Effect of Transplanted Pieces from Non-X-irradiated Worms on Irradiated Ones in *Dugesia japonica*

Hisao Sugino, Yoshinobu Okuno and Jun Yoshinobu

(Department of Biology)

(Received Sept. 7, 1970)

**Introduction**

Fresh water planarians lose their ability to regenerate when they are irradiated with X-ray. However, when a non-irradiated piece is grafted on an irradiated worm, regeneration ability is restored. Two conflicting hypotheses have been proposed concerning this phenomenon; Dubois (1949) and Teshirogi (1963, 1964) thought that neoblasts migrated from the non-irradiated region to the wound surface to form the regenerating bud, whereas Kishida and others (1966) thought that cells of the irradiated region acquired regenerating ability by a factor released from the healthy tissue. No direct evidence is available for migration of neoblasts through irradiated region to the wound surface. Betchaku (1968) suggested that neoblasts gathered passively to the wound surface by constriction of wound and by the movement of gastrodermal cells. We also observed (unpublished) in an *in vitro* culture that neoblasts do not locomote as a whole, although their processes dilated and contracted slowly. On the other hand, for the second hypothesis it is necessary to assume a new mechanism induced by the grafting of healthy tissue that reactivates the irradiated region. In order to elucidate this problem the authors conducted the following experiments.

**Material and Methods**

The freshwater planarian, *Dugesia japonica* Ichikawa et Kawakatsu was collected in the following localities.

1) Osaka Prefecture (Ushitaki) in May, 1966.
2) Nagano Prefecture (Otaki-mura) in April and September, 1966. The worms were kept in the laboratory in glass vats filled with filtered tap water (pH 7.0~7.2), at temperatures between 18°C and 25°C in the dark. They were fed with chicken liver once a week, and the water in the vats was changed three times a week.

For experiments healthy worms of uniform size that have not undergone fission recently were selected. They were starved for at least one week before use. X-ray was irradiated on the whole worm at doses from 1,500r to 5,000r. The condition for irradiation of 3,000r was 20kV, 20mA with filters of 1.0 mm Cu and 0.5mm Al, and the distance between the X-ray source and the worm was 30cm. Irradiation was continued for 20 minutes at a dose rate of 150r per minute. The worms were kept in a Petri dish, 8.7cm in diameter, containing 20cc water, fifty worms at a time.

The worms were operated, after they have been anesthetized with 0.3% chloretone or ice, with ophthalmic scalpel under binocular microscope upon wet filter paper. The anesthetization with ice gave better results.

The worms were fixed for examination by the Bouin's solution or Nozawa's solution, and dehydrated with ethanol. They were cut in section of 7μ thick in paraffin, and stained with Giemsa's solution.

Experiments and Results

1. Regeneration after X-ray irradiation

The worms from the two localities both produced regenerating bud one or two days after decapitation, and regenerated eyes in 3 to 4 days, restoring normal shape in 6 to 7 days.

When decapitation was performed 3 days after X-ray irradiation at doses of 3,000, 4,000 and 5,000r, no worm ever produced the regenerating bud, necrosis developed gradually along the antero-posterior axis, and the worms died within 20 days in the case of the worms from Otaki, or within 30 days in the case of the worms from Ushitaki.

One worm from Otaki, that was decapitated 3 days after irradiation of 1,500r, formed a regenerating bud 2 days after the operation, but this regenerating bud was decomposed within 3 days.

Histological examination of tissues after 5,000r irradiation was carried out. The worms were fixed every 3 days after irradiation.

In non-irradiated controls, the neoblasts were larger than other cells, in
the shape of a spindle, a pear or a ball. When stained with the Giemsa’s solution, their nuclei stained far fainter than the cytoplasm (Fig. 1).

After X-ray irradiation, neoblasts decayed gradually (Fig. 2, 3, 4). However, many morphologically almost normal neoblasts were observed near the nerve cords, brain and pharynx, although large ones were rare (Fig. 5). Moreover some neoblasts were still observed until just before the death of the worm (Fig. 4, 6, 7).

Cells other than neoblasts also decayed significantly, and their number decreased. The number of rhabdites also decreased.

2. Extirpation of dorsal epithelium of irradiated worm

Dorsal epithelium in the prepharyngeal region after irradiation at 5,000r was removed. Irradiation at this dose caused delay in the healing of the wound surface, and even when healing did take place, it was merely a joining of old epithelium, little or no regeneration of epithelium being observed. Most of the worms gradually decayed from the wound surface. Epithelium of the joined region often disintegrated by the action of Bouin’s solution used for fixation, suggesting that the epithelium of this region was thin and weak.

Already thirty minutes after removal of epithelium a few neoblasts were seen to have migrated to the wound surface (Fig. 8). Also some neoblasts were observed to have aligned at the wound surface, and some specimens showed figures that as if new epithelium were beginning to be formed (Fig. 9). Five hours after transversal section the old epithelium was extending to the wound surface. However, completed new epithelium could not be observed when observation was continued to later times (Fig. 10).

3. Transplantation of non-irradiated tissue to irradiated worm

A square hole 1 mm wide by 1 mm long was cut in the prepharyngeal region of a worm irradiated with X-ray at a dose between 1,500r and 4,000r, and a small piece taken from corresponding region of a non-irradiated worm was grafted into this hole. After joining of the small piece to the host has occurred, the worm was cut transversally at a position 1 mm anterior to the graft (Fig. 11).

Of 16 successful cases, 6 formed a regenerating bud after decapitation. The regenerating bud was formed between the 4th and the 6th day after decapitation, which is from 2 to 4 days later than in normal regeneration. Moreover, the regenerating bud did not extend to the whole cut surface, but was limited to a width corresponding to that of the graft until comparatively later times, when the regenerated part covered the whole cut surface (Fig. 12a, b). At any rate it took considerably longer for the regenerating
bud to become a complete head, than in the case of normal regeneration.

Those cases that did not form a regeneration bud after decapitation died within 40 to 50 days. Some of the cases that formed regenerating bud showed decay of the irradiated host, and some even died. These were cases where the graft was too small compared with the irradiated host.

Histological observation on a specimen that regenerated a head on the 14th day after decapitation revealed numerous neoblasts in the grafted piece, as well as in the regenerating bud that seem to have multiplied. The graft and the regenerating bud were separated by irradiated host tissue, which also contained neoblasts similar to those in the graft, and was indistinguishable from the graft. However, irradiated host tissue posterior to the graft showed clear difference from the graft (Fig. 13).

4. Union of irradiated and non-irradiated worms

Pharyngeal region of an irradiated (3,000, 4,000r) worm and of a non-irradiated worm was removed respectively and the postpharyngeal region of one was joined to the prepharyngeal region of the other, as shown in Fig. 14. After joining completed the composite worms were cut at the level shown by the broken line in Fig. 14C and D.

a) Union of irradiated prepharyngeal region and non-irradiated post pharyngeal region

Seven successful cases were obtained when the prepharyngeal region of an irradiated worm and a postpharyngeal region were joined, and the composite worm was cut in the former (Fig. 14C).

The time required for the formation of the regeneration bud after cutting was 4 ~ 5 days at the fastest, and 10 ~ 15 days at the slowest. And it took 20 ~ 30 days for the formation of a complete head. Some regenerating bud showed abnormal morphology, but they also became normal in 20 ~ 30 days. The irradiated part gradually diminished and finally vanished (Fig. 15a. b).

Histological observation was performed at the time when the irradiated part had not been absorbed completely by the healthy tissue, i.e. 20 ~ 24th day after cutting. The regeneration bud contained numerous, comparatively small neoblasts, which must have arisen by recent fission. The irradiated tissue also contained similar neoblasts and was histologically indistinguishable from the regeneration bud (Fig. 16). Non-irradiated tissue contained large, normal neoblasts. This was, therefore, clearly distinguishable from the irradiated and regenerated tissue (Fig. 17). Some figures were formed that suggested the intrusion of the non-irradiated tissue into the irradiated region.
b) Union of non-irradiated prepharyngeal region and irradiated postpharyngeal region

Eight successful cases were obtained when the prepharyngeal region of a normal worm and the postpharyngeal region of an irradiated worm were joined (Fig. 14D).

New tissue appeared between the two regions, that is, from the posterior end of the non-irradiated component. This is thought to be due to the presence of an intact brain and the absence of the pharynx.

Regeneration of tail was considerably delayed; as irradiated tissue was replaced by healthy tissue, regeneration bud was formed. This replacement proceeded to completion within 30-40 days (Fig. 18).

c) Longitudinal union of irradiated piece and non-irradiated piece

In this experiment irradiated and normal worms were sectioned longitudinally along the median line, joined, and were decapitated after healing was complete (Fig. 19).

Four successful cases were obtained, but in one of them the joining was incomplete. Formation of regeneration bud after decapitation took place on the second day, which is not slower than with normal worms. Regeneration bud was formed not only from the non-irradiated side, but also from the irradiated side (Fig. 20a). Complete head was formed within 14 days. Irradiated tissue diminished gradually (Fig. 20b), and was finally replaced by healthy tissue.

Histological examination showed that the irradiated region contained fewer cells, rhabdites and neoblasts than normal region (Fig. 21).

Considerations and Conclusions

In Dugesia japonica Ichikawa et Kawakatsu regeneration is completely inhibited by X-ray irradiation above 3,000r. However, when a piece of non-irradiated worm is grafted to an irradiated host, capacity for regeneration is restored to the irradiated host. Some authors (Dubois, Teshrogi) consider that regeneration bud is formed by neoblasts from the healthy graft that has migrated to the wound through the irradiated region. Other authors (Kishida and others) opposed to this view.

In our experiments, the number of neoblasts of irradiated worms decreased very much, and those that were observed showed significant decay (Fig. 4,7). But even just before death neoblasts had not disappeared completely, although
large intact neoblasts of the normal shape were rare (Fig. 6, 7).

When the dorsal epithelium of the prepharyngeal region was removed from an irradiated worm, neoblasts aggregated at the wound surface and were arranged in the form of an epithelium 30 minutes after cutting (Fig. 9). However, later developments into regeneration of new epithelium did not take place (Fig. 10). These observations may be interpreted to be due to the damage inflicted upon the neoblasts by irradiation. When a small piece was grafted from a healthy worm to an irradiated host (Fig. 11), the regeneration bud formed in the irradiated part did not extend over the entire wound, but was limited until quite later times to a width corresponding to that of the graft (Fig. 12a). This was as if the regeneration bud was formed by the neoblasts that migrated from the healthy graft. So far as these results are concerned, the view of Dubois and others seems to have been confirmed.

Later the irradiated tissue was absorbed by healthy tissue. Histological examination revealed numerous neoblasts not only in the graft but also in the regeneration bud, and the two appeared quite similar (Fig. 13). Also the region between the two was indistinguishable from them.

At this stage of regeneration (17 days after cutting), the irradiated region seems to have been completely replaced by healthy tissue, and the regeneration bud completed.

In the experiment of joining of irradiated prepharyngeal region with non-irradiated postpharyngeal region (Fig. 14), or vice versa, regeneration bud was formed beyond the irradiated part (Fig. 15a). In such cases the time required for its formation was 4 ~ 5 days at the fastest and 10 ~ 15 days at the slowest.

The irradiated tissue and the regeneration bud formed anterior to it both contained comparatively small neoblasts that seem to have undergone fission recently. Thus they were histologically quite indistinguishable (Fig. 16). However, the posterior non-irradiated region was clearly distinguishable from either (Fig. 17).

If neoblasts from non-irradiated tissue migrate by ameboid movement through the irradiated tissue to the cut surface to form the regeneration bud, as proposed by Dubois, then the neoblasts in both regions ought to appear much more alike, contrary to our observations. We propose that neoblasts do not migrate directly by ameboid movement, but reach the cut surface after repeated fission and multiplication. Such replicated cells should be called "differentiating cells" (Morita, 1967) rather than neoblasts.

When parts of irradiated and non-irradiated worms were joined longitudinally
Effect of Transplanted Pieces from Non-X-irradiated

side by side (Fig. 19), regeneration bud was formed on the entire cut surface, not only from the non-irradiated part but also from the irradiated region (Fig. 20a) and very swiftly after decapitation. In this case the neoblasts of the irradiated region may have recovered from the irradiation damage by the influence of the adjacent healthy tissue, as maintained by Kishida and others. However, in this experiment the healthy region was larger than in other experiments, taking a direct part in regeneration, and the healing surface was large along the median line. These factors might have affected the results of this experiment.

The fact that irradiated region is gradually replaced by healthy tissue (Fig. 20b, 21) seems to contradict the view that neoblasts of irradiated region recovered under the influence of healthy region. Therefore we propose that when an irradiated worm and a non-irradiated worm are grafted together, the tissue of the healthy worm proliferates and invades the other worm on the expense of the material derived from the damaged worm, which had lost the capacity for growth and multiplication. The epidermis of the irradiated worm, however, do not change its appearance rapidly, but are gradually replaced by the epidermis from the healthy worm in spite of a rapid replacement of the internal tissue. This would result in an outward appearance that looks as if only neoblasts from healthy tissue migrated through the tissue of the irradiated worm to form a blastema at the cut surface. If such a worm had been kept alive for a long time, its whole body would have been replaced by tissue derived from the healthy worm, as in the case of union of a prepharyngeal piece of Kyūshū strain and a postpharyngeal piece of Kiso strain (Sugino,1969). The cutting of the irradiated region only accelerated such a change by stimulating regeneration.

**Summary**

1. Upon X-ray irradiation at a dose over 3,000r regeneration of tissue of freshwater planarian, *Dugesia japonica* Ichikawa et Kawakatsu was completely inhibited, and the worm decayed resulting in death. But even just before death some neoblasts remained.

2. When the anterior part of an irradiated worm was joined to the posterior part of a non-irradiated worm, a cut was made in the anterior part, a head regenerated at the anterior cut surface to form a normal worm. Neoblasts from the non-irradiated component multiplied by division and invaded the
irradiated component, replacing the irradiated tissue.

3. When an irradiated worm and a non-irradiated worm were sectioned longitudinally along the median line, and corresponding parts were exchanged, and subsequently the head removed, healthy tissue invaded the irradiated component, resulting in a normal worm.

4. The irradiated region between the regeneration bud and non-irradiated region contains neoblasts and "differentiating cells" derived from them, just like the regeneration bud. From this and other data we propose that neoblasts do not migrate directly by ameboid movement, but reach the cut surface after repeated fission and multiplication.

5. The possibility that irradiated neoblasts recover under the influence of healthy tissue is excluded.

Acknowledgement The authors wish to express sincere thanks to Dr. Atsuhiko Takeda, Radiation Center of Osaka Prefecture, and Dr. Iwashiro Oki, Osaka Prefetural Institute of Public Health, for their kind guidance on the techniques of X-ray irradiation.

References


Effect of Transplanted Pieces from Non-X-irradiated

Explanation of figures

Fig. 1. Neoblasts in normal worm.

Fig. 2. Neoblasts 3 days after 5,000r irradiation (Worm from Ushitaki). Decay of cytoplasm is beginning.

Fig. 3. Neoblasts 9 days after 5,000r irradiation (Worm from Ōtaki). Decay is beginning, and stain not clear.

Fig. 4. Neoblasts 14 days after 5,000r irradiation (Worm from Ōtaki). Cytoplasm has decayed completely.

Fig. 5. Neoblasts around nerve, 9 days after 5,000r irradiation (Worm from Ushitaki), n, nerve.

Fig. 6. 14 days after 5,000r irradiation just before death (Worm from Ōtaki). Neoblasts are not normal but have not decomposed.

Fig. 7. 23 days after 5,000r irradiation, just before death (Worm from Ushitaki). Neoblasts are beginning to decompose.

Fig. 8. Dorsal epidermis was removed within 24 hours after 5,000r irradiation. The specimen was fixed 30 minutes after the operation (Worm from Ushitaki). Neoblasts have aggregated at the wound surface and are beginning to be lined up.
Effect of Transplanted Pieces from Non-X-irradiated

Fig. 9. Same as in Fig. 8. Neoblasts are lined up at the wound surface and new epidermis is beginning to be formed.

Fig. 10. Dorsal epidermis was removed 7 days after 5,000 r irradiation. Fixed 2 days after operation. A thin epidermis–like layer seems to have extended from the old epidermis. The wound has almost healed.

Fig. 11. A small piece from the healthy worm was grafted on the irradiated worm. A, healthy worm, B, irradiated worm. ph, pharynx. Broken line shows the position of cutting.

Fig. 12. A small piece from normal worm (from Ushitaki) was grafted on worm (from Ōtaki) irradiated with 4,000 r X–ray. The regenerating bud has a width corresponding to that of the graft, as seen in Fig. 12a. In Fig. 12b tissue of irradiated region is beginning to be decomposed.

a. 16 days after decapitation.

b. Same worm, 29 days after decapitation.

Fig. 13. A small piece from normal worm was grafted on host irradiated with 1,500 r X–ray. Fixed 17 days after decapitation. Note the difference in the tissues of the graft and the irradiated host. ih, X-irradiated host, g, grafted piece, b, regenerating bud.

Fig. 14. The anterior and posterior parts of irradiated and normal worms were exchanged. The streaked region was derived from irradiated worm. Broken lines show the position of cutting.

Fig. 15. Prepharyngeal region of irradiated worm and postpharyngeal region of normal worm were joined.

a. 27 days after decapitation. Eyes have regenerated and irradiated tissue is being absorbed.

b. 64 days after decapitation. Irradiated tissue has been completely absorbed.
Fig. 16. Prepharyngeal part of 4,000r irradiated worm (Ushitaki) and postpharyngeal part of normal worm (Otaki) were joined, 24 days after joining. Irradiated region and regenerating bud are similar in appearance. i, irradiated region, b, regenerating bud, e, eye.

Fig. 17. Similar to Fig. 16, but here the difference between irradiated and healthy region is clear. h, healthy host tissue, i, X-irradiated region.

Fig. 18. Prepharyngeal part of normal worm (from Ushitaki) and postpharyngeal part of 4,000r irradiated worm (from Otaki) were joined.
   a, 8 days after removal of tail. New tissue has formed between the two regions.
   b, 23 days after removal of tail. Irradiated tissue is being absorbed, and tail is regenerating.

Fig. 19. Normal and irradiated worms were cut longitudinally along the median line and reunited with parts exchanged. Streaked regions are derived irradiated worm. Broken lines show position of cutting.

Fig. 20. Parts of 4,000r irradiated and normal worms were joined side by side. Dark region was irradiated.
   a, 3 days after decapitation. Regenerating bud has formed from the anterior end.
   b, 15 days after irradiation. Normal head has formed and irradiated tissue is gradually diminishing.

Fig. 21. Parts of irradiated worm (from Ushitaki) and normal worm (from Otaki) were joined side by side. 24 days after joining. The right thin part was derived from irradiated worm, its epidermis was thinner, and it contained fewer normal cells.