The Effect of X-Irradiation on 5-Hydroxytryptamine (Serotonin)
Contents in the Small Intestines of Experimental Animals

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ABSTRACT

The influence on 5-Hydroxytryptamine (5-HT) contents of small intestine of X-irradiation (500-2,000 r whole-body) was studied in rats, mice, guinea-pigs and rabbits. In order to examine the relationship between effect on 5-HT contents and intestinal injury by radiation, mean survival time and incidences of diarrhea were also observed in each species following irradiation.

5-HT contents of small intestine were remarkably reduced by exposure. The depressed level of 5-HT continued 48 hrs. There were great differences in normal contents of 5-HT and reduction rate after exposure among these species. It was found that the species which have high 5-HT contents and large reduction rate after exposure were sensitive to radiation.

The radiation sensitivity of intestine seemed to be closely related with its endogenous 5-HT. Its possible relationship was discussed.

INTRODUCTION

It has been well known that the 5-HT is one of the most protective agents against irradiation of mammals, and many reports concerning with its mechanism of protection have been published1-5).

5-HT differs from other protective agents such as AET or MEA in that this substance has been recognized as a active substance in the animal body and that it has powerful pharmacological action6).

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THE EFFECT OF X-IRRADIATION

It is an interesting problem to examine the correlation between endogenous 5-HT and irradiation. Melching reported the change of 5-HT contents in brain, spleen and liver of mice in 2 hrs after exposure, but Randic did not find any significant change of 5-HT contents in rat's brain. Endogenous 5-HT contents of intestinal tract occupies 85% of the total body amount, and intestinal tract was found to be the main source of 5-HT by Bertaccini. Many reports are available on the pharmacological action of 5-HT to intestinal function.

From these reasons noted above, it is assumed that the 5-HT plays a role in the manifestation of radiation effect on intestinal function. In fact, Van Den Brenk et al. recognized that 5-HT depressed the incidence of diarrhea, a main symptom of intestinal injury of radiation, protecting animals from "intestinal death".

In this paper, the effect of radiation on endogenous 5-HT in small intestine of four species was studied.

MATERIALS AND METHODS

Animal: Animals used were all adult males. Wistar strain rats (250-350 g), RF strain mice (25-33 g), guinea-pigs (370-450 g), and rabbits (2.5-3.0 kg) randomly mated within each stock in our institute were exposed to whole-body x-irradiation.

Irradiation procedure: X-ray apparatus was operated at 200 Kvp 25 ma; 0.5 mm Cu and 0.5 mm Al filters added; H.V.L. 1.2 mm Cu; target distance to bottom of box, 100 cm; and dose rate 20.0 r/min. (measured in phantom). The animals to be irradiated were placed in a wooden box, divided into 8 compartments for mice and rats, and 4 compartments for guinea-pigs; and one compartment for a rabbit. During exposure, integral doses were measured by Siemens Universal Dosimeter.

Bioassay of 5-HT: After single irradiation, animals were kept in the cage, fed on normal diet and sacrificed according to the experimental schedules. The small intestine were removed immediately for the 5-HT assay. 5-HT from intestinal tissue was extracted by the procedure adopted by Feldberg & Toh with small modification. In this method, recovery was about 85%.

The assay of 5-HT was performed by using rat stomach fundus strip (Vane 1957) in rat uterus Ringer (Gaddum 1949) at 37°C in 10 ml. organ bath with bubbling oxygen and containing atropine $1 \times 10^{-6}$ gm/ml and mepyramine $1 \times 10^{-7}$ gm/ml. In order to depress the spontaneous movement, the strip was stocked in ice cold place for one or two days before assay.

Assay was continued by the method of bracketing the unknown between known doses. Contractions produced by sample or standard solution were drawn for 90 sec. These contractions were completely abolished by BOL (2-bromo-lysergic acid diethyamide) $1 \times 10^{-5}$ gm/ml. In this assay, 15% error was permitted. Contents of 5-HT in tissue were expressed as µg of 5-HT creatinine sulphate.

RESULTS

1. Change of 5-HT Contents following whole-body X-Irradiation
produced on 5-HT content with the rather rapid recovery.

2. Species Difference in 5-HT Contents and Radiation Effect

The second experiment was tried to found the correlation between the radiation effect on 5-HT contents and the clinical symptom of radiation.

Four species of animals, rats, mice, guinea-pigs and rabbits which have different radiosensitivity of intestine were used. Small intestine of these animals were investigated physiopathologically.

Mean survival time and incidence of diarrhea after irradiation of 1,000 r and 1,500 r were selected as marks of intestinal injury. These doses were considered the dose which produces "intestinal death" for mammals.

The results were shown in Table 1. and 2. These doses were found to induce intestinal death always in rats and mice, not always in guinea-pigs and none in rabbits.

| Table 1. Mean Survival Time after Irradiation |
|------------------------|---------|--------|--------|--------|
| Dose       | Rat     | Mouse  | Guinea-pig | Rabbit |
| 1500 r     | 3.3 days| 5.0 days| 8.4 days | >20.0 days |
| 1000 r     | 4.0     | 7.0    | 8.3     | >30.0 |

Each value showed the mean of ten animal.

| Table 2. Incidence of Diarrhea and Average Days of Its Appearance after Irradiation |
|------------------------|---------|--------|--------|--------|
| Dose       | Rat     | Mouse  | Guinea-pig | Rabbit |
|            | I. D. (%)| A. D. | I. D. | A. D. | I. D. | A. D. | I. D. | A. D. |
| 1500 r     | 100     | 2.0    | 100   | 4.0   | 80    | 6.4   | 0 not observed |
| 1000 r     | 100     | 3.0    | 100   | 4.0   | 40    | 6.5   | 0     |

I. D.: Incidence of diarrhea %.
A. D.: Average days of appearance of diarrhea after irradiation.
Each value showed the mean of ten animals.
Table 3. Species Difference in the Effect of X-irradiation on the 5-HT Contents of Small Intestine

<table>
<thead>
<tr>
<th>Species</th>
<th>Contents of 5-HT µg/g</th>
<th>Reduction Rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control m.v. ± s.e.</td>
<td>2000 r, 3 hrs m.v. ± s.e.</td>
</tr>
<tr>
<td>Rat</td>
<td>6.34 ± .30</td>
<td>0.92 ± .12</td>
</tr>
<tr>
<td>Mouse</td>
<td>5.61 ± .41</td>
<td>2.98 ± .46</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>4.42 ± .41</td>
<td>2.36 ± .05</td>
</tr>
<tr>
<td>Rabbit</td>
<td>5.06 ± .72</td>
<td>4.43 ± .16</td>
</tr>
</tbody>
</table>

* (1 - Contents in Irrad.) × 100

Table 3 showed the contents of 5-HT in non-irradiated and irradiated animals. This result showed that there are great species differences in both normal 5-HT contents and reduction rate after irradiation among these species.

DISCUSSION

It was found that the contents of 5-HT of intestine decreased shortly after exposure, and the reduction was remarkable, and was kept up for a long time. These results differed from the reports of Melching et al\(^7\) and Randic\(^8\) in other tissues. Though many investigators support that 5-HT may have an important role in the intestinal function\(^16\)-\(^19\), meaning of reduction of 5-HT contents by irradiation reported here is not clearly explainable.

It was shown by Feldberg\(^15\) that the amount of 5-HT contained in intestine occupied the most part of its total amount in the whole-body. Bertaccini\(^10\) reported that intestine was the only source of 5-Hydroxyindoleacetic acid (5HIAA) of blood and urine, which was considered to be a metabolite of 5-HT. Van Den Brenk\(^9\) succeeded in depressing the radiation diarrhea in rats by pre-treatment 5-HT. These facts and results of our study suggest the possible role of 5-HT in radiation injury of intestine.

It was found that the tendency of radiosensitivity among these species shown in Table 1. and 2. were correlated to the contents of 5-HT in Table 3. This fact supports that the decrease of 5-HT contents is one of the important factors which influence the radiation effect on intestinal function.

It is reasonable to suppose that the decrease of 5-HT contents is due to over-release of endogenous 5-HT from the tissue. This is supported by the reports as follows: Melching\(^7\) found the increase of excretion of 5HIAA in blood and urine after exposure, Bertaccini\(^10\) described that intestinal tract was the main source of 5HIAA in blood and urine from the experiment of total or partial removal of intestine. Furthermore, in our laboratory, similar gross findings at autopsies as in the irradiated animals were observed in the non-irradiated animals treated with reserpine, known as a powerful releaser of endogenous 5-HT, and Nembutal (sodium pentobarbital). It had been found that 5-HT contents of intestine decreased remarkably at that period after Nembutal injection\(^30\). In these three treated groups, a similar decrease of intestinal tonus were observed.

From the discussion noted above, it is probable that x-irradiation induces an over-release of 5-HT followed by a lowered level of 5-HT contents which result in
keeping the intestine from normal physiological function such as motility, tonus, absorption and so on.

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