Experimental Studies on Continuous Intra-Arterial Infusion of Anti-Tumor Agents (with Special Emphasis on Histochemical Changes)

by

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Received for Pubulication Jun 8. 1966

INTRODUCTION

To improve the surgical rate of patients with malignant disease very often surgery, radiation and chemotherapy are used in combination. To enhance the action of cancer chemotherapeutic agents and to prevent or control the systemic toxicity by appropriate antidote intra-arterial infusion is utilized. Most of the agents used for this form of therapy are 5-fluorouracil, 5-fluoro-2'-deoxy uridine, Methotrexate.

The intra-arterial infusion was introduced by Klopp[38] and improved by Brennan[39], Westburg[40], Duff[41], Trussel[42], Sullivan[43] and many others. In this country, since Shiraha[44] first used intra-arterial infusions of Nitromine and Mitomycin C, many investigators[45)-53)] have proved both experimentally and clinically that the administration of anti-tumor agents by protracted continuous infusion into an artery supplying the localized malignant tumor has a better antitumor effect than could be expected from the systemic administration of the same anti-tumor agent.

In his histochemical studies, Campbell found high β-glucuronidase activity in animal tumors. Takamatsu et al. noted increased phosphatase activity in tumor tissue and Pearson[24] reported high alkaline phosphatase activity in the livers of rats fed DAB. Since then the study of various enzymes in tumor tissue has progressed rapidly.

This paper describes the author's studies of different enzyme levels in normal tissue and the changes induced by the continuous intra-arterial infusions of various anti-tumor agents. The author investigated the therapeutic effect of the continuous intra-arterial infusion of various anti-tumor agents in rabbits with Brown-Pearce (B. P) carcinoma[34] transplanted into their gastric submucosa. An attempt was made to correlate the anti-tumor effects and the changes in various enzyme activities in the tumor tissue as well as the normal tissue.

I. EFFECT of CONTINUOUS INTRA-ARTERIAL INFUSION of ANTI-TUMOR AGENTS (ATA) on the NORMAL TISSUES in EXPERIMENTAL ANIMALS

1) EXPERIMENTAL MATERIALS and ANIMALS

A) Agents: Nitrogen Mustard N-Oxide (HN_2), Endoxan (EX), Chromomycin

A_3 (CrA_3) and Mitomycin C (MMC) were used.

B) Dose schedules: The maximum infusion dose was 1.8 mg/kg/day for HN_2, 4 mg/kg/day for EX, 15 γ/kg/day for CrA_3 and 0.4 mg/kg/day for MMC.

C) Animals: 15 mature rabbits (body weight 2.5～3.5 kg) were divided into 5 groups regardless of sex.

2) EXPERIMENTAL METHODS

Rabbits were laparotomized under intravenous anesthesia with 40 mg/kg of pentobarbital sodium, a polyethylene (PE) catheter (inside diameter of 0.5 mm) was inserted and fixed in the left gastroepiploic artery, and a continuous infusion 5% glucose solution was started. Heparin was not used because of its effect on tissue enzymes.

The above doses of HN_2, EX, CrA_3 and MMC were given by dropwise infusion continuously for 7 days starting one day after operation. The animals were sacrificed by i.v. air injection on the 9th day, and speciments of stomach, liver, kidneys and small intestine were frozen immediately in dryiced-acetone and cut in sections about 10 μ thick in a cryostat at −20°C. Under the same condition, tissue sections were stained with succinic dehydrogenase (SDH), leucine aminopeptidase (LAP), β-glucuronidase (β-GL) and acid & alkaline phosphatase (Ac- & Al-Ph).

Staining was done by the post azo coupling dye method; SDH was demonstrated by the method of Nachlas et al. (1957). Speciments were incubated for 20 minutes with nitro BT as the substrate; LAP was demonstrated by Seligman's method (1957), and the tissue was incubated for 2 hours with L-leucyl-β-naphthylamide as the substrate and Fast blue B as the azo coupler; β-GL was demonstrated by the method of Seligman et al., with the incubation period of 6 hours with 6-bromo-2-naphthyl-β-D-glucopyruronoside as the substrate and Fast blue B as the azo coupler; Ac-Ph was demonstrated by Burstone's method, with the incubation period 6 hours with naphthol AS-BI phosphate as the substrate and Red violet LB salt as the azo coupler; and Al-Ph was demonstrated by Pearse's method, with incubation period of 2 hours with sodium α-naphthyl phosphate as the substrate and Fast black B as the azo coupler.

The enzymatic activity was estimated by the inspection of 2～3 sections from each tissue and the sections were stained under uniform conditions. The results are summarized on tables.

3) RESULTS

Macroscopic findings: All anti-tumor agents caused ulceration ranging from 0.5～1.0 cm in diameter and 0.3 cm depth with intense edema of the surrounding mucosa. No perforations were observed. The control animals which were infused with physiological saline solution did not have any ulceration.

Histochemical findings: SDH activity was low or absent in the infused area with all of the ATAs. Serial sections away from this area showed gradually increasing SDH levels and at the distance of appropriately 1.0 cm SDH levels were normal. SDH activity in the liver was higher than in the controls after infusion with HN_2 and CrA_3; it remained unchanged with MMC and was lower with EX; in the kidneys and small intestine it

remained normal.

LAP activity in the gastric mucosa was absent or low in the infused area and as well as in the remaining portions of the stomach except after EX infusion. In this case there was definite activity outside of the ulceration; normal LAP activity was seen in the liver, kidneys and small intestine with all agents (Table 1).

β-GL activity was lowered generally by all the ATAs and disappeared entirely in the infused area, except after MMC infusion; it was lower than normal in the liver, kidneys and small intestine.

Ac-Ph activity, on the other hand, was higher than normal in the infused area by all of the ATAs and was normal in the not infused regions. In the liver, Ac-Ph activity was absent in the hepatic cells of both infused and control animals, and was only 1+ in the adjacent part to the blood vessels; it was normal in the kidneys and small intestine, except that it was lowered with MMC infusion (Table 2).

Al-Ph activity was also high in the infused areas with any of the ATAs and was higher in the regions further from the infused area; it was normal in the liver, kidneys and small intestine (Table 3).

Table 1. Succinic dehydrogenase and Leucine aminopeptidase activity in the normal tissues after ATA infusions

		SDH activity					LAP activity				
		Control	HN_2	EX	CrA_3	MMC	Control	HN_2	EX	CrA_3	MMC
Stomach	Gastric mucosa	2+	2+	1+	2+	1+	2+	−	2+	2+	2+
	Chief cells	2+	2+	1+	2+	1+	2+	1+	3+	2+	1+
	Parietal cells	2+	2+	1+	2+	1+	2+	1+	3+	2+	1+
	Infused part	2+	−~1+	−	−~1+	−	2+	1+	−	−~1+	−~1+
Liver	Hepatic cells	2+	3+	1+	3+	2+	−~1+	−~1+	1+	−~1+	−~1+
Kidney	Cortex	2+	3+	2+	2+	2+	2+	2+	2+	2+	2+
	Malpighian corpuscle	−	−	−	−	−	1+	1+	1+	1+	1+
	Proximal convulsion	2+	3+	2+	2+	2+	2+	2+	2+	2+	2+
	Distal convulsion	2+	3+	2+	2+	2+	2+	2+	2+	2+	2+
	Collecting tube	2+	3+	2+	2+	2+	1+	1+	1+	1+	1+
	Outer zone of medulla	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
	Inner zone of medulla	−	−	−	−	−	−	−	−	−	−
	Papilla	−	−	−	−	−	−	−	−	−	−
Small intestine	Epithelium villi	2+	1+	1+	2+	1+	2+	2+	2+	2+	2+
	Lieberkuhn's gland	1+	1+	−	1+	−	1+	1+	1+	1+	1+
	Tunica propria	−	−	−	−	−	1+	1+	1+	1+	1+
	Tunica muscularis	−	−	−	−	−	1+	−	−	−	−

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

Table 2. β-Glucuronidase and Acid phosphatase activity in the normal tissues after ATA infusions

		β-GL activity					Ac-Ph activity				
		Control	HN_2	EX	CrA_3	MMC	Control	HN_2	EX	CrA_3	MMC
Stomach	Gastric mucosa	2+	1+	1+	1+	1+	1+	2+	3+	2+	2+
	Chief cells	2+	1+	1+	1+	1+	2+	3+	3+	2+	2+
	Parietal cells	2+	1+	1+	1+	1+	2+	2+	3+	2+	2+
	Infused part	2+	−	−	−	1+	2+	3+	3+	3+	3+
Liver	Hepatic cells	2+	1+	1+	1+	1+	−	−	−	−	−
Kidney	Cortex	2+	2+	1+	1+	1+	2+	2+	2+	2+	1+
	Malpighian corpuscle	1+	1+	1+	1+	1+	−	−	−	−	−
	Proximal convulsion	2+	1+	1+	1+	1+	2+	2+	2+	2+	1+
	Distal convulsion	2+	1+	1+	1+	1+	2+	2+	2+	2+	1+
	Collecting tube	1+	1+	1+	1+	1+	2+	2+	2+	2+	1+
	Outer zone of medulla	1+	1+	1+	1+	1+	−	−	−	−	−
	Inner zone of medulla	−	−	−	−	−	−	−	−	−	−
	Papilla	−	−	−	−	−	−	−	−	−	−
Small intestine	Epithelium villi	2+	1+	1+	1+	1+	2+	2+	3+	2+	1+
	Lieberkuhn's gland	2+	1+	1+	−	1+	1+	2+	3+	2+	−
	Tunica propria	2+	−	1+	−	−	−	−	−	−	−
	Tunica muscularis	2+	−	1+	−	−	−	−	−	−	−

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

Table 3. Alkaline phosphatase activity in the normal tissues after ATA infusions

		Al-Ph activity				
		Control	HN_2	EX	CrA_3	MMC
Stomach	Gastric mucosa	1+	3+	3+	3+	3+
	Chief cells	2+	3+	3+	3+	3+
	Parietal cells	2+	3+	3+	3+	3+
	Infused part	2+	3+	2+	3+	2+
Liver	Hepatic cells	2+	2+	2+	3+	2+
Kidney	Cortex	2+	2+	2+	2+	2+
	Malpighian corpuscle	1+	1+	1+	1+	1+
	Proximal convulsion	1+	1+	1+	1+	1+
	Distal convulsion	1+	1+	1+	1+	1+
	Collecting tube	1+	1+	1+	1+	1+
	Outer zone of medulla	1+	1+	1+	1+	1+
	Inner zone of medulla	−	−	−	−	−
	Papilla	−	−	−	−	−
Small intestine	Epithelium villi	2+	2+	2+	2+	2+
	Lieberkuhn's gland	1+	1+	1+	1+	1+
	Tunica propria	1+	1+	1+	1+	1+
	Tunica muscularis	1+	1+	1+	1+	1+

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

II. CONTINUOUS INTRA-ARTERIAL INFUSION of VARIOUS ANTI-TUMOR AGENTS in ANIMALS with TRANSPLANTED BROWN-PEARCE CANCER

1) EXPERIMENTAL MATERIALS and ANIMALS

A) Agents: In additions to the above listed agents Methotrexate (Mtx) in doses of 2 mg/kg/day was used.

B) Animals: 18 mature rabbits (body weight 2.0～2.5 kg) were divided into 6 groups regardless of sex.

Prior to B. P cancer transplantation, arteriograms[37] were made and the stomach was outlined by barium in order to determine how much of the stomach was supplied by the artery into which the PE catheter was inserted (Fig. 1).

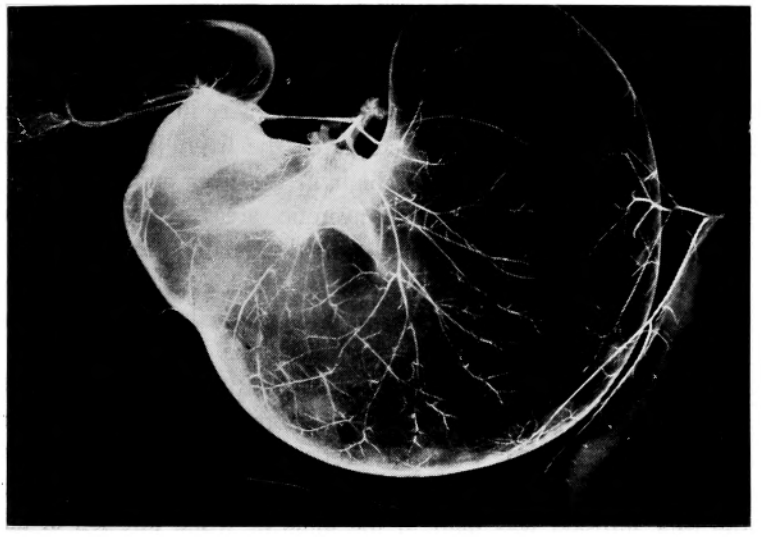

Fig. 1 Arteriogram of stomach. Capillary artery is seen.

2) EXPERIMENTAL METHODS

Homotransplanted B. P cancer was removed after about 3 weeks. The necrotic part was discarded and the proliferating cancer was ground. An emulsion[35)36)] was made with one gram of cancer tissue in one ml of physiological saline solution, and one ml of this emulsion was mixed with 100～200 U of Penicillin and 10～20 mg of Streptomycin. About one ml of this mixture was injected into the gastric submucosa of the rabbit and the transplant was allowed to grow for about 3 weeks. At that time the abdomen was opened again and the growth of the B. P cancer was examined. A PE catheter was inserted about 1 cm in the left gastroepiploic artery and fixed. Starting on the day of the catheter insertion the infusion was carried out in each animal continuously for 8 days. On the 10th day, the animals were sacrificed by i.v. air injection, and SDH, LAP, β-GL and Ac-Ph & Al-Ph activity in the transplanted gastric cancer, liver, kidneys and small intestine was determined.

3) RESULTS

All animals infused with ATAs were in better general condition than the non-treated control group. There was little or no decrease in appetite, less weight loss and longer survival.

Macroscopic findings: The size of the tumors remained essentially unchanged in animals infused with EX, CrA_3, or MMC. The central part of every tumor was necrotic, and only occasional lymphnode metastases were observed. Liver metastases were found in one animal infused with EX or CrA_3. The tumors in all animals infused with Mtx were almost twice as big and there were lymphnode and liver metastases. After HN_2 infusion the tumors became very small and the surrounding tissue showed cicatrization; no metastases were found. The majority of tumors in all groups became necrotic, but no gastric perforations occurred.

Histochemical findings: SDH activity in the control animals (not infused with ATA) was 1+～2+ in the tumor cells, (−) in the stroma and necrotic areas, 2+～3+ in normal areas, 3+ in the liver and small intestine and 2+ in the kidneys.

In animals infused with any of the ATAs SDH activity in the tumor cells was reduced to (±)～1+ with coresponding lower activity in the liver, kidneys and small intestine except that after Mtx infusion it remained high in the liver and kidneys.

LAP activity in both tumor cells and necrotic areas of control animals was (−); and 1+ in the stroma; it was a little higher in the small intestine but low in the liver and kidneys. LAP activity slightly decreased by all ATAs with the exception of HN_2 where it remained unchanged. The MMC infusion caused higher levels in the kidneys and the Mtx infusion higher levels in the small intestine (Table 4).

β-GL activity in the control animals was 2+～3+ in the tumor cells, 2+ in the stroma, 1+ in the necrotic areas and 3+ in the normal areas; in the liver, kidneys and small intestine it was 3+; i.e. it was high every where except in the stroma and in the necrotic areas. β-GL activity with HN_2 infusion remained unchanged in all tissues, and its activity after Mtx infusion was high in the kidneys, while infusion of all other ATAs lowered β-GL activity in tumor tissue, liver, kidneys and small intestine.

Ac-Ph activity in the control animals was (−)～1+ in the tumor cells, (−) in the

Tabie 4. Succinic dehydrogenase and Leucine aminopeptidase activity in the transplanted tumor tissues and other tissues after ATA infusions

		SDH activity						LAP activity					
		Control	HN_2	EX	CrA_3	MMC	Mtx	Control	HN_2	EX	CrA_3	MMC	Mtx
Stomach	Gastric mucosa	2+～3+	2+	1+	−	1+	−	2+	2+	1+	2+	2+	2+
	Chief cells	2+～3+	2+	1+	2+	2+	1+	2+	2+	1+	1+	1+	1+
	Parietal cells	2+～3+	2+	1+	2+	2+	2+	2+	2+	1+	1+	1+	−
	Tumor cells	1+～2+	1+	1+	±	±	1+	−	−～1+	−	−	−	−
	stroma	−	−	−	−	−	−	1+	−	−	−	−	−
	necrotic part	−	−	−	−	−	−	−	−	−	−	−	−
Liver	Hepatic cells	3+	1+	1+	1+	2+	3+	−～1+	−	−	−～1+	1+	1+

Kidney	Cortex	2+	1+	1+	1+	2+	3+	2+	−	1+	2+	3+	2+
	Malpighian corpuscle	−	−	−	−	−	−	1+	−	1+	1+	2+	1+
	Proximal convulsion	2+	1+	1+	1+	2+	3+	2+	1+	1+	2+	3+	2+
	Distal convulsion	2+	1+	1+	1+	2+	3+	2+	1+	1+	2+	3+	2+
	Collecting tube	2+	1+	1+	1+	2+	2+	1+	1+	1+	1+	2+	1+
	Outer zone of medulla	2+	1+	1+	1+	1+	1+	1+	−	−	−	1+	1+
	Inner zone of medulla	−	−	−	−	−	−	−	−	−	−	−	−
	Papilla	−	−	−	−	−	−	−	−	−	−	−	−
Small intestine	Epithelium villi	3+	1+	1+	2+	1+	2+	3+	2+	1+	1+	1+	3+
	Lieberkuhn's gland	3+	1+	1+	1+	−	1+	2+	1+	1+	1+	−	2+
	Tunica propria	−	−	−	−	−	−	2+	1+	1+	1+	−	1+
	Tunica muscularis	2+	−	−	−	−	1+	2+	−	−	−	−	2+

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

Table 5. β-Glucuronidase and Acid phosphatase activity in the transplanted tumor tissues and other tissues after ATA infusions

		β-GL activity						Ac-Ph activity					
		Control	HN_2	EX	CrA_3	MMC	Mtx	Control	HN_2	EX	CrA_3	MMC	Mtx
Stomach	Gastric mucosa	3+	2+	1+	1+	2+	1+	−	2+	−	1+	−	−
	Chief cells	3+	2+	1+	1+	2+	1+	−	−	−	−	−	−
	Parietal cells	3+	2+	1+	1+	2+	1+	−	1+	−	1+	2+	−
	Tumor cells	2+～3+	1+	1+	1+～2+	1+	1+	−～1+	1+	−	1+	2+	−
	stroma	2+	1+	1+	1+	1+	1+	−	−	−	1+	1+	−
	necrotic part	1+	−	−	1+	−	−	−	1+	1+	1+	1+	−
Liver	Hepatic cells	3+	2+	1+	1+	1+～2+	2+	−	−	−	1+	−	−
Kidney	Cortex	3+	2+	2+	1+	2+	3+	2+～3+	1+	1+	2+	2+～3+	1+
	Malpighian corpuscle	2+	1+	1+	1+	1+	2+	−	−	−	−	−	−
	Proximal convulsion	3+	2+	2+	1+	2+	3+	2+	1+	−	2+	2+	−
	Distal convulsion	3+	2+	1+	1+	1+	3+	2+	1+	−	1+	2+	−
	Collecting tube	2+	1+	1+	1+	1+	2+	2+	1+	−	1+	2+	−
	Outer zone of medulla	1+	1+	1+	1+	1+	1+	−	−	−	−	−	−
	Inner zone of medulla	1+	−	−	−	−	1+	−	−	−	−	−	−
	Papilla	−	−	−	−	−	−	−	−	−	−	−	−
Small intestine	Epithelium villi	3+	2+	1+	1+	−～1+	1+	3+	2+	−	−	1+	−
	Lieberkuhn's gland	3+	2+	1+	1+	−～1+	1+	3+	2+	1+	2+	2+	−
	Tunica propria	2+	2+	−	−	−	−～1+	−	−	−	−	−	−
	Tunica muscularis	3+	2+	−	−	−	−	−	−	−	−	−	−

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

necrotic areas and stroma, and slightly elevated in the small intestine. After MMC infusion it was 2+ in the tumor cells and 1+ in the necrotic areas and stroma; after CrA_3 infusion it was 1+ in the tumor cells, stroma and necrotic areas. CrA_3 infusion lowered Ac-Ph activity in the kidneys and small intestine (Table 5).

Al-Ph activity in the control animals was 1+ in the tumor cells, 2+ in the stroma and necrotic areas, 1+ in the normal areas, and 3+ in the kidneys and small intestine. After ATAs infuson it was 1+～2+ in the tumor cells and stroma the same as in the control animals, but it was high in the necrotic areas, except after CrA_3 infusion. Only after MMC or Mtx infusion was it as high as 3+ in the kidneys (Table 6).

Table 6. Alkaline phosphatase activity in the transplanted tumor tissues and other tissues after ATA infusions

		Al-Ph activity					
		Control	HN_2	EX	CrA_3	MMC	Mtx
Stomach	Gastric mucosa	1+	2+	1+	1+	3+	3+
	Chief cells	1+	2+	2+	2+	1+	1+
	Parietal cells	1+	3+	3+	2+	3+	3+
	Tumor cells	1+	2+	2+	1+	1+	1+
	stroma	2+	1+～2+	1+～2+	1+	1+～2+	1+～2+
	necrotic part	2+	3+	3+	1+	3+	3+
Liver	Hepatic cells	2+	2+	2+	1+	3+	2+
Kidney	Cortex	3+	2+	2+	2+	3+	3+
	Malpighian corpuscle	1+	1+	1+	1+	1+	1+
	Proximal convulsion	3+	2+	2+	2+	3+	3+
	Distal convulsion	3+	2+	2+	2+	3+	3+
	Collecting tube	3+	2+	3+	3+	3+	3+
	Outer zone of medulla	2+	2+	2+	2+	2+	3+
	Inner zone of medulla	−	−	−	−	−	−
	Papilla	−	−	−	−	−	−
Small intestine	Epithelium villi	3+	2+	−	2+	1+	2+
	Lieberkuhn's gland	3+	2+	2+	2+	2+	1+
	Tunica propria	2+	−	−	1+	−	−
	Tunica muscularis	−	−	−	−	−	−

Symbols indicate : 3+ highest activity, 2+ moderate activity, 1+ slight activity, − no activity, ± more slight activity.

Histological findings in H-E stained section : In the control animals the transplanted cancer showed vigorous proliferation, with penetration of the muscularis mucosae and infiltration into the mucosal surface; several mitotic figures were seen in the tumor cells (Fig. 2 & 3). After infusion of HN_2 only a few tumor cells could be seen and these showed degeneration and were surrounded by small round cells (Fig. 4 & 5). After EX infusion there was a marked decrease in number of the tumor cells and an increase of the stroma was observed (Fig. 6). CrA_3 infusion caused pycknotic changes, vacuole formation, disappearance of nucleoli and infiltration of small round cells (Fig. 7). After

MMC infusion there was marked vacuole formation in the tumor cells as well as a great increase of fibrous stroma (Fig. 8). After Mtx infusion, although the tumor appeared macroscopically larger and lymphnode metastases were present, a reduction of the tumor cells with corresponding increase in the stroma was observed microscopically (Fig. 9).

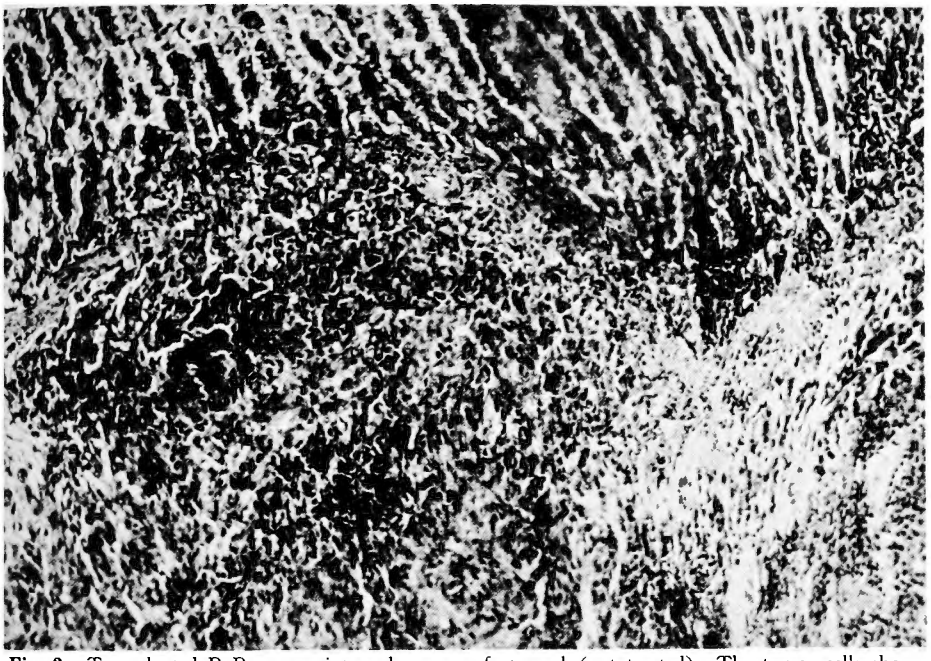

Fig. 2 Transplanted B. P. cancer into submucosa of stomach (not treated). The tumor cells show vigorous proliferation with penetration of the muscularis mucosae. H-E ×100

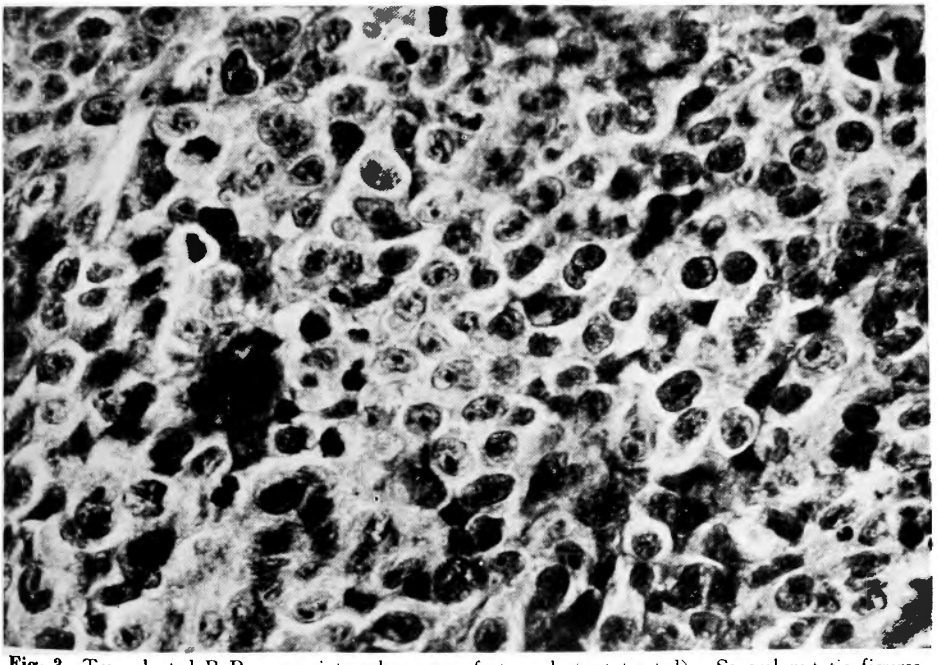

Fig. 3 Transplanted B. P. cancer into submucosa of stomach (not treated). Several mitotic figures are seen in the tumor cells. H-E ×400

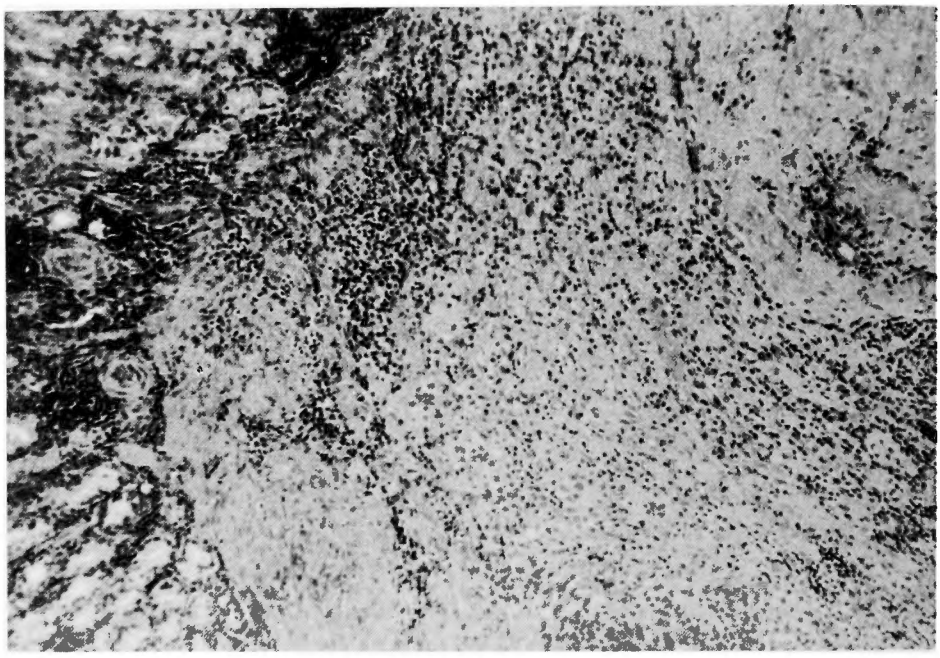

Fig. 4 Transplanted B. P. cancer into submucosa of stomach attacked with HN_2 infusion. Only a few tumor cells could be seen, small ·ound cells and fibrous stroma are increasing. H-E ×100

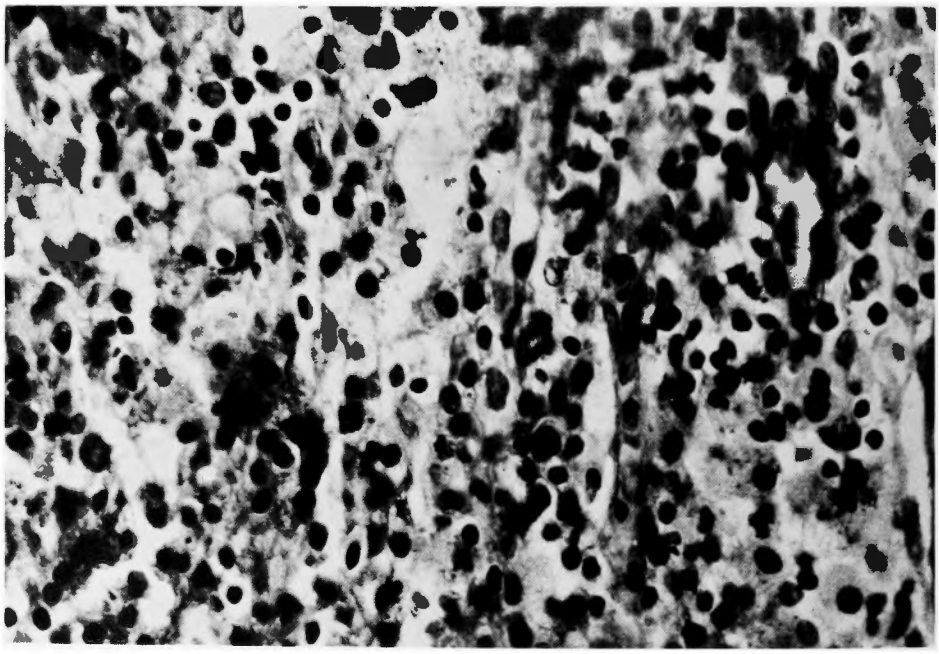

Fig. 5 Transplanted B. P. cancer into submucosa of stomach treated with HN_2 infusion. Tumor cells are degenerated. H-E ×400

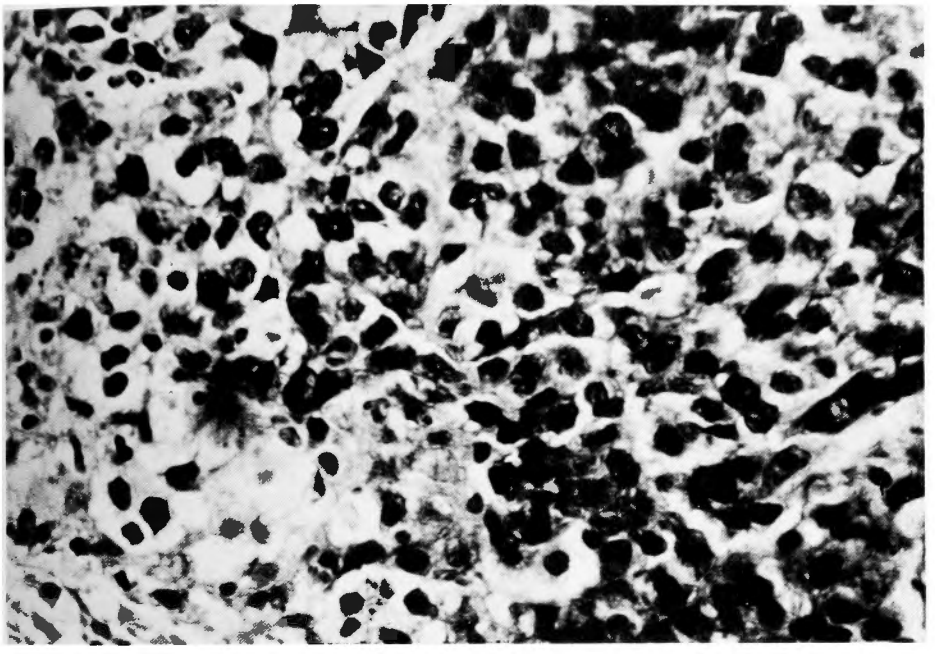

Fig. 6 Transplanted B. P. cancer into submucosa of stomach treated with EX infusion. Tumor cells are decreased and degenerated. H-E ×400

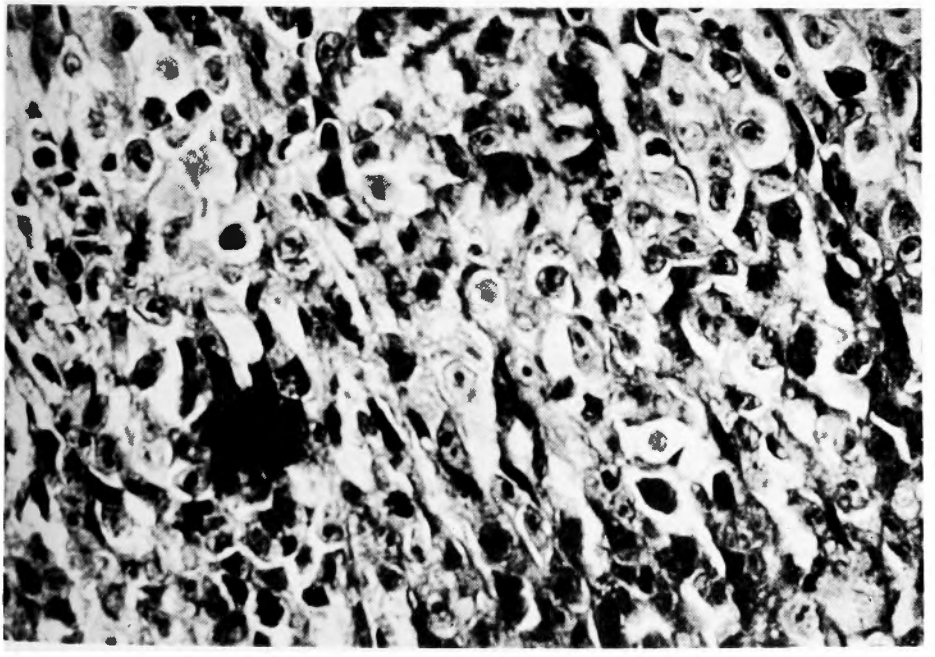

Fig. 7 Transplanted B. P. cancer into submucosa of stomach treated with CrA_3 infusion. Rycknotic changes and vacuole formation in the cytoplasm are seen. H-E ×400

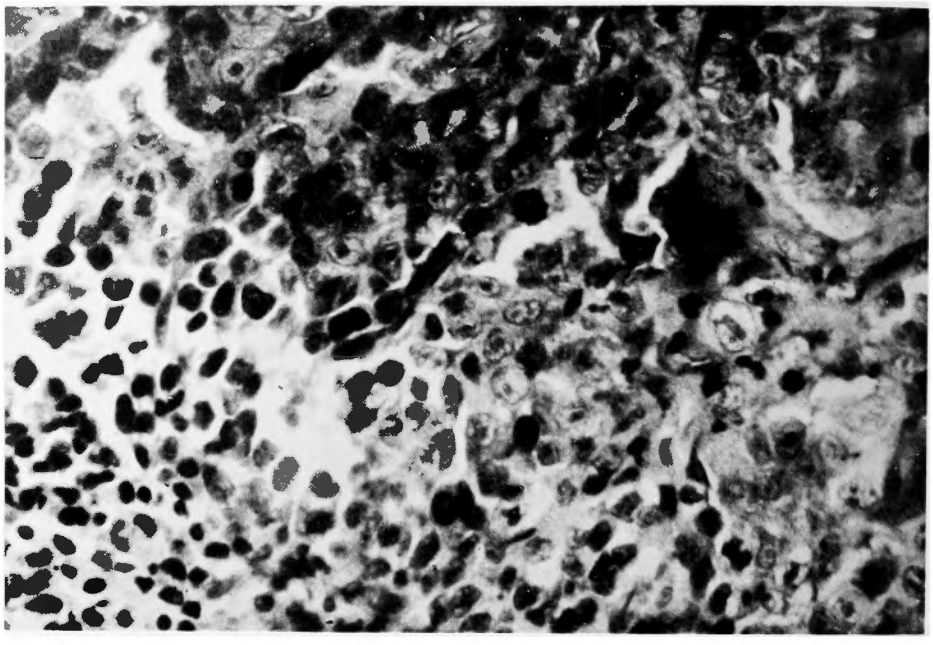

Fig. 8 Transplanted B. P. cancer into submucosa of stomach treated with MMC infusion. Infiltration of small round cells and increasing of the stroma are seen. The tumor cells are decreased and degenerated. H-E ×400

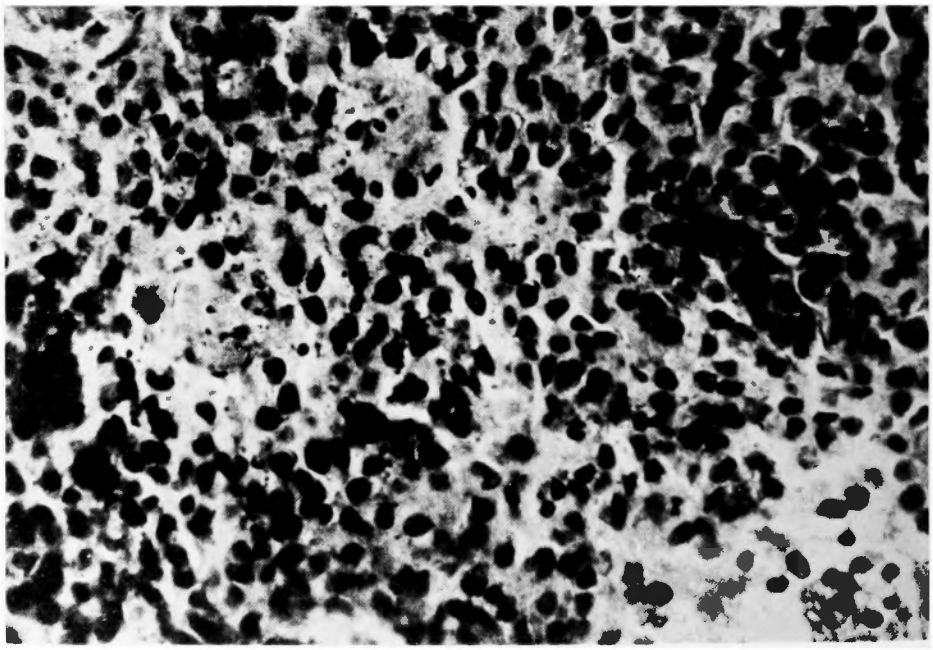

Fig. 9 Transplanted B. P. cancer into submucosa of stomach treated with Mtx infusion. Degenerated tumor cells are seen. H-E ×400

DISCUSSION

SDH is clearly related to mitochondria, which play a great role in metabolism[2)4)5)]. Kajiwara[32)] and Sato[33)] observed in Yoshida-sarcoma treated with CrA_3 that the electron density was lowered and the protoplasmic space of nuclear membrane increased. This electronmicroscopic findings showed that CrA_3 decreased SDH activity in mitochondria.

It has been reported that MMC has no influence on SDH activity in the hay bacillus[31)]. Thus, MMC probably does not inhibit SDH activity directly. It is inferred that HN_2 and Mtx also act on mitochondria and therefore lower SDH activity. Although EX is a "masked compound" and is activated only in the liver, it suppressed the SDH activity in the gastric epitheial cells only in the area infused. Of course, its lowering action of SDH activity in the liver was much higher than that of the other ATAs.

Wattenberg[6)] has stated that SDH activity is decreased in hyperplasia and increased in cancer of the large intestine[3)]. Rutenburg[1)] found no SDH activity in animal sarcoma 37, Bagg in lymphosarcoma, Walker carcinoma 256, or cancer of the large intestine, breast or ovary.

In this experiment, SDH activity was present in the tumor cells and the increased SDH activity in the liver and small intestine was lowered equally by all of the ATAs except Mtx. These findings indicate that SDH activity is probably inhibited not only in the tumor cells, but also in other organs. This phenomena do not always coincide with reduction of tumor size and necrosis of the tumor cells. It is of interest that in the case of HN_2 infusion local SDH activity remained almost at normal levels but the tumor size was reduced.

Aoki[20)] found that LAP activity was high in the proliferating areas of ascitic hepatic cancer, Yoshida's sarcoma and Quinon cancer and low in the degenerated necrotic areas; a similar tendency was noted in human hepatic cancer. Glenner[8)] observed LAP activity in the tumor cells of adenocarcinomas of the stomach and large intestine.

On the other hand, Burstone[7)] observed a high LAP activity in the stroma in adenocarcinoma of the liver and large intestine, and Monis[29)] reported a high activity in the stroma of gastric adenocarcinoma and no activity in the tumor cells. In the present experiment no LAP activity was noted in the transplanted tumor cells but a slight activity in the stroma and an increased activity in the mucosa of the small intestine was observed.

Levvy[9)] found high β-GL activity in juvenile cells and damaged tissue, and Campbell[10)] noted that it was low in Rous sarcoma and squamous cell carcinoma of the mouse, but increased in anaplastic areas of breast cancer in mice. Cohen[12)], Fishman[11)30)], Braun-Falco[13)], Monis[14)], Koike[16)17)], Kijima[19)] and Kawase[28)] observed a rising β-GL activity in the tumor tissues of tumor bearing animals and in human cancer. However, Millis and Smith found adecrease in β-GL activity in hepatic cancers of rats. In the present experiment, β-GL activity in the tumor cells was nearly as high as in normal gastric mucosa and higher in the liver, kidneys and small intestine; all ATAs lowered β-GL activity in the tumor cells. This findings suggests that β-GL activity, which is activated by DNA[18)], may be inhibited by the interference of DNA synthesis by HN_2, CrA_3 and MMC.

A high Ac-Ph activity was observed by Lemon et al.[21)] in adenocarcinoma of the

colon, but Greenstein[54] found decreased Ac-Ph activity in transplanted cancers of mice. Aoki et al. and Reiner[22] stated that Ac-Ph activity rises as the tumor cells degenerate and become necrotic. In the present experiment, no change in Ac-Ph activity was noted in the tumor tissue or in several organs before or after the infusion with any of the ATAs. On the basis of the present experiment the author concludes that Ac-Ph activity in either tumor tissue or other organs is not a useful index of drug effect.

Mellors and Sugiura[23] found a high Al-Ph activity in hepatic cells injured by butter yellow. Al-Ph activity in the epithelium of bile-ducts involved in DAB-hepatic cancer showed a high activity with the progression of malignancy. It rises in the stroma, infiltrating cells and necrotic tissue as the tumor developes, but is lower in the tumor cells [24][25][27]. Similarly, Monis-Rutenburg[15], Aoki et al.[20] and Pearson et al.[26] stated that Al-Ph activity was not found in the cancer cells of tumor bearing animals or human cancers but that it was high in the areas of degeneration and necrosis. In the present experiment, the author obtained similar results except CrA_3 infusion. Only EX was destroyed and activated in the position $\rangle N\text{-}P\langle$ or $-P\langle^{O}_{O}$ by phosphamidase or phosphatase respectively in the liver. The high Al-Ph activity in infused areas forced the author to conclude that EX might be also activated by the tissue.

SUMMARY

Continuous intra-arterial infusions with the anti-tumor agents HN_2, EX, CrA_3, MMC and Mtx were instituted in normal stomachs and stomachs with transplanted B. P cancer, and their influence on SDH, LAP. β-GL, Ac- & Al-Ph activities in normal tissue and tumor tissue were investigated.

1) SDH activity in normal gastric tissue was lowered by each ATA; lowering or disappearance of SDH activity in transplanted tumor did not always coincide with the response to the ATA; it became negative in degenerated and necrotic tumor cells.

2) LAP activity was lower in both normal and tumor tissues by ATA infusion.

3) β-GL activity tended to be greatly inhibited by each ATA. It was lower in degenerated and necrotic tumor cells by all ATAs and decreased also in the liver, kidneys and small intestine. These changes in β-GL activity are considered to correlate well with the effectiveness of ATAs.

4) Ac-Ph activity did not change in tumor tissue. It is not an useful index of the effectiveness of ATAs.

5) Al-Ph activity rose as the cell was damaged by ATAs, but its rise has not always correlated with response to the ATAs.

The author wishes to express his deep gratitude to Prof. Dr. Chuji Kimura for his kind guidance and to instructor Ryo Inouye, who has consistently providing warm encourangement and guidance.

Part of the material of this paper was presented at the second and third congress of the Japan Society for Cancer Therapy, Chiba Nov. 19～20, 1964 and Sendai Nov. 2～3, 1965.

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和文抄録

各種抗腫瘍剤の動脈内持続注入に関する実験的研究
(特に組織化学的研究)

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悪性腫瘍の治療として,手術療法,放射線療法,化学療法等の単独或はこれらの併用による方法が一般化しているが,最近手術に併せて化学療法が用いられ,後者のうちでも Klopp がはじめて動脈内挿管投与を試みて以来全身投与よりも局所大量衝撃投与が盛んに用いられるようになつて来た.また薬剤の投与方法及び動脈内挿管方法も幾多の改良がなされ,更に注入動力も携帯化されるようになり全く安全かつ有効な方法として広く用いられている.

一方,動物腫瘍,人腫瘍における組織酵素の研究が多方面から論ぜられ,その消長が重要視されるようになつた.そこで著者は各種抗腫瘍剤 (ATA) として Nitromine (HN_2), Endoxan (EX), Chromomycin A_3 (CrA_3), Mitomycin C (MMC), Methotrexate (Mtx) を用い成熟家兎の動脈内持続注入により正常組織における琥珀酸脱水素酵素 (SDH), ロイシンアミノペプチダーゼ (LAP), β-グルクロニダーゼ (β-GL), 酸及びアルカリフォスファターゼ (Ac-& Al-Ph) 活性がどのような消長を示すかを検索した.この知見に基づいて更に Brown-Pearce 癌 (B. P. 癌) の胃移植癌に対する各 ATA の動脈内持続注入による治療効果を検討し,この効果が腫瘍組織ならびに他臓器における前記各酵素の消長と平行するか否かを検索し,次の結果を得た.

1) B. P. 胃移植癌注入例全群とも無処置対照群に較べ全身状態良好で食欲減退は全くないか,あつても軽度であり延命効果も優つていた.EX, CrA_3, MMC 注入例では注入前に較べて腫瘍の大きさに著変はみられないが腫瘍の中心部はいずれも壊死に陥つていた.リンパ節転移は全くないかまたは軽度であつた.Mtx 注入群では全例とも腫瘍は約1.5倍になつておりリンパ節転移,肝転移が認められた.HN_2 注入群では腫瘍は非常に小さくなつており周囲組織は瘢痕化し転移は認めなかつた.

2) 正常組織の SDH 活性は各 ATA 注入により一様に低下した.B. P. 癌胃移植癌においては SDH 活性の低下乃至消失は ATA の効果と必ずしも一致しなかつたが腫瘍細胞が変性壊死に陥ると共に陰性化した.肉眼的によく奏効したと思われる HN_2 注入例は局所 SDH 活性は正常組織にほぼ近い活性を示した.

3) LAP 活性の消長は正常組織,腫瘍組織ともに ATA の注入により活性の低下をみた.効果がみられなかつた Mtx 注入例は腫瘍組織は活性の低下をみたが,小腸において他の ATA 注入例に較べて逆に活性の上昇を認めた.

4) β-GL 活性は各 ATA により強く阻害される傾向が認められた.腫瘍細胞の変性壊死化で活性の低下をみると共に肝,腎,小腸の活性もこれと平行して低下した.この消長は各 ATA の効果とよく一致する.

5) Ac-Ph 活性は各 ATA の注入で,正常組織では細胞の壊死化と共に活性の上昇を認めたが腫瘍組織に関してはその変動がみられず各 ATA の効果判定の指標とはならない.

6) Al-Ph 活性は正常組織においては各 ATA によつて細胞が障害される程度が強いほど活性の上昇を認めた.しかし腫瘍組織ではその成績がまちまちであり,各 ATA の治療効果とは必ずしも一致しなかつた.