dithiocarbamide dinitrate used in experiment 1. e) was very effective for the depigmentation of the eye, some worms were treated with 10^{-4}M solution of thiocarbamide nitrate. On the fourth day after the treatment, the eyes of all the worms were of type N. On the twenty-eighth day after the treatment, the occarance of types A, B, C and D were found to be 32%, 30%, 28% and 2.5% respectively. These resuelts are almost the same as in the case with thiocarbamide, as is shown in Table 2.

Table 2. Depigmentation of planarian eye treated with the solution of thiocarbamide.

Types of depigmented eye	Days after treatment					
	1	7	14	18	21	28
N	100.0	50.1	38.8	25.8	0.0	0.0
A	0.0	45.0	53.8	62.5	56.3	23.8
B	0.0	3.8	3.8	6.3	31.3	31.3
C	0.0	0.0	2.5	2.5	8.8	46.3
D	0.0	0.0	1.3	1.3	2.5	3.8
E	0.0	0.0	0.0	0.0	1.3	5.0

b) *Treatment with carbamide, guanidine and carbamide nitrate.* Since thiocarbamide $SC(NH_2)_2$ was effective for the depigmentation of the eye, carbamide $OC(NH_2)_2$ and guanidine $NH_2C(NH_2)_2$ which resemble it in chemical structure were tested. Carbamide nitrate which is a salt of carbamide was tested too. In each test solution, the worms survived over thirty days without food, but no depigmentation of the eye occurred.

c) *Treatment with phenylthiocarbamide, phenylthiocarbamide chloride and phenylthiocarbamide bromide.* It is well known that phenylthiocarbamide (PTC) causes depigme-

ntation of the embryo in some vertebrates and invertebrates. In the present experiment, the worms had been reared without food for thirty-five days prior to the treatment with 10^{-4}M solution of PTC. As is shown in table 3, on the seventh day after the treatment, types N and A appeared in 91.8% and type B in 6.3%. On the fourteenth day after the treatmant, the rate of the appearance of the eyes having normal pigment

Table 3. Depigmentation of planarian eye treated with the solution of phenylthiocarbamide and phenylthiocarbamide chloride.

Test chemicals	Types of depigmented eye	Days after treatment*				
		7	14	18	21	28
PTC	N	38.8	15.0	15.0	0.0	0.0
	A	55.0	61.3	51.3	22.5	2.5
	B	6.3	13.8	20.0	31.3	13.8
	C	0.0	6.3	6.3	21.3	48.8
	D	0.0	2.5	2.5	5.0	10.0
	E	0.0	1.3	5.0	20.0	35.0
PTC-chloride	N	41.2	25.0	16.7	0.0	0.0
	A	55.0	47.1	31.3	16.7	15.2
	B	3.8	17.6	29.2	18.3	24.3
	C	0.0	2.9	10.4	28.3	27.3
	D	0.0	2.9	4.2	6.7	0.0
	E	0.0	4.4	8.4	30.0	30.3

* The worms used were reared without food during 35 days prior to the treatment.

mass (N) was reduced to 15.0%, while that of the completely depigmented eyes (E) increased to 1.3% from zero on the seventh day. On the twenty-first day after the treatment, type N was not found at all, while type A was occured in 22.5%, types B and C in 52.6% and types D and E in 25%. On the twenty-eighth day after the treatment, type A was reduced to 2.5% and type E increased to 35%.

When the worms were treated with 10^{-4}M solution of PTC-chloride, the depigmentation of the eye proceeded at the same speed as in the case with PTC, but on the tenth day after the treatment, the heads of some worms were so disintegrated that their eye types could not be determined.

The effect of 10^{-4}M solution of PTC-bromide was the same as that of PTC or PTC-chloride.

In the above experiment, since the worms were left starving for as many as sixty-three days, it seemed to be necessary to examine the effect of starvation on the depigmentation of the eye. As is shown in table 4, after the starvation of the worms for sixty-three days the occurance of types N and A were 50% respectively, types B, C, D and E being not observed. The effect of starvation itself on the depigmentation of the eye was recognized as early as at fourty-two days after initiation of the starvation. However, the percentage of type N was not decreased even after more prolonged starvation. That is, on the fourty-

Table 4. Depigmentation of planarian eye due to starvation during long term.

Types of de-pigmetned eye	Days after starvation					
	42	49	53	56	63	138
N	54.1	50.0	52.6	42.2	50.0	19.0
A	46.9	50.0	47.4	57.8	50.0	9.5
B	0.0	0.0	0.0	0.0	0.0	4.8
C	0.0	0.0	0.0	0.0	0.0	11.9
D	0.0	0.0	0.0	0.0	0.0	7.1
E	0.0	0.0	0.0	0.0	0.0	47.6

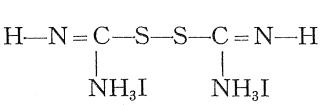
ninth day after initiation of the starvation type N was found in 50.0% and type A in 50.0%. But on the fifty-third day type N was increased to 52.6% and type A was decreased to 47.4%. It seems likely that depigmentation and recovery occurred alternately in the course of the starvation. But, finally, as is shown in Table 4, on the one hundred and thirty-eighth day from initiation of the starvation the depigmentation became so prominent that completely depigmented eyes were found in 47.6%.

d) *Treatment with cystine, allinamine, allithiamine, and monoiodoacetic acid.* It has been well known that each of these three chemicals has -s-s- bond, blocking the reaction of SH group, in the same manner as monoiodoacetic acid. The reason for the use of these present chemicals is that the salts of dithiocarbamide have also -s-s- bond. But in 10^{-4}M solution of any of them even at thirty days after the treatment, the depigmentation of the eye did not occur.

Discussion

In our previous works it has been shown that when planarian was reared in the aqueous solution of thiocarbamide containing iodine (Kido and Kishida, 1960) or some other oxidizers (Kishida, 1960), the pigment cells of the eye disintegrated and the pigment mass of the eye dispersed into fragments which would be absorbed into intestinal tracts, and finally the disappearance of the eye-pigment occurred. The present experiment now demonstrates that the salts of dithiocarbamide are specific agents destructive to the pigment cells of planarian eye.

What is the nature of such destructive action of the salt of dithiocarbamide ? According to Marshall(1902), the chemical structure of dithiocarbamide diiodide was proved as follows.



In the present experiment, it was found that the time required for the complete disappearance of the eye-pigment of all the worms treated with the salts of dithiocarbamide was the same, regardless of the kinds of salts. On the other hand, thiocarbamide nitrate was

less effective for the depigmentation of the eye than thiocarbamide, and carbamide nitrate was not effective at all. Accordingly, it is assumed that disappearance of the eye-pigment should not to be ascribed to the action of halogens or acids incorporating in dithiocarbamide. It may be imagined that -s-s- bond acts to damage the pigment cell of the eye as was suggested in the previous paper. Concerning this point, cystine, allinamine and allithiamine which have -s-s- bond and monoiodoactic acid which blocks SH group in the living cell were tested in experiment 2, d). However, none of them was effective to cause the depigmentation of the eye. From this fact, it seems likely that -s-s- bond itself is not indispensable to the depigmentation of the eye, though Harada and Kato (1960) claimed with *Philosamia cynthia ricini* that SH compound was responsible for the formation of melanosis in body fluid. But, the informations that dithiocarbamide is produced by incorporation of two molecules of thiocarbamide and that the salt of the former was the most effective agent for the depigmentation of the eye may be useful for answering the quenstion of the chemical reaction of the agent.

Carbamide and guanidine which resemble thiocarbamide in chemical structure were not effective. These chemicals are different from thiocarbamide in the point that do not contain sulphur. The role of sulphur in the depigmentation of the eye cannot be deduced from the present experiment.

Phenylthiocarbamide (PTC) was more toxic to planarian body than thiocarbamide. But no definite difference between the effects of PTC and thiocarbamide on the depigmentation of the eye was recognized, so far as the tests were conducted at such concentrations of PTC that were below sublethal dose. In the control experiment in which the worms were cultured in tap water for a long term without food, some eye-pigments disappeared. Berninger (1911) observed the similar phenomenon in *Dendrocoelum lacteum*. The question how starvation causes the depigmentation of planarian eye will be left for future study. However, when the worms are reared for a long term in the test solution without food, the effect of the starvation should be taken into consideration in evaluating that of the test solution.

Summary

1. The present experiment was carried out in order to verify our previous assumption (Kido and Kishida, 1960) that the salt of dithiocarbamide might be effective to cause the depigmentation of the eye of *Dugesia gonocephala*. In addition, the effect of some related chemicals to thiocarbamide and dithiocarbamide were also tested concerning the depigmentation of the eye.

2. The worms were treated with five salts of dithiocarbamide prepared in our laboratory. These chemicals displayed stronger effect than thiocarbamide. Among these salts of dithiocarbamide, no difference was recognized regarding the time required for the depigmentation.

3. The effect of thiocarbamide nitrate was almost the same with that of thiocarbamide. Carbamide, guanidine and carbamide nitrate were not effective.

4. Phenylthiocarbamide showed almost the same degree of effectiveness with thiocarbamide in the same concentration, as long as the worms were survived. Phenylthiocarbamide chloride and-bromide were more toxic to the worm body than phenylthiocarbamide and these three chemicals were almost the same in their effect upon eye-pigment.

5. Cystine, allinamine, allithiamine and monoiodoacetic acid were not effective to cause the depigmentation of the eye.

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