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Effect of X-irradiation on the regeneration of the planarian, *Dugesia japonica*.

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It has been well established that the planarians lose their regenerative power by the x-irradiation as well as in other animals. Recently, the procedures of the local irradiation and of coupling the irradiation and the transplantation have given us important information, which become available for disclosing the mechanism of the planarian regeneration (Wolf and Dubois, 1948; Dubois, 1949; Lender, 1962; Wolf, 1962). It should be, however, in mind that the susceptibilities on the irradiation in different species of planarians and the regenerative powers which occur in the different periods from the irradiation to the dissection of the worms, are not always identical. Under the situation, the present experiments were undertaken in order to know as exactly as possible the effects of the x-irradiation on the regeneration of *Dugesia japonica* which has been scarcely used in this line of the experiment, and furnish the evidences necessary for the further studies.

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Material and Methods

The material used was sexual form of *Dugesia japonica* collected in the vicinity of Kanazawa City. The worms were starved for five to ten days before use. Throughout the experiment they were kept without giving any food at 21—23°C and reared water was daily changed.

The worms were irradiated with 0.5, 1, 2, 3, 5KR of x-rays in a petri-dish under the following conditions: 200 KVP, 20mA with 0.9mmCu+0.5mmAl filters, 1.45mmCu of half value layer, 30cm of focus-material distance and 173R/min. of dose rate. The worms were almost transversally cut off at a level of the postcephalic

region and subsequently observed on the head regeneration from the rear pieces with binocular microscope. The criterion of the regeneration was in the appearence of the eyes. The regeneration rate is represented in the following formula:

$$\text{Regeneration rate}(\%)=\frac{\text{Number of worms with regenerated eyes}}{\text{Number of experimental worms}}\ \text{x}100$$

Furthermore, there were compared with the sizes of the regeneration blastemata in the irradiated and non-irradiated worms and the numbers of the regenerated eyes in a specimen were calculated.

For the histological observation the worms were fixed in Helly's solution, and transverse or holizontal sections in 8μ thickness were prepared by the ordinary technique and by the counter staining with Delafield's haematoxylin and eosin.

Results

Experiment 1. Effects of whole body irradiation.

The worms were x-irradiated with doses of 500, 1000, 2000, 3000 and 5000R respectively and they were then transferred in fresh water.

On sixth or seventh day after the irradiation, in some of the worms the subepidermal pigment of the head was so concentrated that the head appeared in black as symptom of the necrosis. The reduction of the apical part of the head and auricles occurred in following that the eyes sank into the deep site of the body. After all the head region began to disintegrate. The disintegration partially occurred not only in the head region but also was sometimes observed in the region of the copulatory organ, in the tip of the tail and in the lateral margin of the body.

Table 1. Conditions of the worms on 12 days after irradiation with various doses.

Dose (R)	Exp. worms	Normal	Necrotic	Occurring of necrotic worms (%)
500	16	13	3	19
1000	14	3	11	79
2000	16	2	14	88
3000	12	1	11	92
5000	15	0	15	100

As is shown in Table 1, the more the doses were increased the more abundant the necrosis occurred and all worms fell in necrosis with 5000R. On 14 days, in the worms irradiated with 500R and 1000R the necrotic damage did not still proceed. Whereas, in the worms irradiated with 2000R and 3000R necrotic rate was increased in 100% respectively. All necrotic worms fell in disintegrated death in three or four weeks after the irradiation.

Experiment 2. Regeneration of the worms decapitated after irradiation with various doses.

The worms were irradiated with doses of 500, 1000, 2000, 3000 and 5000R, respectively and then cut off at a level of the postcephalic region to observe the regeneration from the rear pieces. In addition, the decapitation was undertaken at respective interval of 24 hours, 48 hours, and 14 days after the irradiation.

Table 2. Effect of dissection after irradiation

A. Decapitation at 24 hours after irradiation with various doses.

Dose(R)	Exp. worms	Non-regenerated	Regenerated	With two eyes	With one eye	Regen. rate (%)
500	48	11	37	24	13	77
1000	42	16	26	9	17	62
2000	48	28	20	2	18	42
3000	38	35	3	0	3	8
5000	43	41	2	0	2	5

B. Decapitation at 48 hours after irradiation with various doses.

500	41	25	16	9	7	39
1000	31	29	2	0	2	6
2000	42	41	1	1	0	2
3000	48	48	0	0	0	0
5000	41	41	0	0	0	0

C. Decapitation at 14 days after irradiation with various doses.

500	38	0	38	37	1	100
1000	25	11(7)	14	13	1	56
2000	27	27(19)	0	0	0	0
3000	21	21(13)	0	0	0	0
5000	26	26(19)	0	0	0	0

The numerals within ( ) indicate the number of the worms which have died without regeneration through the experiment.

(a). Regeneration of the worms decapitated at 24 hours after the irradiation. As is shown in Table 2A, in the case of 500R, the regeneration rate was 77% and there appeared in more worms with two eyes than those with one eye in the regenerates. In the cases of 1000R and 2000R, the rates indicated 62% and 42%, respectively and most of all specimens produced one eye. In the cases of 3000R and 5000R, the rates

were 8% and 5% respectively and the blastema sizes of each case were very small and one eye appeared in all of the specimens.

(b). Regeneration of the worms decapitated at 48 hours after irradiation. As is shown in Table 2B, in the case of 500R, the regeneration rate was 39% and the worms with two eyes were more or less large in number than those with one eye. In the case of 1000R, the rate was 6%, and all of the regenerated worms produced one eye. The size of the blastema was very small as described in experiment 2(a). In the cases of 3000R and 5000R, no regeneration occured entirely in all worms.

(c). Regeneration of the worms decapitated at 14 days after irradiation. As is shown in Table 2C, in the cases of 500R and 1000R the rates were 100% and 56%, respectively, and most of the regenerates produced two eyes. In 2000R and upwards, however, no regeneration occurred.

Experiment 3. Regeneration of the worms decapitated at various times before and after 5000R-irradiation.

It was revealed that in the above experiments the regeneration rate was varied on the different intervals between x-irradiation and decapitation, even if the worms were treated under the same doses. The present experiment, accordingly, commented

Table 3. Effect of dissection after and before irradiation with 5000R.

A. Decapitation at various times after 5000R-irradiation.

Periods from irradiation to decapitation (hours)	Exp. worms	Non-regenerated	Regenerated	With two eyes	With one eye	Reg. rate (%)
2	71	38	33	16	17	46
6	67	47	20	7	13	30
18	87	76	11	3	8	13
24	85	80	5	2	3	6
36	42	41	1	0	1	2
48	103	103	0	0	0	0
72	56	56	0	0	0	0

B. Irradiation with 5000R at various times after decapitation.

Periods from decapitation to irradiation (hours)	Exp. worms	Non-regenerated	Regenerated	With two eyes	With one eye	Reg. rate (%)
2	19	6	13	6	7	68
6	17	2	15	15	0	88
24	71	0	71	71	0	100
48	20	0	20	20	0	100

upon detail as to effect of the intervals. In both experimental series before and after 5000R-irradiation the cutting level was the same as in the experiment 2. The worms were observed on 9th day after decapitation. Non-irradiated worms decapitated as control at the same time regenerated two eyes on fourth day after the decapitation.

(a). Regeneration of the worms decapitated at various intervals after 5000R-irradiation. As is shown in Table 3A, in the worms decapitated at 2 hours after the irradiation the regeneration rate was 46% and the regenerates with two eyes were almost same in number as those with one eye. On 6 hours and 18 hours, the rates were 30% and 13%, respectively. On 24 hours, the rate was about 6% as same as in the experiment 2, and the regenerates with one eye were more abundant than those with two eyes. In the case of 36 hours, the rate was less than 2% and it was noteworthly that no regenerates developed the blastema, but produced one eye in the old tissue. In the cases of 48 hours and 72 hours, no regeneration occurred. These results indicated that, in according to protract the intervals between irradiation and decapitation, the regeneration rate and the size of the blastema were decreased.

(b). Regeneration of the worms decapitated at various intervals before irradiation As is shown in Table 3B, in the worms irradiated at 48 hours and 24 hours after decapitation the regeneration rates were 100%, respectively and the size and feature of regenerated parts were almost equal with those of the control. On 6 hours, the rate was 88% and all of the regenerates were with two eyes. On 2 hours, the rate was 68%, and the regeuerate with two eyes were same in number as those with one eye. These results indicated that the more the intervals between decapitation and irradiation became short the more the regeneration rate decreased, and, in addition, the abnormal regenerates with one eye tended to increase in number.

It should be noted that the regeneration rates of the worms dissected after and before the irradiation, even if the interval were equal, were different from one another. In addition, whenever the worms were normally regenerated with heavy doses, they

Table 4. Effects of redissection after irradiation

A. Irradiation with 5000R on 24 hours after decapitation, and then redissection at the portion immediately posterior to the primary cut surface.

Periods from irradiation to redissection (hours)	Exp. worms	Non-regenerated	Regenerated	With two eyes	With one eye	Reg. rate (%)
2	90	54	36	15	21	40
6	85	56	29	6	23	34
18	102	96	6	1	5	6
24	99	92	7	2	5	7
36	46	43	3	0	3	7
48	90	88	2	1	1	2
72	36	36	0	0	0	0

B. Dissection on different periods before irradiation of 5000R and then redissectiou on 48 hours after the irradiation

Periods from dissection to irradiation (hours)	Exp. worms	Non-regenerated	Regenerated	Reg. rate (%)
24	40	40	0	0
48	58	58	0	0

became necrotic and fell in death after all.

Experiment 4. Regeneration of the worms, primarily dissected before irradiation and secondarily redissected at various intervals after 5000R-irradiation.

The experiment was carried out in order to know what effects of the dissection brought about the worms which were previously irradiated to loss the regenerative power.

(a). The worms were irradiated with 5000R on 24 hours after decapitation and then at 2, 6, 18, 24, 36, 48 or 72 hours after the irradiation cut again at a level posterior to about 1-2mm from the cut surface. The administrated worms were observed on the 9th day after the dissection.

As is shown in Table 4A, in the worms dissected at 2, 6, 18, 24 and 36 hours after the irradiation respective regeneration rates were 40, 34, 6, 7 and 7% and even in any cases the regenerates with one eye were more abundant than those with two eyes. On 48 hours, the regeneration rate was 2% and no regeneration occurred except two worms of which one was with one eye and the other was with two eyes without appearance of the blastema. Accordingly, all eyes in these two worms were developed in old tissue. At 72 hours, the rate was 0%.

(b). The anterior pieces of the worms cut at the pharyngeal base had been irradiated on 24 and 48 hours after the dissection, and then they were decapitated on 48 hours after the irradiation. The cut surface anterior to the pieces thus made was made after irradiation and the cut surface posterior to the same pieces was made before irradiation, from which the small blastema had been sometimes appeared at the time of the irradiation. They were observed on 9th day after the dissection.

As is shown in Table 4B, in the worms irradiated at 24 or 48 hours after primary dissection the regeneration rate was 0% in either case. But the blastema formed at the posterior cut end and sometimes developed to form the tail and pharynx, although they were later disintegrated.

Experiment 5. Histological observation on the regeneration of the worms dissected at 48 hours after 5000R-irradiation.

The anterior and posterior pieces dissected at a level of the prepharygeal region at 47 hours after 5000R-irradiation and the non-irradiated ones as control, were provided in the experiment.

In both sorts of the non-irradiated pieces, the cut surface was closed by the epithelial cells and the intestinal wound-surface closed itself at 24 hours after the dissection. A large number of the basophilic regenerative cells were accumulated at the cut end in this time and some mitotic figures appeared in the accumulation. On three days, the posterior pieces showed the differentiation of the eyes in the new tissue and the anterior pieces developed a rudiment of the pharynx at the median position of the border between the new and old tissues.

In both sorts of the irradiated pieces, the closures of the epithelial and intestinal wound surfaces were performed at 24 hours after the dissection as well as in the cases of thenon-irradiated pieces. While, even at any time after dissection, the accumulation of the basophilic regenerative cells and mitotic figures were not clearly recognizable in both sorts of the pieces, and eye and pharynx were not definitely developed.

Discussion

Some authors reported the relationship between the doses of x-irradiation and the time from x-ray treatment to the dissection of the planarians, necessary for the complete inhibition of the regeneration; *i. e.*, with *Dugesia lugubris*, 5000-8000R, 24-26 hours (Dubois, 1949): with *Bdellocephala brunnea*, 8000R, 3 hours (Teshirogi, 1963): with *Planaria subtentaculata*, 8000-10250R, 48 hours (Chandebois, 1964). The present experiment showed with *Dugesia japonica* that the regeneration of the worms was completely inhibited by dissection on 48 hours after the irradiation with 3000-5000R of x-rays. The result different from the above authors' will cause differences of the planarian species and conditions of the irradiation. The dissected worms tended to recover the regenerative power with the doses of 500 and 1000R. Similar findings have been shown in other species (Strandskov, 1937; Dubois, 1949). The regeneration of the worms irradiated with 3000-5000R, if they were cut in shorter times than 48 hours after the irradiation, could not be compltely inhibited. The phenomenon thus regenerated will not be described in the recovery of the regenerative power but rather that the regenerative power inflicted with heavy stimulus of x-rays exerted their ability during the latent time till the irradiation effect emanated, because the worms thus hardly regenerated fell after all in necrotic death as well as non-regenerated worms. From the present experiment the latent time will be at least maintained during 24-48 hours. It has been assumed that regenerative cells were of higher susceptibility and inflicted selectively specific damages with x-irradiation (Curtis and Hickman, 1926; Curtis, 1928; Dubois, 1949; Lender and Gabriel, 1961; Lender, 1962; Wolff, 1962), and Chandebois (1964, 1965) has also a similar thought as to Type-1 cells.

Another consideration, however, against the conception of the regenerative cell or neoblast as stated by the above authors will be offered in x-irradiation experiment. Cells, instead of the neoblasts, which are derived from the original tissue cells involv-

ed with the dissection effect participate in the blastema formation, as suggested by Kido (1967). The x-irradiation will affect unknown substance(s) related to convert the original tissue cells into regenerative cells. The regenerative substance(s) is disturbed with high doses of the x-irradiation, but displays its activity during the latent time. Either opinion is different from one another as to origin of the regenerative cells but the phenomenon given rise in the effect of x-irradiation is identical.

While, the regeneration rate was higher in the case of the worms dissected at 2 hours before the x-irradiation than that of the worms dissected at the corresponded time after the x-irradiation (Table 3). The reason will be thought in two, *i.e.*, 1) the neoblasts have been in some degrees in differentiated aspect during 2 hours before the x-irradiation. It is, however, wonder whether the neoblasts would be so fast differentiated that they were aboidable from the heavy stimulation of the x-irradiation, though Sengel (1960) stated that planarian blastema was differentiated in the most early stage of the regeneration, 2) the blastema formation does not firstly depend upon the neoblasts but upon the original tissue-cells converted to the regenerative cells. Accordingly, the regenerative substance(s) prepared in the original tissue-cells during 2 hours before the x-irradiation will precede further going with only their own restricted agency.

From the present experiment there are no evidence to verify either the former or the latter.

Summary

1. When whole body of *Dugesia japonica* was irradiated with 5000R of x-ray, all worms administrated fell in necrosis on 12 days after the irradiation.
2. Regeneration of the worms dissected on 48 hours after the irradiation of 5000R were completely inhibited, and the more the intervals between the irradiation and the dissection became short the more the regeneration rates increased.
3. When irradiated with 5000R at intervals of 24 hours or upwards after the dissection of the worms occurred in the regeneration with 100%, but the regenerates fell in death after all. At the less intervals than 24 hours the regeneration rates were gradually decreased.
4. Redissection of the dissected worms irradiated with 5000R gave no significant result for the regeneration.
5. Histological observation of the worms dissected at 48 hours after 5000R-irradiation was performed. The wound surfaces of the epithelium and intestinal tissue were closed with their own tissues as well as in non-irradiated worms dissected. At any time after the dissection, accumulation of the basophilic regenerative cells and mitotic figures were not distinctly recognizable.

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