Chemotherapy of Ehrlich's Ascites Tumor with Cyanide and Ether Combinations.* (24943)

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The early research of Warburg indicates that a general biochemical characteristic of tumors as opposed to normal tissues is their high rate of anaerobic fermentation accompanied by low efficiency of oxidative phosphorylation(1). Warburg contended that this is the result of irreversible impairment to oxidative phosphorylative capacity of the original malignant cell during carcinogenesis, and that oxidative metabolism in malignant cells may approach an irreducible minimum. Weinhouse showed however, that damage to oxidative capacity of the tumor cell is not as clear cut as seems from Warburg's data(2). He demonstrated that CO_2 production from glucose or fatty acids is similar in malignant cells and normal tissues. In addition it has been shown that effects of inhibitors such as fluoride or dinitrophenol are similar in normal tissues and tumors(3). Quastel showed that respiration and phosphorylation in tumor cells are tightly coupled(4). If Warburg's theory is accepted as a working hypothesis the large quantitative differences in oxidative capacity between normal and tumor cells could form an excellent basis for chemotherapy. Use of the cyanide ion in cancer chemotherapy is not new. Older research demonstrated that this ion seemed to have a differential inhibitory effect upon malignant tissues(5,6,7), but the margin of safety appeared so low that interest was lost. Our data represent the effects of sublethal doses of cyanide given to tumor bearing animals under the influence of ether anesthesia.

Methods. Our animals were male Swiss mice obtained from Taconic Farms. Weight averaged 20 g $\pm$ 10%. The animals were injected intraperitoneally with Ehrlich's ascites tumor cells obtained from stock inoculated 8 days previously. The cells were withdrawn under light ether anesthesia using No. 18 needle and 2 ml syringe. Tumor cells were centrifuged 2 minutes in a centrifuge, peritoneal fluid was decanted, and the cells resuspended in 10 $\times$ dilution of normal saline, and immediately injected. Therapy was reserved in Group 1 until ascites became grossly evident at fifth day post inoculation. Control and experimental animals were selected from inoculated mice at random. Treatment was as follows: Experimental mice were subjected to an anesthetic concentration of ether in a bell jar, and when no longer responsive to painful stimuli were injected intraperitoneally with 0.75 or 1.5 mg/kg of sodium cyanide in normal saline. The volume injected was 0.01 ml/10 g of mouse. Animals were allowed to recover from ether anesthesia. Control animals were not subjected to sham procedure with ether and normal saline because this regimen slightly decreased survival time of animals. Time intervals from inoculation to institution of treatment and doses were varied from group to group.

Results. In Group 1 an extremely large inoculum was utilized, (8 x 10^7 cells), and treatment was established, as schematically represented in Fig. 1 and Table I. In addition the control group consisted of 40 animals as opposed to 10 experimental mice.

Group 2 is represented in Fig. 2 and in Table I. Treatments were instituted at times noted on Fig. 2 and in the Table. There were 10 mice/experimental procedure.

Group 3 is presented in Table I and Fig. 3. Dosage was increased to 1.5 mg/kg NaCN. Treatment was instituted at 24, 48, and 120 hours post inoculation. A further group also received multiple treatment on first, third, and fifth day after implantation of tumor. Ten mice were used in each condition.

Discussion. These data demonstrate that significant increases in survival time of mice

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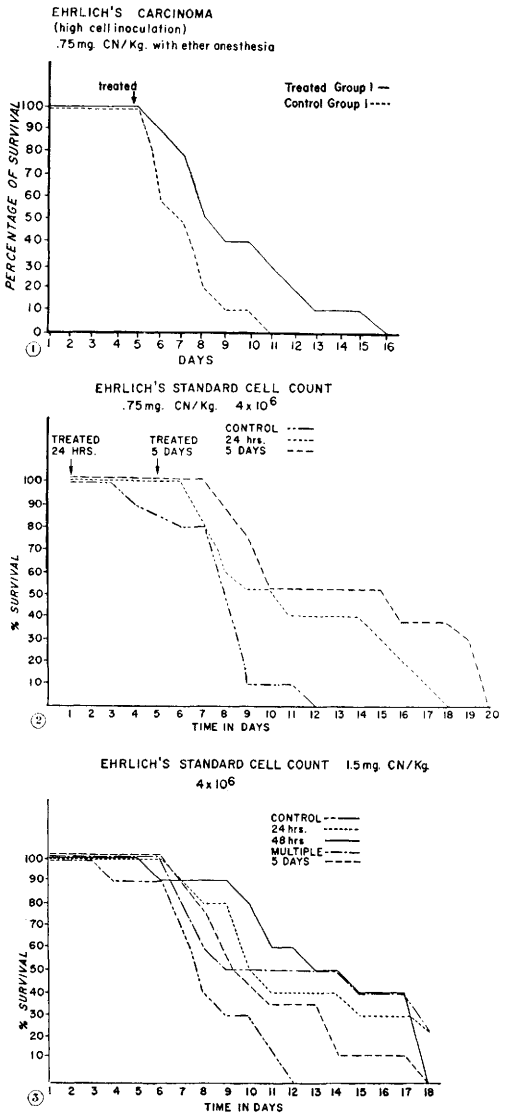

FIG. 1. Survival curves for Group I showing comparison between control Ehrlich's ascites mice and animals treated with 0.75 mg/kg cyanide and ether anesthesia (8×10^7 cell inoculation).

FIG. 2. Survival curves for Group II (4×10^6 cell inoculation) showing comparison between control animal survival and that of animals treated at 24 hr, and others treated at 5 days post inoculation with 0.75 mg/kg sodium cyanide and ether anesthesia.

FIG. 3. Group III (4×10^6 cell inoculation) survival curves comparing survival of control animals with that of animals treated at 24 hr, 48 hr, 5 days, and a series treated at 1, 3, and 5 days post inoculation with 1.5 mg/kg cyanide and ether anesthesia.

with Ehrlich's ascites carcinoma may be obtained with sodium cyanide and ether as chemotherapeutic agents. We consider cyanide-ether combinations superior to cyanide alone because our pilot studies demonstrated 57% average increase in survival time over animals given cyanide alone. In addition, we demonstrated a decrease in acute toxicity of cyanide under anesthesia which will be reported elsewhere.

As to the mechanism of action of these agents, these data do not give definite answers. Several possibilities may be pointed out. The action of cyanide may be a corollary of Warburg's hypothesis. However, it should be pointed out that cyanide blocks many enzyme systems, and although inhibition of cytochrome oxidase is the most evident of these actions, inhibition of another enzyme system may be responsible for increase in survival time. Chance has shown that addition of glucose to Ehrlich's ascites causes rapid acceleration of respiration, and that reduced pyridine nucleotide, flavoprotein, cytochromes *b* and *c* and in some cases cytochrome *a* are more oxidized during glucose activated phase of respiration(8). It is suggested that the well known hyperglycemic action of ether may be related to synergism between cyanide and ether in treatment of this tumor. The respiratory enzymes thus activated by release of endogenous glucose may then be more vulnerable to the action of the cyanide ion. In addition the use of ether may extend the margin of safety in use of cyanide. The possibility that malignant cells killed by cyanide may serve as antigenic material also exists. Finally, it should be noted that Ehrlich's ascites tumor can be inhibited by a variety of compounds seemingly unrelated in mechanism of action or chemistry(9).

Experiments involving the combination of cyanide, ether, and inhibitors of DNA synthesis, and also the use of cyanide and ether in treatment of spontaneous tumors of dogs are in progress.

Summary. It has been demonstrated that sodium cyanide given intraperitoneally at several sublethal dosage levels during ether anesthesia is capable of significantly prolonging survival time of mice inoculated with Ehrlich's ascites carcinoma.

TABLE I. Alteration of Survival Time in Ehrlich's Ascites Mice Produced by Cyanide-Anesthesia Treatment.

Group No.		Dosage (mg/kg)	Cells/mouse	Avg survival (days)	Increase in survival (days)	% increase
I	Control	.75	8×10^7	6.2	0	0
	5 days	"	"	8.8	2.6	42.0
II	Control	.75	4×10^6	8.1	0	0
	24 hr	"	"	9.8	1.7	21.0
	120 "	"	"	10.4	2.8	35.0
III	Control	1.5	"	8.1	0	0
	24 hr	"	"	12.7	4.6	56.8
	48 "	"	"	13.8	5.7	73.7
	120 "	"	"	11.1	3.0	37.0
	27, 72, 120 hr	"	"	12.8	4.7	64.1

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Adrenal Cortical and Body Temperature Responses to Repeated Endotoxin Administration. (24944)

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It is difficult to speculate logically on the mechanism of adrenal cortical stimulation following endotoxin, inasmuch as fever and hypotension may be responsible. Previous studies indicate that the adrenal cortical stimulating effect occurs before fever(1), but most animals that reveal pituitary-adrenal activation following endotoxin also develop fevers. The present studies were set up to produce tolerance to fever-promoting effect of endotoxin. The ease of producing tolerance to various responses following endotoxin varies considerably, and it was possible that fever-tolerance might occur before loss of pituitary-adrenal stimulation.

Methods. Adult mongrel dogs ranging 8-25 kg were used. Cannulation of right lumbar vein(2) was performed according to the technic of Hume and Nelson, and intermittent samples of adrenal venous blood were obtained. Cortisol concentrations in the adrenal venous blood were determined by modification of method of Silber and Porter(3) as described by Peterson, Karrer and Guerra(4). Purified endotoxin of Boivin type derived from *E. coli* was prepared by method of Spink and Anderson(5). Endotoxin was prepared in 2 concentrations in normal saline, one of 1 mg/cc, the other 0.1 mg/cc. All endotoxin injections were by intravenous route and given in approximately 30 seconds time. Twenty-five units of ACTH were given intravenously at conclusion of some experiments. Rectal temperatures were taken, and in some experiments arterial blood pressures were obtained by cannulating femoral artery with polyethylene catheter connected to mercury manometer.

Results. The adrenal cortical and fever responses to large doses of endotoxin are given in Table I. Significant adrenal cortical responses were observed in all animals except

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