EFFECTS OF X-IRRADIATION UPON RAINBOW TROUT (*SALMO IRIDEUS*)

I. Influence on the feeding activity in rainbow trout fry*

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As an important symptom of X- and γ-irradiation injury, reports have been published on disturbance of appetite, food intake and water balance in the laboratory mammalian animals such as rat, mouse, rabbit, dog and monkey[1)-15)]. In most of these studies, loss of body weight has been found to be closely associated with the reduction of food intake. Although precise studies in this field have been made using laboratory animals, no information is available on the effects of X-irradiation on fish feeding. In this paper the authors have dealt with changes in the feeding activity of the rainbow trout fry after X-irradiation. Information was obtained by using a revised type of the automatic feeding recorder which has been previously reported by the authors[16)17)]. The present experiment was not only designed for the study of fish feeding, but also for the studies of the effects of X-irradiation on the various organs of the fishes. This paper is the first of series in which it is proposed to present those studies.

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MATERIALS AND METHODS

A preliminary experiment was performed in October, 1955, though it was not designed for the study of fish feeding. Rainbow trout fry measuring 37–72 mm in total length were used. The radiation factors were as follows: 180 KVP, 3 ma; 0.1 mm Cu filter; target distance 40 cm, including 2.5 cm of the depth of water in a glass dish of 22 cm diameter in which the fishes were contained to be irradiated; diameter of the field size 25 cm; dose rate measured in air 33 r/min. During the periods in which all the irradiation procedure ends, the water temperature was maintained, by addition of ice fragments, at about 12°C which is identical with that of the well-water in the rearing pond. The fishes were divided into five groups, each consisting of 30 – 44 individuals: (1) single exposure, (a) 300 r, (b) 600 r, (c) 1200 r; (2) three times repeated exposures of 200 r with the interval time of a week; and (3) the control non-irradiated. After irradiation these fishes were reared over a period of 115 days in a tank which was equally partitioned into five compartments by fine brass wire screens. The size of each compartment of the tank was 185 cm long, 32 cm broad and 45 cm

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deep. The depth of the water was maintained at about 35 cm, a sufficient volume of well-water flowing into the tank constantly.

As indicated in Table 1, the rainbow trout fry is resistant enough even to the radiation dosage as high as 1200 r. However, even in the 300 r group some distinct effects were found in the reproductive organs, kidney tubules, intestinal epithelium and blood cell constituents[18].

Table 1. Survival of rainbow trout fry after X-irradiation; experimental period: 115 days

Dose r	No. of fish irradiated	Time of death in days (No. of fish died on that day)
300	40	11(1), 14(1), 44(1), 53(2), 59(1), 67(3), 76(1), 101(1)
600	40	11(1), 33(1), 35(1), 59(1), 92(1), 93(1)
1200	44	1(1), 10(2), 17(1), 26(1), 32(1), 35(1), 38(1), 39(1)
200 × 3	30	6(2), 12(1), 28(1), 54(1), 62(1)
Control	40	11(1), 14(1), 15(1), 45(1), 72(1)

In view of the results of the preliminary experiment, the exposure of rainbow trout fry to low dosage of X-ray was tried again on August 10, 1956, to ascertain its effects on the organs above noted, together with changes of the feeding activity. The fishes used were 2550 in number, which had been artificially fertilized and reared for about three months in a small tank of well-water set in a greenhouse of the Faculty of Fisheries. The average values of the body length and body weight of 12 fish sampled at random at the time of irradiation were 42–57 mm and 1.3–3.5 g. All of the fish were divided into three groups, two for exposure to 100 r and 500 r, and the third the non-irradiated control. The radiation factors were almost the same as those of the preliminary experiment, except that throughout the periods of X-irradiation procedure the water containing the fish was gently bubbled by supplying oxygen. After irradiation the three groups of fish were transferred for rearing into an out-door concrete pond which was partitioned into three compartments by fine brass wire screens. Out of the three groups of the irradiated and control fishes, 30 each individuals of uniform body weight were carefully selected and transferred into the equally partitioned respective compartments of a tank set in a greenhouse which was designed for the feeding experiment. The size of each compartment was 185 cm long, 53 cm broad and 45 cm deep. As in the preliminary experiment well-water was flowed into the tank constantly, to maintain the depth at about 35 cm. Feeding records were taken continuously more than 100 days after irradiation, though all the data are not presented in this paper. To maintain the water quality in good conditions throughout the periods of X-irradiation procedure, the fishes were fasted for about 24 hours before irradiation. After irradiated, the fishes to be used in the feeding experiment were transferred into their respective compartments of the tank and further fasted for about 20 hours there. So, these fishes were first supplied bait after about 44 hours, and then, the feeding records were taken.

The principle of construction of the feeding recorder is identical with that of the type previously reported[16,17]. That is, the bait is suspended in a tank of water to make the fish peck it. By the pecking of the fish the bait box vibrates, and so the slender aluminum bar which is set vertically to the box swings like a pendulum and touches the horizontally placed aluminum bar; then the electric current is closed, and thus, a feeding mark is recorded on the kymograph by a magnetized lever. The parts of contact in both slender aluminum bars are plated especially with silver. The bait was prepared mincing the following materials: boiled intestine of domestic animals, boiled potato, fresh beef liver, dried crustacean larvae and rice-bran. The bait was changed twice a day for fresh material at about 9 a. m. and 7 p. m. which correspond to the times when the active feeding of the fishes terminates temporarily considering from their diurnal rhythm. Further, it was previously ascertained that disturbance of the feeding by the exchange of the bait is slight as reported in the experiment with the goldfish[17].

RESULTS

General observations

Besides the experiments for the sake of the feeding record, some observations were made on the behaviour of both irradiated and control fishes reared in the out-door pond. The most noticeable behaviour of both groups 100 r and 500 r treated, was that they appeared to be in an inactive state during a period of more than one week following irradiation. The pond is partitioned longitudinally into the three compartments, each size being of 445 cm long, 110 cm broad and 100 cm deep. The depth of the water was maintained at about 75 cm, well-water flowing into the respective compartments separately. Near the outflow-side of the pond, a transverse partition of fine brass wire screen is set to be common to the three longitudinal compartments. All the irradiated fishes of each group were found to be crowded in a mass so as to cause their bodies to adhere to the corners of the transverse partition, while the control fishes were observed to swim about vigorously except the first day on which they were transferred into this pond. It was often observed that some irradiated fishes swam out towards the center of the compartment against the current, but they returned immediately to the original situations as if frightened at some phenomena occurring in the upper stream of the pond. Possibly this unusual behaviour might be in part due to depression of their swimming activity following irradiation. If this is true, and further, if any materials contributing to the buoyancy of their bodies were absent, the irradiated fishes might be drifted away by current.

Depression and recovery of the feeding activity

To learn the daily standard deviation of the feeding frequency within the groups, a test was made on the three groups of control fishes for ten consecutive days in the feeding experiment tank and also in the other small tank. From these experiments, it was found that coefficient of variance in the daily feeding frequency is of the order of 12 - 26 % if the individual fishes are selected to be of uniform body weight.

The feeding frequencies of the irradiated and control groups are given in Table 2. To determine the approximate day number following irradiation on which its influence is statistically significant, comparison of the daily difference in the feeding frequency was made between the control and each irradiated group for each three adjacent days in

Table 2. Daily feeding frequency post-irradiation

Days Post-irradiation	Feeding frequency Control	100 r	500 r
1	113+	74+	233+
2	87	69	73
3	332	173	39
4	211	74 *	41 *
5	299+	72+ *	37+ *
6	369	101 **	33 *
7	233	34	16 *
8	212	168	14 **
9	217+	133+	27+ *
10	190	57 *	93 *
11	131	48 *	20
12	232+	117+	34+ *
13	193	175	29
14	166	110	93 *
15	214	43	64 *
16	298+	145+	177+ **
17	326	278	210
18	381	441	120
19	182+	276+	40+ *
20	317	236	132 *
21	420	248	146
22	369	284	329
23	264	243	431
24	311	236	200
25	359+	422+	187+

The mark + means that the recording was interrupted in part in the course of the experiment on that day.
*, ** see the explanation in text.

a series, i. e., *days 1-2-3, 2-3-4, 3-4-5*, etc. In this table, an asterisk indicating statistical significance was put on each middle day of the three adjacent ones tested; for instance, if the test on the feeding frequency of *days 3-4-5* showed to be significant more than 95% of probability, an asterisk was put on *day 4*. As the test was made by such a method, for instance, the feeding frequency on *day 1* of the 500 r group is not shown to be significant though it might be highly significant actually. The percent changes of the feeding frequencies in the irradiated groups from the control are illustrated in Fig. 1. The general pattern of the irradiation effects on the feeding activity will be easily seen from this figure.

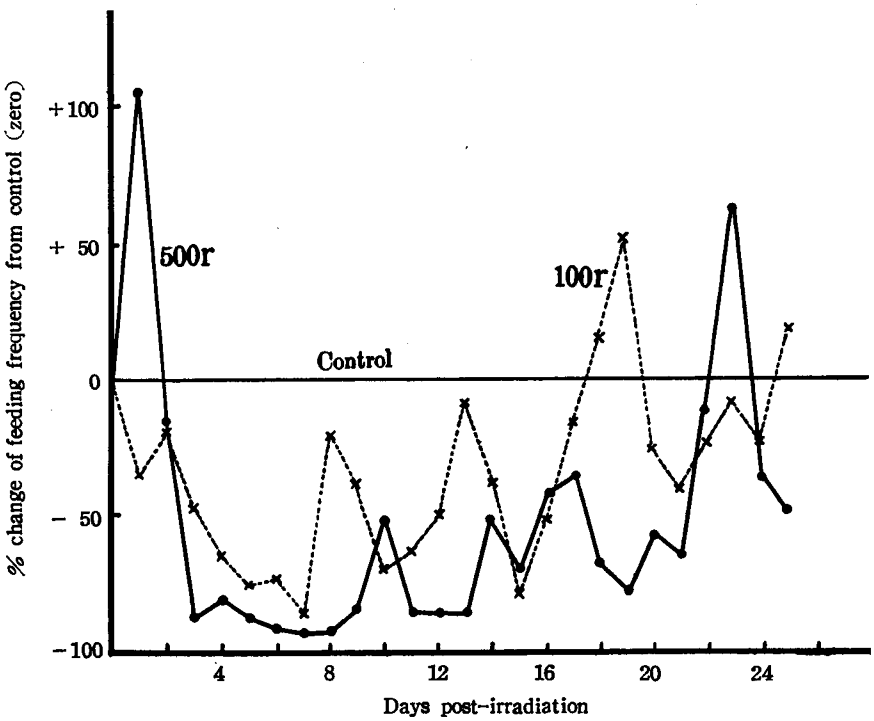

Fig. 1. Percent change of the feeding frequency in the irradiated fishes from the control (zero); 500 r •——• and 100 r ×……×

The feeding records of the 500 r group were begun 18 hours after irradiation. Consequently, although the changes in the period immediately after irradiation are not available here, it is presumable from the records of Fig. 2 that the relatively marked increase of the feeding in this group began to occur in the beginning of the afternoon of *day 1*, namely within 24 hours after irradiation. It is noteworthy that such a supracontrol feeding was recorded on *day 1* in the 500 r group. A severe depression of feeding was found first to occur on *day 3*, showing −88% from the control. Thereafter, the depression continued until *day 9*, with the range of −81～−93% from the control.

First recovery occurred on *day 10* with -51% from the control. The second depression continued during the period of *days 11-13* with a magnitude of about -85%

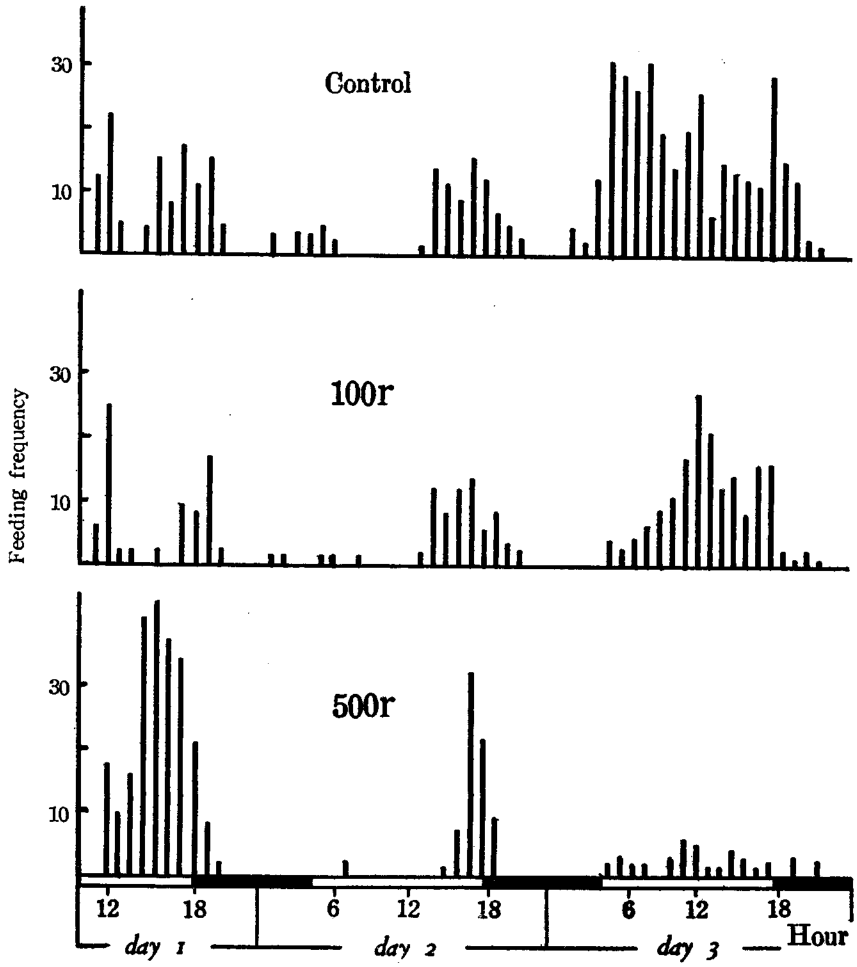

Fig. 2. Diurnal rhythms of the feeding activity in the three groups: control, 100 r and 500 r, in the period of *days 1 - 3*

from the control. The second recovery was found to occur on *days 14-17*. This recovery may be said to be relatively marked as is also seen in Table 2. After the slight depressions following this second recovery, a very distinct recovery was recorded on *days 22* and *23*. The feeding frequency of the latter day was far beyond the control level. On the basis of these observation it can be said that the feeding activity of the 500 r group almost returns to the normal level by about 3 weeks after irradiation, though the recovery is not yet complete.

In the fishes of the 100 r group, the inhibition of the feeding activity was far slighter in comparison with that of the 500 r group. However, the irradiation effects were clearly found as is shown in Fig. 1 and Table 2. That is, the feeding frequency declined with a slow declivity until *day 7*. The first but very distinct recovery occurred on *day 8* with the feeding 79% of the control. Although the second depression continued during the period of about *days 10* and *11*, the feeding activity almost returned to the control level on *day 13*.

Diurnal rhythm of the feeding activity

Correlation between the diurnal rhythm of the feeding activity in the rainbow trout fry and the environmental factors will be studied in future. Here, only the effect of X-irradiation on the rhythm of the said activity is analyzed. To show the general appearance of the effect, the results of *days 3–22* were illustrated in Fig. 3, A – D, dividing these days into the four periods, *days 3–7*, *8–12*, *13–17* and *18–22*. In these figures, the diurnal rhythm of the feeding activity in each group is expressed as the hourly average in each period of the days above cited. From these figures, the recovery process in the diurnal rhythm of the said activity will be clearly seen, together with the fact that following irradiation the rhythm is not so severely distorted as the activity itself is highly affected. Such a relationship will be judged also from the rhythms illustrated in Fig. 2. It is clearly seen from Fig. 3 that the feeding activity in the control group shows a distinct diurnal rhythm. Especially, the rhythms shown in Fig. 3, B and C may be said to be typical. Namely, the two peaks of the feeding activity are found daily, one at daylight and the other before sunset. Of these two peaks, the latter one occurs invariably, usually with a higher activity than the former. Generally saying, the feeding begins actively towards daylight, then decreases gradually until about 9–11 a. m., thereafter increases at the beginning of the afternoon, and reaches the peak in the time before sunset. The said activity in the night-time is usually very feeble, at least, during the present experimental period. As is clear in Fig. 3, the diurnal rhythms of the irradiated groups are found in general to be closely parallel to that of the control. The rhythm of the 500 r is shown to be indistinct in the first period of *days 3–7*. However, it will be seen from Fig. 2 that the relatively active feedings occur in the peak time of evening although the feeding activity itself is severely depressed. In the second porid of *days 8-12*, the peak in the time of evening becomes distinct even in the 500 r group. The diurnal rhythm in the 100 r group appears to be almost identical to that of the control, though the feeding frequency is not yet so. No difference of the diurnal rhythm of the feeding activity was found between the two groups of 100 r and 500 r in the third period of *days 13–17*. Consequently, so far as only the diurnal rhythm is concerned, it may be said that 500 r group almost returned to normal in this third period. In the last period of *days 19–22*, the rhythms of all the three groups are almost the same, though the feeding frequency in the 500 r group is apparently still depressed slightly.

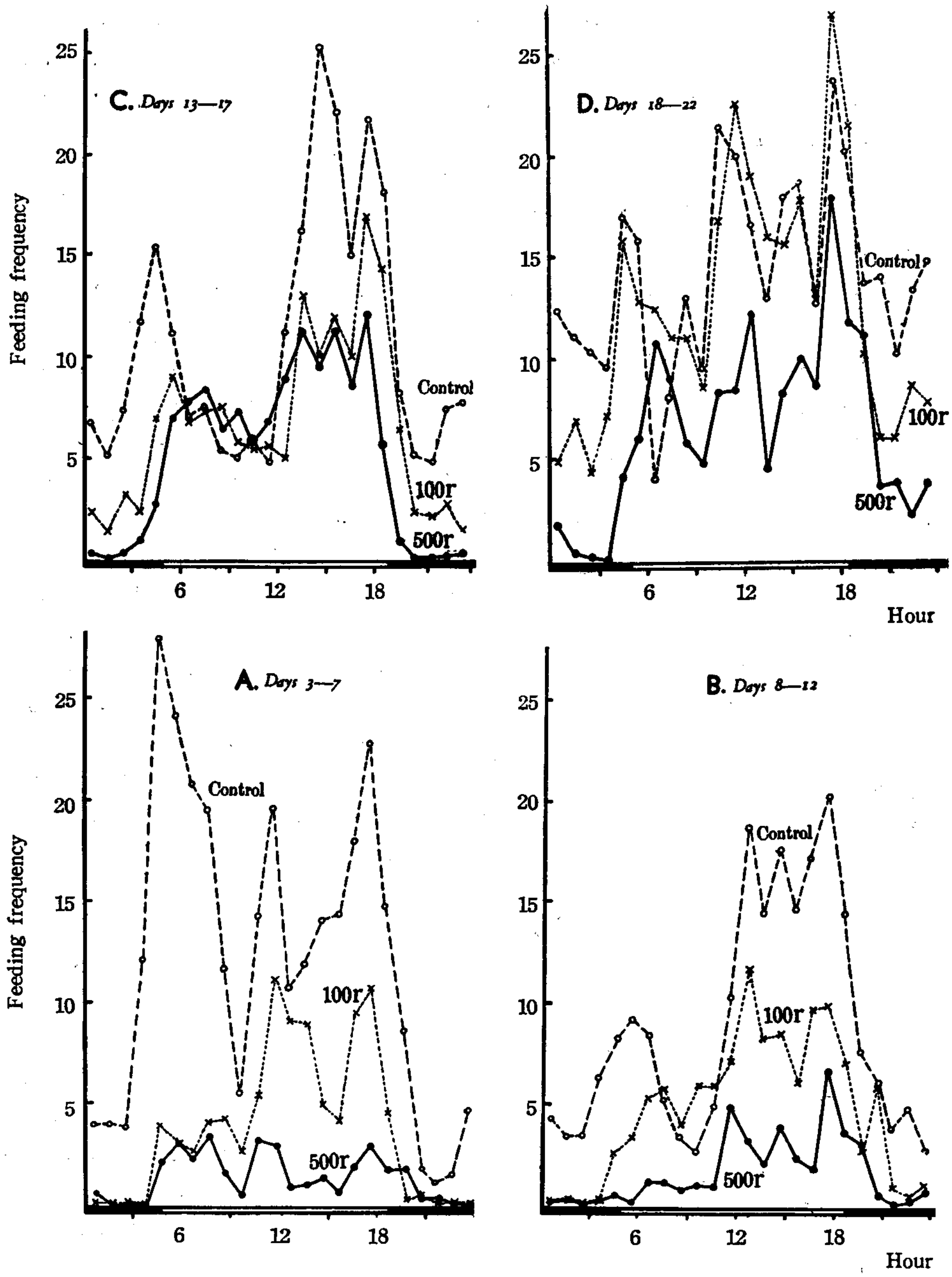

Fig. 3. Comparison of the diurnal rhythms of the feeding activity in the three groups: control, 100 r and 500 r, illustrated as the hourly average of each period of *days 3-7* (A), *8-12* (B), *13-17* (C) and *18-22* (D)

DISCUSSION

Susceptibility of the gastrointestinal tract to X- and γ-irradiation has been well established on the laboratory mammalian animals[1)15)]. Although no literatures concerning the effect of X-irradiation on fish feeding were available here, it is noteworthy that the present findings on the rainbow trout fry were in general agreement with those which have been reported by the previous workers on the mammalian animals.

Supracontrol feeding was found to occur in the 500 r group of the fishes on *day 1* after irradiation. It seems that such a relatively marked increase of food intake following irradiation has not been reported in the other animals. It has been generally recognized that following irradiation a marked increase of water consumption occurs in rats on *day 1* and often does again after several days, for instance, when food intake returned to normal[4)-6)]. Although further experiments are necessary before usual occurrence of supracontrol feeding in the irradiated fishes can be definitely asserted, it is biologically of much interest when considered from the facts that the experimental animal used is an aquatic one, fish, and also that such an increase was found only in the fishes irradiated with a relatively higher dosage, similar to the case of the water consumption in rats[7)].

It has been reported that the threshold dosage of X-ray for eliciting anorexia and loss of body weight is as low as 25 r or 50 r in rats[6)]. So, it may not be a surprising fact that in the rainbow trout fry a slight depression of the feeding activity was found following the exposure of 100 r. The magnitude and duration of the inhibition in the feeding activity after irradiation is also in agreement with the findings in the mammals, being dependent upon the radiation dosage to which the fishes were exposed. In most of the mammalian animals, the initial change of the gastrointestinal tract such as decrease of food intake, anorexia, increase of intestinal motility and delayed gastric emptying activity has been found to occur immediately after or as early as 24 hours or so after irradiation. Being different from the terrestrial animals, in the case of the aquatic fishes the effects of handlings such as netting, transfer and transport of them for irradiation must also be taken into consideration, in addition to the effect of irradiation itself. So, it seems that in the present experiment various kinds of stresses resultant from the handlings might affect the fishes in many ways[19)20)]. Swift *et al.*[10)] have emphasized that gastric emptying in rats is found also to be delayed significantly by some stress-producing treatments other than radiation. In fact, the feeding activity of the control group in the present experiment was found to be distinctly depressed by the handlings until *day 2*. Analysis of the initial change of the feeding activity in these fishes is difficult as stated above. However, considering from the records of the said activity taken 18 – 22 hours after irradiation, it can be said that marked changes relative to the control group occurred first on *day 1* and *day 3* in the 500 r group and on *day 4* in the 100 r group.

Similarly as in the case of the mammalian animals, the recovery of the feeding activity in the irradiated fishes was found to occur exponentially, accompanying on the graph with a wavy process of repeated decreases and increases. If the wavy fluctuation on the way to recovery is neglected, it seems that the recovery of the feeding activity occurs approximately corresponding to the exponential curves calculated from the following equation:

$$R=-ae^{-kt} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (1)$$

where R means magnitude of change of feeding frequency from the control (zero) or magnitude of recovery, a magnitude of the greatest inhibition, t day number in the recovery process or t_0 the day showing the greatest inhibition, and k means recovery coefficient.

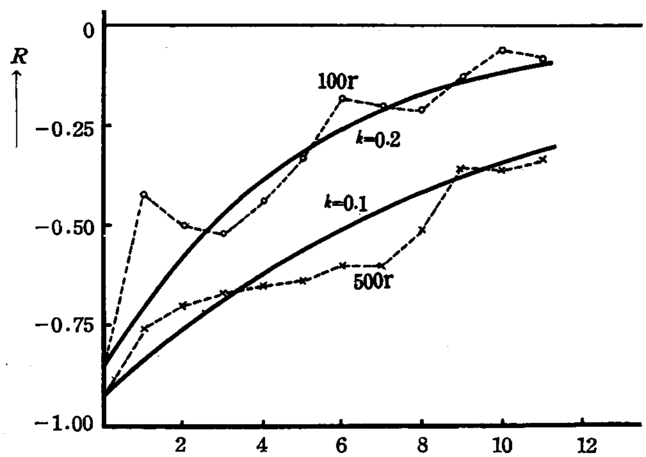

Fig. 4. Recovery curves of the feeding activity experimental and calculated; t_0 corresponds to *day 7* in the 100 r group and to *day 8* in the 500 r group

In the present experiment, the greatest inhibition of the feeding activity occurred on August 17, *day 7* in the 100 r group, and on August 18, *day 8* in the 500 r group, the depression from the control showing -0.86 and -0.93 (-86 % and -93 %) respectively. The curves of the solid lines in Fig. 4 were obtained introducing these values into a, and 0.2 in the 100 r group and 0.1 in the 500 r group into k, of equation (1). Those of dotted lines show the moving 7-day average obtained from the differences of the feeding frequency from the control (cf. Fig. 1). It can be said that in both groups of 100 r and 500 r the two curves experimental and calculated are in agreement with each other, respectively. That is, the approximate process of recovery of the feeding activity in the two groups of rainbow trout fry after irradiation may be inferred respectively from the following equations:

$$R=-0.86e^{-0.2t} \qquad (100\ r)$$

$$R=-0.93e^{-0.1t} \qquad (500\ r)$$

If the recovery coefficient, k, is widely utilizable as a characteristic in the study of X-irradiation injury, approximate recovery process of some function or response in a given animal irradiated may be expressed succinctly by it.

It seems to be important from the fisheries point of view that inactive state of the fishes continued for at least several days following irradiation. This is true because, if similar phenomenon occurs widely in fishes, those which have suffered some radiation injury might be drifted far away by current during the period of their inactive state.

The fact that no difference in the number of dead individuals was found beween the control and 1200 r groups seems to show that rainbow trout fry have a greater resistance to X-irradiation than goldfishes have[21)-24)]. This greater resistance may be associated with the low water temperature in which these cold water fishes were reared, similar to the findings in infant mice that the lowerd environmental temperature decreases the mortality following irradiation[25)]. However, the problem of survival rate in relation to low temperature appears to be somewhat complicated in the study of X-irradiation[25)26)].

SUMMARY

1. Using an automatic recording apparatus, the effect of X-irradiation on the feeding activity was studied in the rainbow trout fry.

2. In the 500 r group, a supracontrol feeding was recorded on the first day following irradiation. The marked depression of feeding occurred first on the third day and continued until the 9th day, the change in this period showing a range of -81~-63% from the control. On the 22nd day, the feeding activity of this group almost returned to the control level, followed by a wavy process of repeated decreases and increases.

3. A slight depression of the feeding was found in the 100 r group. A relatively marked depression occurred first on the 4th day after irradiation, showing -65% from the control. The depression continued until the 7th day, on which the greatest inhibition of -86% from the control was recorded. Feeding returned to the control level on the 13th day, accompanying also with a wavy process on the way of recovery course.

4. Diurnal rhythm of the feeding activity in the irradiated fishes was not so severely distorted as the said activity itself was highly affected. Consequently, the recovery of the former occurred a little earlier than that of the latter.

5. Exponential recovery process of some function or response in a given animal irradiated will be given by the following equation: $R=-ae^{-kt}$; where R means magnitude of change of feeding frequency from the control (zero) or magnitude of recovery, a magnitude of the greatest inhibition, t day number in the recovery process or t_o the day showing the greatest inhibition, and k means recovery coefficient. On the basis of this idea, approximate process of recovery of the feeding activity in the irradiated rainbow trout fry will be inferred from the following equations: $R=-0.86e^{-0.2t}$ in the 100 r group and $R=-0.93e^{-0.1t}$ in the 500 r group.

6. Following irradiation the fishes of both 100 r and 500 r groups showed an inactive state for at least several days. This fact seems to be important from the

fisheries point of view, because fishes which have received some radiation injury might be drifted away by current during the period of their inactive state.

7. It was found that the rainbow trout fry are resistant even to the radiation dosage as high as 1200 r. It can be said that this fish has a greater resistance to X-irradiation than goldfish has.

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