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Effect of a nucleotide mixture (Nucleton) on the depression of
DNA synthesis by ionizing radiations in
regenerating mouse liver.*

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INTRODUCTION

Restorative effect of nucleotides has been reported for survival of singly irradiated mice by Maisin (1) and survival time of repeatedly irradiated mice by Sugahara (2). In order to explore the biochemical mechanism of the effect, nucleic acid metabolism in regenerating liver was studied using ^{32}P as a tracer. An alkaline hydrolysed product of yeast RNA (Nucleton) which is reported to bear ribonucleotide has been used, since it was demonstrated previously that the mixture was most effective for survival mixture time among various kinds of nucleic acid precursors so far studied. Restoration from radiation-induced depression of DNA metabolism was observed with the administration of the mixture.

MATERIALS AND METHODS

a. Partial hepatectomy

Regenerating liver in the mouse was obtained after several trials. Thus the method will be described in some detail.

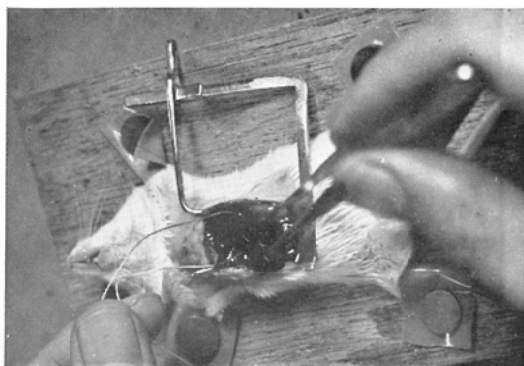
Female mouse of dd/YF line obtained from Funabashi Farms, Chiba was used at 60 days of age. After Nembutal anaesthesia, the abdomen from the sternal region down was shaved and sterilized with 70% alcohol. The abdominal skin was exfoliated by means of a slightly curved transverse incision of about 2 cm long about 0.5 cm below the processus xyphoideus. Blood vessels running longitudinally in the muscle tissue about 0.5 cm from the midline were ligated above and below the scheduled incision. This procedure is important to reduce bleeding during and after the operation. After opening the peritoneal cavity by transverse incision, the operation field was kept open as widely as possible by mean of an eye speculum as shown in Fig. 1. After the radices of the left lateral and right median lobe of the liver as shown in Fig. 2 were ligated with No. 1 silk ligature respectively, the lobes were resected. During the ligation, great caution should be paid not to tear off the liver tissues and ligate the gallbladder together.

After completing the resection the abdomen was closed by silk sutures, the peritoneum and muscle layer and the skin layer with a continued suture respectively.

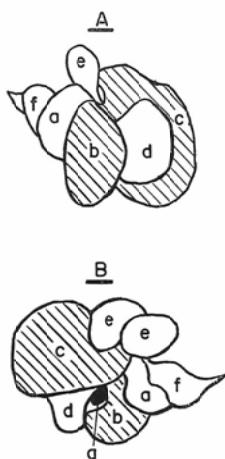
b. Analytical procedures

Twentyfour hours after intravenous injection through the tail vein of ^{32}P $2\mu\text{Ci/g-body weight}$ at various times after partial hepatectomy the mice were sacrificed by cervical dislocation and the liver was

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Legend for Fig. 1 The mouse under operation, its abdominal cavity being kept open by an eye speculum.



Legend for Fig. 2 Gross anatomy of the mouse liver

- A. The mouse liver seen from above
 B. The mouse liver seen from below
 a. Right, external lobe b. Right, internal lobe
 c. Left, external lobe d. Left, internal lobe
 e. Processus papillaris f. Processus caudatus
 g. gall bladder

taken out for chemical analysis.

ASP (acid-soluble phosphorus), LP (lipid phosphorus) RNA-P and DNA-P fractions were obtained by the method of Tyner, Heidelberger and Le Page (3) with a slight modification after homogenizing the resected liver in 5ml of 0.6N cold perchloric acid. Radioactivity of ^{32}P in each fraction was measured with an endwindow G.M. counter, after drying under an infra-red lamp, and the amount of phosphorus was assayed by a modification of Fiske-Subbarow method.

c. Irradiation and treatment

A whole-body irradiation of 800R was given to partially hepactomized mice 6 hours after the operation. A 0.1 ml solution of the nucleotid mixture as mentioned below was administered subcutaneously every 24 hours until sacrificing the animal. For the control, the partially hepactomized mice were kept

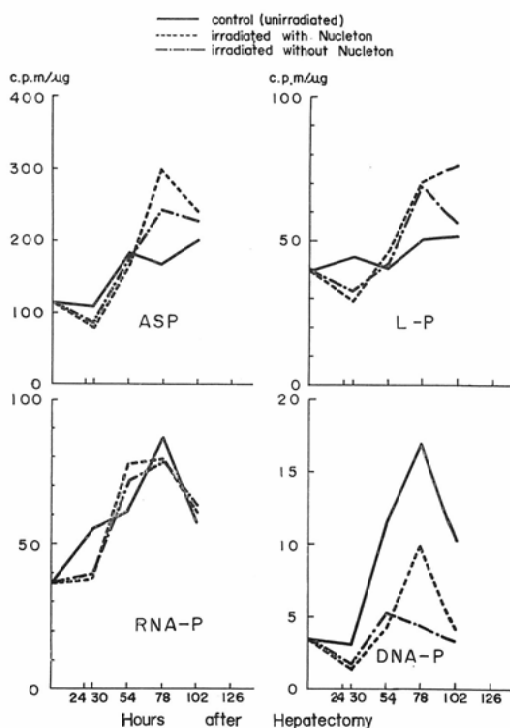


Fig. 3 Phosphorus metabolism in regenerating mouse liver irradiated with 800 R six hours after partial hepatectomy

without the administration. Mice were sacrificed at 30, 54, 78 and 102 hours after the partial hepatectomy. Total 200 mice were used for the experiment.

d. Chemicals

A nucleotide mixture (Nucleton) is an alkaline hydrolysed product from yeast RNA produced by Daigo Nutritive Chemicals Ltd. It is reported that it consists mainly of 3' (or 2')-ribose-nucleotides of four kinds, i.e., adenylic, guanylic, cytidylic and uridylic acids of approximately equal amounts. It was available as a 2 ml solution in an ampulla which contained 50 mg of the mixture. The solution was diluted 10 fold with physiological saline for injection.

RESULTS

Changes in specific activity of ^{32}P in the regenerating liver at various times after partial hepatectomy and the effect of irradiation and Nucleton-treatment on them are shown in Fig. 1. In unirradiated regenerating liver slight rise of the incorporations of ^{32}P to ASP and LP and remarkable rise of those to RNA-P and DNA-P with the maximum at 78 hours after hepatectomy were observed. Irradiation with 800R 6 hours after hepatectomy does not affect the RNA metabolism significantly. Incorporation of ^{32}P to ASP and LP was rather accelerated by irradiation. DNA metabolism seems to be completely inhibited with the irradiation of 800R.

Nucleton seems to have only a slight effect on ASP and LP metabolism, possibly depressing the rise of incorporation induced by radiation. However, the treatment with Nucleton significantly restore the DNA metabolism from the inhibition induced by radiation.

DISCUSSION

Increased survival after single whole-body irradiation with alkaline hydrolysed products of yeast RNA was reported by Maisin (1) Sugahara (2) demonstrated quantitatively the restorative effects of various nucleic acid precursors using survival time under repeated exposure as criteria. A nucleotide mixture (Nucleton) which consists of four kinds of 3'-nucleotides was shown to be most effective among precursors so far studied. It is the aim of the present paper to explore the biochemical mechanism of the effect. Nucleic acid metabolism has been widely studied as a biochemical effect of ionizing radiation. Depression of DNA metabolism by radiation has been well demonstrated in various organs, especially in regenerating rat liver at its early stage of regeneration.

In mice, regenerating liver obtained by surgical resection has not been widely studied because of the difficulty of resection. At 6 hours after partial hepatectomy the liver was assumed to be at its preparative stage for DNA synthesis as extrapolated from the data on regenerating rat liver (4). The stage may be that of enzyme formation and sensitive to radiation.

The present results indicate that DNA synthesis is depressed by radiation but RNA synthesis not. The results are in accord with those by workers reporting the increased incorporation of precursors into RNA rather than decreased following irradiation (5).

It may be concluded that the administration of the nucleotide mixture is effective for the restoration from the depression of DNA synthesis induced by ionizing radiation. Since the depression is assumed to be the inhibition of enzyme formation, the effect may be related to the enzyme formation. But the possibility that the increased DNA synthesis observed reflects the mitotic activity and thus the effect of

the mixture is on some other mechanism related to mitotic activity other than DNA metabolism cannot be dismissed.

It is wellknown that various precursors are incorporated into RNA and DNA as demonstrated by radioactive tracer technique. However it is assumed by Heidelberger (6) that nucleotides should be dephosphorylated before entering into the cell. Any way, the effect of Nucleton may be interpreted in two ways; nutrient supply for synthesis and restoration of disturbed metabolic control. Comparative studies on the effects of various kinds of precursors may favor the elucidation of the mechanism.

SUMMARY

Restoration from radiation-induced depression of DNA synthesis was observed in regenerating mouse liver with the administration of an alkaline-hydrolysed product of yeast RNA (Nucleton). The surgical procedure to obtain regenerating liver in the mouse was described in some detail. Biochemical mechanism of the restoration was discussed.

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ハツカネズミ再生肝における放射線による DNA 合成抑制に対するヌクレオチド混合物（ヌクレトン）の効果

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酵母 RNA のアルカリ加水分解物であるヌクレオチド混合物（ヌクレトン）がハツカネズミ再生肝にみられる DNA 合成の X 線による抑制から回復させることを示した。なおハツカネズミにおける再生肝を作るための手術方法についてや、詳細に述べた。