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EFFECTS OF IRRADIATION ON CANCER TOXIN IN TUMOR-BEARING HOSTS

SHOJI SUGA

1st Department of Internal Medicine Nagoya University School of Medicine (Director: Prof. Dr. Susumu Hibino)

ABSTRACT

Irradiation effects on cancer toxin, "toxohormone", in tumor-bearing hosts were investigated.

Crude toxohormone fractions were prepared from preoperatively Co^{60}-irradiated human gastric cancer by the method of Yunoki and Griffin.

1) It was demonstrated that Co^{60}-irradiation reduced the liver catalase depressing action of the crude toxohormone fractions, but the yield of the fractions was less markedly affected.

2) Amberlite XE-64 column chromatography of the fractions revealed the decreased amount of the TH2 fraction which was reported to possess the most potent catalase depressing action.

3) Histological observations of the Co^{60}-irradiated cancer were: a) focal necrosis and/or fibrosis of the tissue; b) degenerative changes of cancer cells, which varied in severity.

Irradiation effects on cancer toxin were then investigated in connection with the tumor growth, using mice bearing a diploid Ehrlich ascites tumor.

1) In tumor-bearing mice, a decrease in liver catalase activity was observed 10 hours after irradiation with 200 or 500 r of X-rays. This decrease was ascribed to an enhanced release of the cancer toxin from the irradiated tumor cells.

2) After 500 r, the arrest of cell division occurred, whereas the average cell volume continued to increase. This increase in volume might be due to an increase in new cell substance. On the other hand, the estimations of liver catalase activity demonstrated an increase in activity 48 hours after irradiation.

3) This later increase in liver catalase activity was also observed frequently after exposure to 200 r. Such irradiation effect was, however, diminished by the concomitant administration of a radioprotective agent, cysteine. On the other hand, no influence of cysteine was observed with regard to the early decrease in liver catalase activity. From these findings, the later increase in liver catalase activity was most likely interpreted to be due to the lowered amount of the cancer toxin after irradiation, which was resulted from a decreased synthesis *de novo* of the toxin. In other words, there was a possible relation of the radiosensitive enzymes with the synthesis of the cancer toxin by the tumor cells.

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INTRODUCTION

Many studies have been reported on the metabolic derangements which produce cachexia and ultimate death in tumor-bearing hosts[1~9]. Such derangements were ascribed to an abstraction by a tumor of some of the essential nutrients of the host, or to a toxic substance elaborated by the tumor[1].

The abstraction of the host nutrients by the tumor[10] or the dislocation of nitrogenous substance from normal to neoplastic tissue was demonstrated[15], but it did not necessarily provide a fully understanding of the metabolic changes in the tumor-bearing host. On the other hand, with regard to the studies on the toxic substance as a possible cause of the cachexia, liver catalase provided a useful system[1]. Liver catalase activity was reported to be depressed in almost all animals bearing tumors of various kinds[1]. Moreover, it was depressed progressively with the growth of the tumor, and restored to the normal level by extirpation of the tumor[6]. In addition, it was reported that the activity was not affected by rapidly growing non-malignant tissue such as an embryo[7]. From these observations and other related facts[8][9], decrease of liver catalase activity in tumor-bearing host was considered to indicate some unique property of the tumor tissue. In 1948, Nakahara and Fukuoka demonstrated a liver catalase depressing action of a substance extracted from human cancer tissues, and thus the concept of a cancer toxin, "toxohormone", was afforded an actual proof[11]. Following their work, every effort was made by many workers for the improvements on the preparation and purification of the toxin[12~15].

The present experiments were undertaken in an attempt to investigate the irradiation effects on the cancer toxin in tumor-bearing hosts. Irradiation is indeed, a clinically well established effective therapy of the patients with neoplastic diseases.

First, the crude toxohormone fractions were isolated from preoperatively Co^{60}-irradiated human gastric cancer by the method of Yunoki and Griffin,[15] and were compared with the untreated toxohormone fractions.

Then, experiments were carried out to elucidate a more precise relation between the growth of tumor and the cancer toxin producing function of the tumor. For this purpose, liver catalase activity in mice bearing a Ehrlich ascites tumor was determined at various time intervals after irradiation, simultaneously with the investigation of the tumor growth.

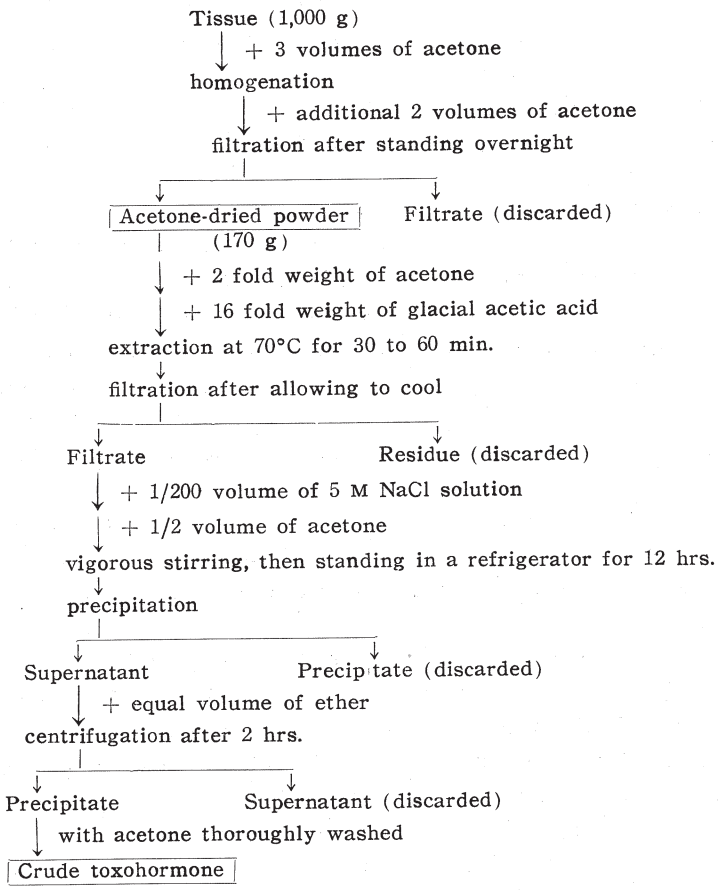
MATERIALS AND METHODS

Materials: For the preparation of crude toxohormone fraction, preoperatively Co^{60}-irradiated human gastric cancers were used as materials. Prior to operation, 200 to 300 r of Co^{60} was irradiated to the patients with gastric cancer everyday over the period of ten to twenty days. The total dose, accordingly,

amounted 3,000 to 6,000 r in each case. In addition, both normal tissue adjacent to cancer and non-irradiated human gastric cancer served as controls respectively.

Preparation of crude toxohormone fractions: Crude toxohormone fractions were prepared from the materials descrived above by the method of Yunoki and Griffin (Table 1).[15] This method was patterned after the procedure of

TABLE 1. Preparation of Crude Toxohormone Fractions



Payne *et al.*[16] for the efficient extraction of corticotropin from pig pituitary glands. In principle, it consisted of the following processes: Tissues were defatted with acetone to give an *acetone-dried powder*, which was then extracted at 70°C with glacial acetic acid. From this solution impurities were precipitated by acetone after the addition of a small amount of salt, and the active fraction then was obtained by precipitation with ether. This preparation was designated

as *crude toxohormone.* A special attention was paid, as to the ether used in this process, for the removal of peroxide which might occur in ether spontaneously with great ease and might exert its potent oxidative action on the toxohormone fractions to inactivate them. For this purpose, ascorbic acid was used. The extraction experiments were repeated five times. In each experiment, samples from three to five different patients with cancer were used as a starting material.

Chromatography of crude toxohormone fractions: For the chromatographical analysis of crude toxohormone fractions, the method of Yunoki and Griffin was employed.[15] A chromatographic column 2.9 cm in diameter and 35 cm high was prepared with Amberlite XE-64 (200 mesh) which was previously equilibrated with N/10 glycine buffer of pH 9.40. Five hundred milligrams of the toxohormone fraction was dissolved in 10 ml of the buffer. After removal of insoluble portion, the supernatant was applied to the column. The column was then eluted with the same buffer. The flow rate was 10 ml/30 min., and the volume of effluent collected per tube was 10 ml. After the elution of the first fraction, the pH of the buffer was gradually increased by the addition of N/10 NaOH through the mixing chamber. Aliquots of the effluent were taken for the determination of protein by the method of Lowry *et al.*[17]

Estimation of liver catalase activity: A modification of the method of von Euler and Josephson was employed for the estimation of liver catalase activity[18].

We had previously investigated the method of preparing liver homogenates which were used as enzyme solution, and obtained the following results.[10] 1) Liver catalase was distributed throughout the whole liver tissue at an equivalent concentration. 2) 0.4% of the total activity was due to the blood components contained in the homogenates. 3) The liver catalase activity associated with relatively stable particles was not measured until these particles were disrupted. For the disruption of these particles, an addition of desoxycholate to the homogenate was available. 4) A Potter Elvehjem homogenizer with a Teflon Pestle was preferable to that of glass made, for the preparation of liver homogenates. From these results, liver catalase activity was estimated by the improved method presented in Table 2.

For the bioassay of liver catalase depressing action of crude toxohormone fractions, the samples dissolved in 0.5 ml of distilled water were injected intraperitoneally into each of 5 mice (*dd* strain males weighing 19 ± 1 g). All mice were sacrificed by decapitation 24 hours after injection, and the liver catalase activities were assayed. The activities were expressed in terms of Kat. F.. Data were also given in percentages, and the values for the normal untreated mice were taken as 100%.

The mice used for the assays were, considering the dietary influence on liver catalase activity, fed a standard diet of Oriental pellets and then were

TABLE 2. Improved Method for the Estimation of Liver Catalase Activity

Fresh liver (100 mg)
↓ + 5 ml of saline solution
homogenation at 0° for 2 min. with a Potter-Elvehjehn homogenizer with a Teflon Pestle
↓ + 10 ml of saline solution
Homogenate (diluted)
↓
1 ml of this homogenate added to 1 ml of 0.6% desoxycholate
↓
Enzyme solution
↓
1 ml of this enzyme solution added to 50 ml of 0.01 N H_2O_2 solution in N/150 phosphate buffer of pH 6.8 at 0°C
↓
Immediately after the additon and vigorous stirring (*i.e.*, at 0 sec.), 5 ml of the reaction mixture pipetted into 5 ml of 2 N H_2SO_4; subsequently, at 60 and 120 sec., each 5 ml of the reaction mixture pipetted into respective H_2SO_4 solution
↓
titration with N/200 $KMnO_4$ of H_2O_2 remaining
↓
Catalase activity*

*Activity expressed as $(\log a/b + 1/2 \log a/c)/2 \times 100$, where a, b, and c are the amount of $KMnO_4$ used at 0, 60, and 120 sec., respectively.

fasted during the 24-hour interval before sacrifice.

Somewhat larger mice, 9 to 11 week old *dd* strain males weighing 22 to 25 g, were employed for the studies of irradiation effects on Ehrlich ascites tumor-bearing animals. They were arranged in groups of 5 mice. each.

Transplantable tumor: A diploid Ehrlich ascites tumor was employed. Ascites fluid was collected from mice inoculated 8 or 9 days earlier. After dilution of the fluid with saline, 5×10^6 tumor cells were injected intraperitoneally into mice in volume of 0.2 ml.

Irradiation: Seven days after inoculation the mice were given a single dose of whole body X-irradiation with 200 or 500 r. X-rays were generated at 200 kV, 20 mA, 0.5 mm. Al 0.5 mm. Cu filtration. The dose rate was 48 r/min..

Total cell number of ascites tumor: Cell counts were done in Bürger hemocytometer after dilution with Türk's solution. Total number was then computed.

Total cell volume of ascites tumor: The hematocrit method was employed for the determination of total volume of cells. Samples of the fluid were centrifuged in a hematocrit tube until the sediment remained constant (about 30 minutes at 3,000 × g.). Total volume of cells was then obtained from the hematocrit value and total volume of the fluid.

Average cell volume of ascites tumor: Average cell volume was calculated by dividing the total volume of cells by the total number of cells. During the entire period of the present experiments, about 15 per cent of the cell population consisted of non-tumorous cells which were generally smaller than the tumor cells. Consequently, the value actually obtained would be somewhat smaller than the value of the tumor cells alone[20].

Cell viability of ascites tumor: Determinations of cell viability were done by the trypan blue staining method[21]. To the ascites was added trypan blue solution. Two hundred cells were counted. The unstained-cell counts were expressed as the per cent of the whole cells (both stained and unstained cells).

RESULTS

I. Crude Toxohormone Fraction Prepared from Preoperatively Co^{60}*-Irradiated Human Gastric Cancer*

TABLE 3. Preparation and Bioassay of Crude Toxohormone Fractions

EXP. No.	Yield (g)	Liver catalase activity		
		Dose (mg)	Kat. F. (mean ± S. E.)	Activity (%)
I	4.32	0	22.1±1.2	100
		10	17.3±1.1	79
		20	17.2±2.0	78
II	4.80	0	23.1±1.7	100
		20	18.2±2.0	78
		40	20.3±2.9	85
III	5.12	0	22.2±0.5	100
		10	19.7±1.3	89
		20	17.5±1.0	78
IV	5.12	0	21.5±1.9	100
		20	14.6±1.1	67
V	5.07	0	21.3±1.7	100
		20	17.7±1.3	83
VI	5.40*	0	23.8±1.2	100
		10	15.2±1.0	64
		20	13.9±1.8	58
VII	5.00*	0	22.1±1.0	100
		10	18.4±1.2	83
		20	19.3±1.5	87

* Values represent the mean of three products.

Yields are expressed in grams of the products obtained from 170 g of acetone-dried powder of respective materials.

Materials used in Exp. No. I to V were preoperatively CO^{60}-irradiated human gastric cancers; those in Exp. No. VI, non-irradiated human gastric cancers; those in Exp. No. VII, normal tissues of human stomch.

Preparation and bioassay of crude toxohormone fractions: These results were presented in Table 3. The yield of the crude toxohormone fractions from non-irradiated cancers was calculated to be 5.40 g (100%), whereas the yields for the irradiated cancers were 4.32 to 5.12 g (80 to 95%). In addition, there was a yield of 5.00 (95%) for the normal gastric tissue. On the other hand, liver catalase activities determined 24 hours after injection with 20 mg of samples decreased to 58% of controls (untreated non-tumor-bearing), for the non-irradiated cancers. However, the values for the Co^{60}-irradiated cancer were 67 to 83% (in most cases 78%). These results demonstrated that the irradiation reduced the liver catalase depressing action of the crude toxohormone fractions, but the yield of the fractions was less markedly affected.

Chromatographical analysis of crude toxohormone fractions: As reported by Yunoki and Griffin, the crude toxohormone fraction of non-irradtiaed cancer was chromatographically separated into three fractions. The first fraction was eluted around pH 9.40, the second fraction around pH 9.50, and the third frac-

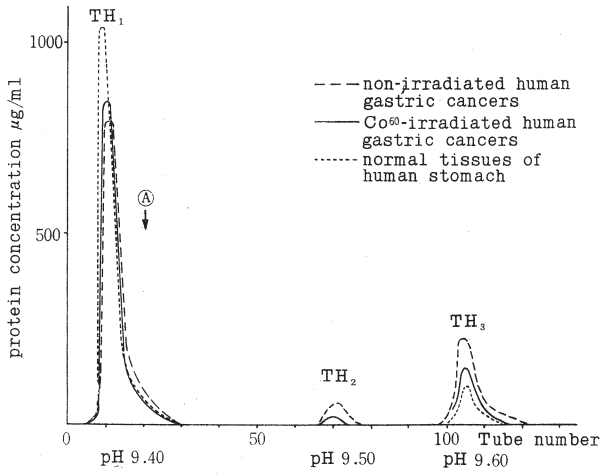


FIG. 1. Chromatographic patterns of crude toxohormone fractions
Sample: 500 mg of the crude toxohormone fractions was dissolved in 10 ml of M/10 glycine buffer at pH 9.4 and after removal of insoluble material, was applied to the column.
Column size; 2.9×35 cm.
Resin: Amberlite XE-64 (200 mesh)
Buffer: M/10 glycine buffer of pH 9.40; Ⓐ; a gradient to more basic buffer by adding 0.1 N NaOH through the mixing chamber
Flow rate: 20 ml/hr. (Volume collected per tube; 10 ml)
Distribution of protein in fractions:

	TH1 (mg.)	TH2 (mg.)	TH3 (mg.)
Non-irradiated human gastric cancers;	32.8	1.7	10.2
Co^{60}-irradiated human gastric cancers;	33.4	0.4	8.0
Normal tissues of human stomach;	36.3	negligible	3.6

tion around pH 9.60. They were designated as TH1, TH2, TH3, respectively. In the present experiments, the distribution of protein in each fraction was: TH1, 32.8 mg; TH2, 1.7 mg; TH3, 10.2 mg. On the other hand, the chromatographical analysis of crude toxohormone fractions of irradiated cancer revealed marked decrease in the amount of the TH2 fraction (0.4 mg). Moreover, the TH3 fraction was also decreased in amount. In addition, the normal gastric tissue was domonstrated to be devoid of the TH2 fraction and the decreased amount of the TH3 fraction was also observed (Fig. 1).

Appendix

Histological observations of preoperatively Co^{60}*-irradiated cancer:* Histological changes observed after Co^{60}-irradiation consisted of: a) focal necrosis and/or fibrosis of cancer tissue; b) a non-specific inflamamtory reaction of the tissue; c) damaged cancer cells with swollen nuclei or with changes in staining properties; d) decrease in number of the mitotic figures of cancer cells.

II. X-Irradiation Effects on Tumor Growth and Liver Catalase Activity in Mice Bearing a Ehrlich Ascites Tumor

Irradiation effects on tumor growth and liver catalase activity in tumor-bearing mice: In tumor-bearing mice with a dose of 500 r, there was a greater decrease in liver catalase activity 10 hours after irradiation as compared with that of non-irradiated groups. The values were 12.5 ± 0.5 (87%) and 14.3 ± 0.9 (100%) respectively. Such a decrease was hereafter referred to as an *early decrease in liver catalase activity.* This decrease was followed by an later increase within 48 hours, showing a value of 16.7 ± 1.1 (114%), which was higher than that of non-irradiated groups (14.7 ± 1.5; 100%). These irradiation effects were quite different from the case of non-tumor-bearing mice in which the liver catalase activity remained unchanged with in 10 hours and was then re-

TABLE 4. Liver Catalase Activity in Normal Mice after Irradiation

X-ray dose (r)	Liver catalase activity Kat. F. (mean ± S. E.)		
	10*	24*	48*
0	27.4±0.9 (100)	25.9±0.5 (100)	25.3±0.9 (100)
200	27.1±0.9 (99)	24.5±0.8 (95)	24.2±0.7 (96)
500	26.8±0.6 (98)	23.7±0.5 (92)	20.8±0.5 (82)

* Time in hours after irradiation.

Data are given in percentages (inparentheses), and the values for the normal untreated mice are taken as 100%.

TABLE 5. Liver Catalase Activity in Tumor-Bearing Mice after Irradiation with 500 r

Treatment	Liver catalase activity Kat. F. (mean ± S. E.)		
	10*	24*	48*
untreated non- tumor-bearing	24.6±0.5 (100)	25.1±0.5 (100)	25.8±0.6 (100)
non- irrad. tumor-bearing	14.3±0.9 58 (100)	14.4±1.1 57 (100)	14.7±1.5 57 (100)
irrad. tumor- bearing	12.5±0.5 51 87	14.6±0.2 58 101	16.7±1.1 65 113

* Time in hours after irradiation.

Values (in parentheses) of untreated non-tumor-bearing mice, as well as those of non-irradiated tumor-bearing mice, are taken as 100% and data for both are caloulated in percentages.

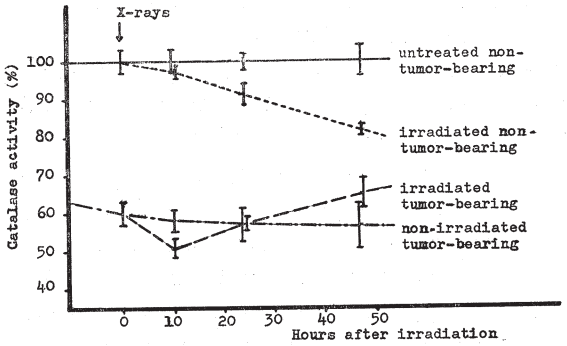


FIG. 2. Liver catalase activity after irradiation with 500 r.

duced to 82% of the control values 48 hours after irradiation with 500 r (Tables 4 and 5, Fig. 2).

The investigation of irradiation effects on tumor growth was carried out simultaneously. As shown in Table 6 and Fig. 3, after irradiation with 500 r, the total number of cells remained almost unchanged during the 48 hour period of time, whereas a considerable increase was observed in non-irradiated groups. It was shown, however, that no detectable change occurred neither in percentage of tumor cells nor in percentage of trypan blue stained cells after irradiation, as compared with those in non-irradiated groups. The percentage of tumor cell population was 85% and the percentage of stained cells was 4% in both irradiated and non-irradiated groups. Moreover, no difference could be distinguished between irradiated and non-irradiated groups with regard to the total volume of cells during the entire observation period. A progressive

TABLE 6. Effect of Irradiation on Tumor Growth (with 500 r)

		8**	10*	24*	48*
non-irradiated	total cell count ($\times 10^8$)	5.3±0.3	6.1±0.6	6.9±0.4	7.9+0.8
	total cell volume (ml.)	0.97±0.03	1.08±0.05	1.25±0.05	1.41±0.11
	average cell volume ($\times 10^3\ \mu^3$)	1.82±0.06	1.78±0.07	1.83±0.07	1.82±0.09
irradiated	total cell count ($\times 10^3$)	5.3±0.3	5.5±0.3	5.8±0.3	6.3±0.4
	total cell volume (ml.)		1.03±0.05	1.24±0.06	1.40±0.06
	average ceil volume ($\times 10^3\ \mu^3$)		1.87±0.03	2.13±0.09	2.23±0.06

** Time in hours before irradiation
* Time in hours after irradiation
Values are the arythmetic meam ±S.E. of 5 mice.

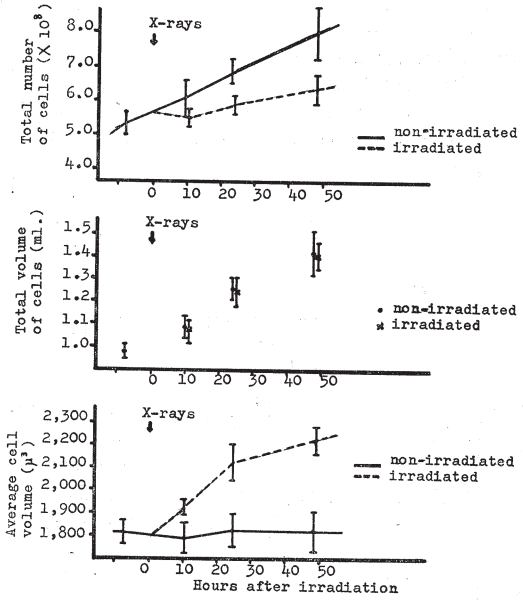


FIG. 3. Effect of irradiation on tumor growth. (with 500 r)

increase in average cell volume, consequently, began immediately after irradiation, and gave a value of 2,230 μ^3 at 48 hours. In contrast, the average cell volume of untreated tumors (1,820 μ^3) was found to remain constant. These results were also presented in Table 6 and Fig. 3.

In an attempt to examine the possible existence of such a X-ray dose as effective for the liver catalase activity without any influence on the growth of tumor cells, the effect of irradiation with 200 r was investigated. The early

TABLE 7. Liver Catalase Activity in Tumor-Bearing Mice After Irradiation with 200 r

EXP. No.	Treatment	Liver catalase activity Kat F. (mean ± S. E.) 10**	6*	10*	24*	48*	72*	96*
I	untreated non-tumor-bearing	27.6±1.7 (100)	29.0±1.5 (100)	27.0±1.9 (100)	27.3±2.0 (100)	27.4±1.4 (100)	29.5±1.9 (100)	28.2±1.5 (100)
	non-irrad. tumor-bearing	1.90±2.2	18.3±1.1 63 (100)	16.7±1.2 62 (100)	17.1±0.7 63 (100)	15.3±1.7 56 (100)	16.3±1.1 55 (100)	15.0±1.1 53 (100)
	irrad. tumor-bearing		16.6±2.1 57 91	14.9±1.4 55 89	16.1±2.2 59 94	18.9±0.8 69 123	19.0±1.0 65 116	14.3±1.6 51 96
II	untreated non-tumor-bearing			25.3±0.9 (100)	26.7±0.6 (100)	26.1±1.1 (100)		
	non-irrad. tumor-bearing			15.9±1.0 63 (100)	17.1±0.9 64 (100)	15.7±0.9 60 (100)		
	irrad. tumor-bearing			13.7±0.3 54 86	15.8±0.8 59 92	15.6±0.6 60 99		

** Time in hours before irradiation

* Time in hours after irradiation.

Values (in parentheses) of untreated non-tumor-bearing mice, as well as those of non-irradiated tumor-bearing mice, are taken as 100% and data for both are calculated in percentages.

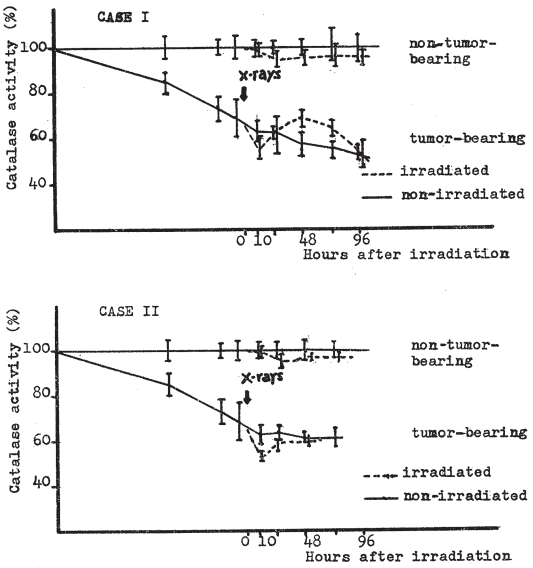


FIG. 4. Liver catalase activity after irradiation with 200 r.

decrease in liver catalase activity was also observed 10 hours after irradiation as in the case of 500 r. After 48 hours, the activity showed the same value as that of non-irradiated groups or much higher values (Table 7; Fig. 4) On the other hand, in non-tumor-bearing mice, X-irradation with 200 r did not affect the liver catalase activity through the whole observation period. (Table 4, Fig. 4). With regard to the ascites tumor, the cell multiplication seemed to slow down for a short period of time after irradiation, but no detectable difference could actually be distinguished between irradiated and non-irradiated groups. The average cell volume at 48 hours after irradiation was 1,840 μ^3 for irradiated groups and 1,830 μ^3 for non-irradiated groups.

Liver catalase depressing action of in vitro irradiated ascites tumor: It was supposed that the early decrease in liver catalase activity after irradiation might be due to an enhanced release of the cancer toxin from the irradiated tumor. In order to examine this possibility, the following experiments were carried out, in which ascites tumor was exposed to 200 r of X-rays *in vitro*. An amount of 2×10^8 of this *in vitro* irradiated tumor was injected into normal recipient mice. A group of mice injected with the same amount of non-irradiated ascites tumor served as controls. A much lowered liver catalase activity was produced by the injection of the *in vitro* irradiated ascites tumor both 10 and 24 hours after irradiation (Table 8). The non-irradiated tumor gave values

TABLE 8. Liver Catalase Depressing Activity of *in vitro* Irradiated Ascites Tumor

Treatment	Liver catalase activity (mean ± S. E.)	
	10*	24*
control (untreated normal)	26.2 ± 0.8 (100)	
injection with non-irradiated-tumor	21.0 ± 1.1 80 (100)	21.7 ± 0.5 83 (100)
injection with irradiated tumor	18.7 ± 1.2 71 89	18.4 ± 1.1 70 85

* Estimations of liver catalase activity were carried out at 10 and 24 hours after injection.

A group of mice injected with the same amount of non-irradiated ascites tumor served as controls.

Values (in parentheses) of untreated normal mice, as well as those of non-irradiated-tumor-injected mice, are taken as 100% and data for both are calculated in percentages.

of 21.0±1.1 at 10 hours and 21.7±0.5 at 24 hours, whereas those for irradiated tumor were 18.7±1.2 and 18.4±1.1, respectively. Moreover, irradiation of the recipient mice alone did not produce any change in liver catalase activity (Table 9).

TABLE 9. Changes in Susceptibility of Recipient Mice to Cancer Toxin by Irradiation

recipient mice	Liver catalase activity Kat. F. (mean ± S. E.)	
control (untreated)	27.6 ± 1.0 (100)	
T-injected non-irradiated mice	24.0±±1.0 87 (100)	
T-injected irradiated mice	25.1 ± 1.1* 91 104	23.7 ± 0.5** 86 99

T: Supernatant fluid of the ascites tumor used as cancer toxin.
* X-ray dose: 200 r
** X-ray dose: 500 r
Values (in parentheses) of untreated normal mice, as well as those of *T*-injected non-irradiated mice, are taken as 100% and data for both are calculated in percentages.

Effect of cysteine on irradiation induced changes in liver catalase activity in tumor-bearing mice: It was of interest to determine whether the injection of cysteine, one of the radioprotective agents[22], might exert its action upon the irradiation effects described above. Mice were given a single dose of 10 mg of cysteine intraperitoneally 10 to 15 minutes prior to irradiation with 200 r. In tumor-bearing mice, it was demonstrated that no increase in liver catalase activity occurred at 48 hours in any instance for the groups irradiated after administration of cysteine, whereas the increase in activity was frequently observed at 48 hours for the groups irradiated without cysteine. The values were: untreated tumor-bearing, 15.8±0.4 (100%); irradiated tumor-bearing, 18.0±1.1 (114%); cysteine administered tumor-bearing, 16.3±1.0 (103%); cysteine administered, irradiated tumor-bearing, 14.9±1.0 (94%). These results were presented in Table 11. In addition, minor influence of cysteine was detectable with regard to the early decrease in liver catalase activity after irradiation. Furthermore, it seemed that the cell growth has not been modified

TABLE 10. Effect of Cysteine and/or Irradiation on Liver Catalase Activity in Normal Mice

Treatment	untreated	cysteine	cysteine and X-rays
Liver catalase activity (mean ± S. E.)	25.7 ± 0.8 (100)	25.4 ± 0.6 (99)	26.0 ± 1.8 (101)

Estimations of liver catalase activity were carried out 48 hours after irradiation with 200 r.
Data are given in percentages (in parentheses), and the values for the normal untreated mice are taken as 100%.

TABLE 11. Effect of Cysteine on the Radiation-Induced Liver Catalase Change in Tumor-Bearing Mice.

Mice	normal	tumor-bearing			
Treatment	untreated	untreated	X-rays	cysteine	crsteine and X-rays
Liver catalase activity Kat. F. (mean ± S.E.)	25.0 ± 0.9 (100)	15.8 ± 0.4 63 (100)	18.0 ± 1.1 72 114	16.3 ± 1.0 65 103	14.9 ± 1.0 60 94

Estimations of liver catalase activity were carried out 48 hours after irradiation with 200 r.

Values (in parentheses) of untreated normal mice, as well as those of untreated tumor-bearing mice, are taken as 100% and data for both are calcurated in percentages.

by cysteine. In normal mice, on the other hand, the injection of cysteine and/or irradiation had no effect on liver catalase activity (Tables 4 and 10).

DISCUSSION

As described in the introduction, the decrease of liver catalase in tumor-bearing hosts has been well established. However, the biological significance of the liver catalase depression itself in tumor-bearing is not clarified, although, catalase, as is well known, destroys the hydrogen peroxide produced by an oxidation by xanthine oxidase or uricase of appropriate substrates. It is, accordingly, considered to be valuable to discuss in some detail on the relation between liver catalase depression and other enzymatic or metabolic changes in tumor-bearing host.

Catalase is one of the hemoproteins, which contains protohemins as prosthetic group of its own molecule. This fact might indicate some possible relation of the cancer toxin, a liver catalase depressing factor, to the metabolism of such hemoproteins as hemoglobin, cytochromes, tryptophan pyrrolase and myoglobin. In fact, decrease of hemoglobin, decrease of cytochrome C and decrease of tryptophan pyrrolase were also observed in tumor-bearing host[23) 24)]. Accordingly, the biosynthesis of hemins which were the common moieties of these enzymes was investigated. In tumor-bearing host, an increased liver porphyrin was reported, indicating the impairment in iron utilization for heme biosynthesis[25)]. In contrast, a decreased δ-aminolevulinic acid dehydrase activity was reported which might, in part, result in a decrease of several hemoproteins[26)]. Moreover, it was demonstrated that iron-protoporphyrin chelating enzyme was not affected by toxormone[27)]. Thus there was some controversy among the observations reported.

With regard to the tryptophan pyrrolase described above, the activity tended to increase the level in the late phase of tumor-bearing[24)]. Considering this fact, the characteristic change of liver catalase activity in tumor-bearing

host was its *progressive* decrease with the growth of tumor or with the irreversible decrease of host weight[9]. Such liver catalase depression must also be considered from the viewpoint of the metabolism of protein moiety of the enzyme. Kawachi studied on the decrease of tryptophan pyrrolase activity in tumor-bearing rats and found the decrement of protohemin IX, and that such decrease of tryptophan pyrrolase activity was not restored to the normal level solely by administration of protohemin IX. From these facts he concluded that the liver tryptophan pyrrolase depression of the tumor-bearing rats was due not only to the decrease of protohemin IX but also to the decrement of apoenzyme (*i.e.*, due to some metabolic impairment of the protein moiety of the enzyme)[24]. It was considered that this might be also the case with heme-containing catalase. Thus the study on the liver catalase has been in good connection with the protein metabolism of tumor-bearing.

On the other hand, a dislocation of nitrogenous substance from normal to neoplastic tissue was demonstrated[28]. Mider[29] illustrated the relationship of nitrogen balance between tumor and tumor-bearing host; "the contribution of ingested nitrogen to the metabolic pool may be adequate to provide protein building blocks for this neoplasm when it comprises up to 10 per cent of the total body weight (rat plus tumor), but larger lymphomas contain more nitrogen than could have been derived from dietary sources". This fact indicated that in the late stage of tumor-bearing, the tumor grows and stores nitrogen at the expense of the host. Thus a negative nitrogen balance was observed in the host, while the tumor persisted in a positive nitrogen balance. This fact was referred to as a "nitrogen trap" by the tumor[29]. An "internal protein deficiency" was the another designation which expressed such metabolic abnormality of tumor-bearing[30]. This concept of the tumor as a nitrogen trap was later confirmed by Lepage *et al.*[28] who investigated the protein turnover of rats bearing Flexner-Jobling carcinoma after injection with C^{14}-glycine. It was demonstrated that the total radioactivity of the liver and kidney proteins in both fasted and fed groups decreased with the time, while the total radioactivity of the tumor continued to increase, *i.e.*, in other words, the nitrogen metabolism of the tumor was essentially a one way passage from normal to neoplastic tissue. An actual use of the tissue proteins by the tumor of the host was mediated by the depletion of the plasma protein, a main source of which was, *via* increased metabolism of the liver, ultimately the muscle system[31].

There is, however, some difficulty in the understanding of the deranged metabolism of the tumor-bearing host as a result of such depletion by the tumor of the host nitrogen alone. Ishikawa and Suda demonstrated, in their experiaments with plasmapheretic rats, the difference in metabolic patterns of proteins, as compared with those of the tumor-bearing rats. An increased synthesis of plasma protein was observed in both plasmapheretic and tumor-

bearing groups, whereas the synthesis of muscle protein was decreased only in the tumor-bearing groups. In contrast, the muscle protein synthesis of the plasmapheretic groups maintained the normal level[30]. These observations might most likely be interpreted that in the tumor-bearing rats, the plasma protein was biosynthesized at such a high rate at the expense of the muscle protein synthesis, and served the building blocks for the tumor. It was also suggested that the tumor might exert its action on the host not only as a nitrogen trap but also through some mechanism which adjust the metabolic pattern of the host in favor of the tumor. They furthermore stated that the administration of non-protein diets to rats could produce no more the same metabolic pattern as that of the tumor-bearing rats[30]. For the protein-free diet fed groups, the synthesis of plasma protein was depressed as well as the synthesis of muscle system in contrast with the increased plasma protein synthesis of the tumor-bearing rats as described above. Moreover, Steward *et al.* demonstrated that the force-feeding to rats bearing the Walker 256 carcinoma did not prevent the loss of carcass weight[32]. These facts might indicate that the metabolic changes underlying in the tumor-bearing host was not due solely to the deficiency of food intake. It was also reported an characteristic feature of the metabolism of carbohydrates in tumor-bearing rats. Tanaka and Suda investigated the liver pyruvic kinase in tumor-bearing rats and reported an increased activity of pyruvic kinase which was due to the increment of the pyruvic kinase of muscle type[37]. The muscle-typed pyruvic kinase was resistant to both hormonal and dietary influences in contrast to the liver-typed which was influenced by those factors with great ease. In other words an poorly inducible pyruvic kinase was increased in the tumor-bearing rats. Such result was also obtained in the non-tumor-bearing parabiotic rats with one partner tumor-bearing. From these observations, it was possibly proposed that the tumor might exert its action on the host actively by some humorally transmissible means. In this respect, the concept of a cancer toxin, "toxohormone", might again be taken into consideration.

Kampfsmidt extracted the toxohormone fraction after the procedure of Nakagawa *et al.* and reported that by the injection of the toxohormone into the normal recipient mice, reproduction of the systemic changes noted in the tumor-bearing host was attained; not only the decreased liver catalase activity but also the serum iron depression, tryptophan pyrrolase depression and the atrophy of the thymus were reproduced[33]. Shiba *et al.* demonstrated in their experiments with an isolated perfused liver the decreased hydrocortisone-inducible formation of tryptophan pyrrolase when the toxohormone was added to the perfusion medium[34]. This fact also showed the reproduction by the toxohormone of the tumor-bearing state in which induction of tryptophan pyrrolase was reduced.

With regard to the preparation and purification of the cancer toxin (*i.e.*,

toxohormone), an improved method was reported by Nakagawa, Ono and Sugimura, Fujii and Yamamura, and Yunoki and Griffin, respectively[13~15]. The most potent preparation ever reported was the TH2 fraction of Yunoki and Griffin, which was obtained by further purification of the crude toxohormone fractions by an Amberlite XE-64 column chromatography[15) 35)]. Its effective dose, by weight, as an liver catalase depressing factor was estimated to be 1 to 5 μg; molecular weight, 4,200 to 6,400; chemical composition, a polypeptide and a phospholipide. Moreover it was reported that acid or enzymatic hydrolysis almost completely destroyed the liver catalase depressing activity[36)].

In the present experiments, we adopted the method of Yunoki and Griffin for the preparation of the crude toxohormone fractions, and Co^{60}-irradiation effects on the toxin in cancerous patients were investigated. The yield of the crude toxohormone fraction from non-irradiated human gastric cancer was almost as good as that reported by Yunoki and Griffin who obtained 5.52 g of the fraction from 170 g of the acetone-dried powder of lung cancer. The crude toxohormone fractions prepared from preoperatively Co^{60}-irradiated human gastric cancer possessed the less effective liver catalase depressing activity, as compared with those from non-irradiated human gastric cancer. Such a decrease in the effectiveness was resulted from the decreased amount of the active (or effective) fraction, TH2. These findings were compatible with the histological observations of the irradiated cancer, which presented the fibrosis of the tissue and degenerative changes of cancer cells.[37)]

Now we come to a different, although related, aspect of the same problem. One might consider that the studies on the relationship between tumor growth and liver catalase depression might give some interesting information on the metabolism of the tumor. In 1954, Lucké *et al.* investigated the quantitative relation between the growth of tumor and the reduction of liver catalase activity and concluded that during the exponential phase of the tumor growth, the toxin was elaborated and released at the steady rate by the tumor cells.[8)] The present experiments also treated this problem, using mice bearing a diploid Ehrlich ascites tumor, after some modification of the tumor growth by X-irradiation. Measuring the amount of the PNA and total nitrogen per cell of a Ehrlich ascites tumor after *in vivo* irradiation with 1,250 r, Klein *et al.*[20)] concluded that despite the absence of cell division, the irradiated tumor cells continued to synthesize new cell substance at about the same rate as before irradiation, resulting in an increase of individual cell volume. Further, they reported that there was no change neither in the percentage of tumor cells nor in the viability of tumor cells. The findings obtained in the present experiments were compatible with their observation. It was evident from the results presented in Tables 8 and 9, that the early decrease in liver catalase activity of tumor-bearing mice was due to an enhanced release of the cancer toxin from the irradiated tumor cells, but not due to an increased

susceptibility of the recipient mice to the toxin by irradiation. The fact that the catalase activity in irradiated tumor-bearing mice increased 48 hours after irradiation might raise a number of interesting considerations. Among the many possibilities responsible for it, it would seem most likely that the increased activity was due to a decreased amount of the cancer toxin at that time of postirradiation. There might be an actual decrease in the new cancer toxin (*i.e.*, decreased synthesis *de novo* of the toxin), or such decreased amount might be due to an enhanced release of the toxin which was previously induced by irradiation. Although possible radiochemical transformations of the toxin might be responsible for it, these were not likely from the facts that there, 10 hours after irradiation, was a more decrease in liver catalase activity, accordingly, an increased amount of the cancer toxin accessible to the liver, and that there was a 48-hour lag phase between irradiation and increment of liver catalase activity. Such lag phase reasonably implied that this irradiation effect on the liver catalase was mediated by impairments of enzyme system related to the production of cancer toxin by the tumor cells. Moreover, the possibility of the decreased *de novo* synthesis of the toxin appeared to be justified from the experiments which showed that the increase in the activity at 48 hours was diminished by the concomitant administration of radioprotective agent, cysteine,[22] devoid of minor influences of cysteine with regard to the early decrease in liver catalase activity. Therefore, it seemed probable that some radiosensitive enzymes such as mercaptoenzymes might be possibly correlated with the synthesis of the toxin by the tumor cells.

Greenstein previously emphasized that the studies on the possible relation of this enhanced toxin producing function of the tumor to the growth property of the tumor might provide an important information for the further understanding of the abnormal metabolism of the tumor.[38] Thus it would be of great interest and importance to determine whether comparable changes in liver catalase activity would also be found in other conditions of tumor growth.

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