ANNOTATIONES ZOOLOGICAE JAPONENSES
Volume 26, No. 1—March 1953

Published by the Zoological Society of Japan
Zoological Institute, Tokyo University

The Effects of Lithium Chloride and Low Temperature on Head Regeneration of *Planaria*[1)]

With 1 Text-figure

Shigehisa MIFUNE

Biological Institute, Faculty of Science, Kyushu University
(*Communicated by* M. ICHIKAWA)

As the results of many investigations concerning the regeneration of *Planaria*, it has long been known that a head regenerates from the anterior cut surface of the posterior long piece, and that this regeneration becomes gradually less frequent as sections are made more posteriorly. This fact seems to show that the gradient of head regenerating potency coincides with the physiological gradient of the body, which decreases in a cephalocaudal direction. This coincidence of the physiological gradient with the regeneration gradient has stimulated many investigators to perform experiments which have been designed to test the effects of various chemicals on morphogenesis. Child (1907) found that after treating a piece of *Planaria* with alcohol, ether, or chloretone, the resulting regenerates gave rise to a small head or sometimes to a cyclops of reduced size.

Among many chemicals so far tested, the action of lithium chloride seems to me very interesting. Brønsted (1942) demonstrated that lithium chloride has no effect on the head regenerating frequency in *Planaria*, but the majority of heads formed are provided with supplementary eyes.

The present paper is also concerned with the action of lithium chloride on head regeneration from the standpoint of the physiological gradient.

I wish here to express my sincere gratitude to Prof. Mamori Ichikawa, Kyoto University, for his kindness in reading the manuscript, and to Prof. Izumi Kawakami, Kyushu University, for his encouragement given to me during this study.

1) Contributions from the Biological Institute, Faculty of Science, Kyushu University, No. 14. This work was made possible by grants from the Government Research Fund for Science.

EXPERIMENTAL

The experiments were performed in the winter of 1949 and in the autumn of 1950. *Planaria gonocephala* collected from the streams near Kyoto was used as the material. Worms measuring 12–15 mm. in length and starved for at least one week were chosen for the experiments. The worms were always decapitated at the level posterior to the head with a dissecting knife, and treated for 4 hours with 1/600 (M/28) lithium chloride solution. The conceneration of the solution and duration of the treatment were just below the lethal level, which were determined by preliminary experiments. After the treatment, the headless worms were removed to a finger bowl filled with tap water, in which the regenerating process took place at room temperature. About 8 individuals were contained in each bowl. A tap water control with the same number of headless worms was always run at the same time. In the winter experiments the room temperature fell from 10°C to 3°C seven days after decapitation.

Two available cases were obtained in the winter experiments. Eight worms were contained in each case.

In one case, worms treated with lithium chloride at 24 hours after decapitation are shown in Fig. 1 A, B, and in another case those treated at 48 hours in Fig. 1 C, D. When the room temperature fell to 3°C as mentioned above, a frost-bite-like injury was observed in them.

Nine days after decapitation, the eye-spots were observed in the new head regenerated from the anterior end in all cases except for D, which had an injury near both ends. As time passed, the injury seemed to become deeper, and a thick layer of slime covered it, while new tissue was observed adjacent to the injury. In addition, a bud of new tissue developed laterally from either side of the middle of the body in specimen A and from one side in specimen B. In specimens C and D, new tissues formed near the tail end grew into a form like the caudal fin of a gold fish.

In specimen A, twelve days after decapitation, the two buds attached laterally in the pre- and postpharyngeal region grew like heads, but each was provided with one eye-spot (Fig. 1, Ab). Moreover, two weeks after decapitation, the tissue of the tail end became a heteropolar head with two eye-spots.

In specimen B, two eye-spots were formed on the regenerated tissue at the head end. The bud of tissue attached laterally in the prepharyngeal region did not show any subsequent visible changes and the newly formed tissue adjacent to the injury seemed to have stopped farther proliferation.

In specimen C, a head with two normal eye-spots was regenerated from the anterior cut surface, but this worm was lost when the water was renewed, and further observation on the caudal buds became impossible.

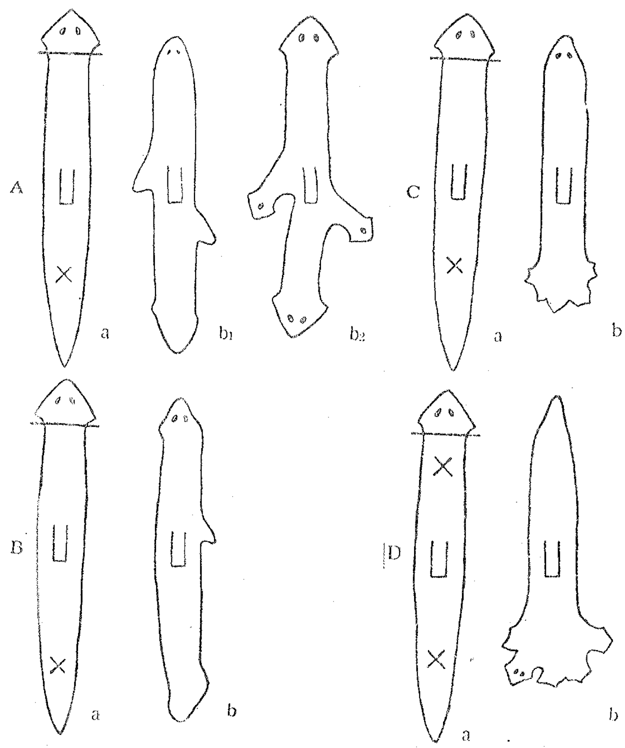

Fig. 1. *Planaria gonocephala* Dugès. a) showing level of section (–) and location of injury (×); and b) showing results obtained from a) of each case.

In specimen D, the regenerated tissue of the tail end grew into five protrusions, each of which became head-like, and one of them developed two eye-spots located rather irregularly.

In the autumn experiments, the effect of low temperature was especially taken into consideration in addition to that of lithium.

From two weeks before decapitation to the end of observation, the worms were always kept at 14–18°C except for a time of low temperature treatment. Twenty-four hours after decapitation, the worms were treated with lithium chloride for 4 hours. Seven days after decapitation, eight of the lithium-treated worms were subjected to low temperature (3°C), in a thermos-jug, for 20 hours. Then they were returned to the original temperature.

In this case, injuries were seemingly limited to the epidermis, and they were distributed uniformly all over the body. The frost-bite-like injury was not observed in any specimen. The worms showed a strong tendency to fissure at the level where the injury occurred, and some which were severely injured by the low temperature were covered with a thick layer of slime, so that further observation of the regeneration process had to be abandoned. At any rate, a heteropolar head was obtained in one of the eight worms subjected to low temperature.

As the injury seemed to be closely related to the malformation above mentioned, the following experiments were made in order to bring out more clearly the factor or factors which produce the injury.

Lithium chloride was dissolved in M/300 Sörensen's buffer solution of various pH values. Twenty-four hours after decapitation, worms were treated at room temperature (14–18°C) with buffered lithium chloride solution or with buffered solution without lithium chloride, for 4 hours. Seven days after decapitation, some of the worms were subjected to the low temperature (3°C) for 12 hours, and the others were left at room temperature. As the control, the third lot of worms, which had been cultured in tap water at room temperature for seven days after decapitation, were subjected to the low temperature without lithium treatment. The results of the three experiments are summarized in the following table.

Table 1.

The effect of lithium chloride solution at various pH values in conjunction with low temperature.

Treatment with LiCl	Exposure to low temp.	Total No. of worm	Number of available cases	Number of injured specimens		
				Preph.	Pharyn.	Postph.
5.2	+	31	11	3	1	0
5.2	–	10	5	0	0	0
5.4	+	16	6	2	1	0
–(5.4)	+	12	8	0	0	0
5.9	+	31	17	3	7	0
5.9	–	10	8	0	0	0
7.7	+	6	3	0	0	0
–(7.7)	+	8	5	0	0	0
8.0	+	?	11	1	0	0
–(8.0)	+	12	8	0	0	0
Tap water	+	15	14	0	0	0

+ represents the cases under treatment. – represents the cases not treated. Numerals in the first column indicate pH value of lithium chloride solution. Preph. prepharyngeal region; Pharyn. pharyngeal region; Postph. postpharyngeal region.

As shown in the table, it is evident that the occurrence of the injury is confined to the experiments in which the worms were treated with lithium chloride and exposed subsequently to the low temperature, regardless of the pH value.

DISCUSSION

Heteropolar head formation has already been demonstrated by Okada and Sugino, 1937 and by Tashirogi, 1951, from short pieces of *Planaria*; the former obtained it without treatment by any chemicals, but the latter produced it by means of lithium treatment. The present experiment verifies the fact that heteropolar head formation is obtainable even of the piece is long. The most noticeable fact is that the formation of biaxial or heteropolar heads occurred only in worms which were successively treated with lithium chloride solution and low temperature. Since such malformation has never been found to take place when the worms were treated either with lithium chloride solution or with cold tap water alone, we are inclined to consider that it is produced by a co-operation between lithium chloride and low temperature. Brønsted (1942) tested the influence of lithium on the regeneration of *Planaria* and drew the conclusion that this chemical is toxic to the worms in such a way that it probably lowers their respiration. On the other hand, according to studies on amphibian embryos, lithium has a tendency to lower glycolysis (Takamoto 1951, Mifune 1952, Kawakami, umpublished). Although the present work contributes nothing to the physiological meaning of the lithium action, it may be allowable to suggest that some kind of physiological disorder has been brought about in the worms by the double treatment. According to the idea of differential susceptibility, the effects are assumed to be the strongest at the level of decapitation, where the metabolic activity is the highest in the headless worms, and the lower the activity physiologically, the less susceptible the region is to the destructive effect of chemicals. However, Child (1911) stated with *Planaria* that the susceptibility is temporarily increased by sectioning, but gradually decreases and finally regains its original state. In this connection my preliminary experiment suggests that the mortality of the pieces was high when they were treated immediately after decapitation with lithium chloride, even at the low concentration of 1/1,200. Therefore, in the present experiments, the treatment with lithium chloride was performed in such a manner as to avoid this period of temporarily high susceptibility. Child showed also that after disappearance of this temporary increase of susceptibility caused by sectioning, the metabolic activity begins to rise again at the time of beginning of regeneration (24-48 hr. after sectioning). Consulting Child's result, the worms in the present experiments seem to have been brought to the experimental conditions when the metabolic activity, viz. susceptibility to the toxic agents of the regenerating region, has increased. If so, the regenerating region of the anterior cut surface may be most strongly affected by lithium chloride in conjunction with low temperature, to produce a levelling of the original gradient. As a result, the posterior region may become isolated from the domain of the original dominancy, and thus the regeneration blastema at the posterior injured region develops a heteropolar head.

Some of the injuries seemed to be caused by the mechanical pressure of the slime layer secreted from the body wall of the worm. In a transplantation experiment on *Planaria*, Gebhardt (1926) observed the appearance of slime layers covering the transplanted region, and the tissue under the slime layer being constricted and torn off by the latter. I observed the same tendency at the injured region. The slime layer, however, did not tear off the tissue, but it seemed to produce surface-wounds in the postpharyngeal or prepharyngeal region of the body, in addition to the anterior cut. The supernumerary heads produced from the middle of the body may be due to these surface wonnds.

Summary

1) Experiments were performed in order to test the action of lithium chloride in conjunction with low temperature on head regeneration of *Planaria*.

2) Double treatment with lithium chloride and low temperature (3°C) produced injuries in several regions of the worms. These injuries did not appear when the worm was treated either with lithium chloride or with cold tap water alone.

3) Polycephalia, heteropolar heads were obtained even when the regenerating piece was long by means of treating the worm successively with lithium chloride and low temperature.

4) The reason why the polycephalia and heteropolar head occurred is discussed from the viewpoint of the physiological gradient.

Literature

Brφnsted, H. V. 1942. Arkiv. f. Zool., 34, B. No. 3, 1.
Child, C. M. 1907. Arch. Entw. Mech., 27.
——— 1911. Arch. f. Entw. Mech., 31.
——— 1928. Protoplasma, 5.
Gebhardt, H. 1926. Arch. f. Entw. Mech., 107: 684-726.
Mifune, S. 1952 Zool. Magaz., 61: 85
Okada, Yô K. and H. Sugino, 1937. Jap. J. Zool., 7: 373-439.
Takamoto, K. 1951. Zool. Magaz., 60: 18.
Tashirogi, W. 1951. Zool. Magaz., 60: 22.