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Glucose as Adjunct to 3.3-Dimethyl-1-Phenyltriazene in its Action upon Sarcoma 180. (24248)

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The characteristic acidity of cells of malignant tumors may make them more susceptible than normal cells to damaging, acid-sensitive chemicals(1,2). One such chemical is 3,3-dimethyl-1-phenyltriazene (PT). It inhibits Sarcoma 180 and other tumors(1,3), perhaps by releasing diazonium ion in a reaction catalyzed by acid(1). To learn whether the increased acidity of tumors might alter the action of PT on Sarcoma 180 in mice it was proposed to inject glucose prior to each day's injection of PT. The administration of glucose is known to increase acidity of tumors (4). This report presents results of such an experiment, together with ancillary observations.

Materials and methods. PT was prepared by the method of Elks and Hey(5). It was characterized by boiling range and absorption spectra in 3 solvents. It distilled at 100-101°C (uncorr.) and 5 to 5.5 mm Hg pressure. Common logarithms of molar extinction coefficients at 225, 285 and 308 mμ were, for PT in 95% ethanol, 3.93, 4.08 and 4.06, respectively. LeFevre and Liddicot(6) reported 3.97, 4.14 and 4.11 as the corresponding values. In iso-octane the logarithms were 3.99, 4.12 and 4.06 at the same wave lengths. In water they were 3.91, 4.10 and 4.10, and a maximum value of 4.12 was obtained at 302 mμ. No comparable data are available. Ultraviolet measurements were made with 1 cm cells and a Beckman DK spectrophotometer. The effect of PT upon tumor growth was measured in female albino mice weighing 17 to 20 g. Each was given by trocar a subcutaneous implant of Sarcoma 180 weighing 7.5 mg average. Injections of PT were begun one day after implantation and continued once daily for 5 days (7 days in preliminary assay). PT was dissolved in peanut oil (1 g/100 ml) and given intraperitoneally. Some of these mice were also injected subcutaneously with glucose in a solution containing 20 g/100 ml in 0.85% sodium chloride. They were given 0.8 ml/mouse/day (8 g glucose/kg) always one hour before PT. On 8th day after implantation of tumor, the mice were again weighed and the external diameters of their tumors were measured. The tumors were then excised, weighed, fixed in formalin, imbedded in paraffin and stained with hematoxylin-eosin for microscopic examination.

Results. The results of a preliminary assay and of the main experiment are presented in Table I. A difference in degree of effect of PT between preliminary assay and main experiment is unexplained. A similar variability is to be found in the data of Clarke *et al.* (1).

To analyze data from the main experiment, that with glucose, measurements of tumor weight and body weight, summarized in Table I, were transformed into a new metric of response by taking the common logarithm of the ratio of each tumor weight to the difference between initial and final body weight after adding 10 g to the difference to make

TABLE I. Effects of PT in Oil and of Glucose, Separately and in Combination, upon Growth of S-180 in Mice.

No. of mice	Daily treatment	Avg body wt (g)		Tumor wt (mg)		Tumor diameters (mm)
		Initial	Final	Avg	Range	
		Preliminary assay				
5	50 mg PT/kg			222	126–317	8.7
5	25 mg "			348	334–354	9.9
5	12.5 mg "			406	217–645	10.5
5	No treatment			455	274–578	10.1
		Main experiment				
10	50 mg PT/kg + .8 ml glucose	18.0	13.2	51	21– 94	5.3
10	50 mg PT/kg	18.0	13.2	79	31–122	5.6
10	.8 ml glucose + .1 ml oil	18.2	18.9	450	297–734	10.4
10	No treatment	18.8	19.6	481	195–835	10.5

it always positive. Transformation was used to produce an approximately additive metric of response adjusted for any general change in body weight. Thus effects on both tumor and body weights were included in a single numerical evaluation; the common alternative is to list them separately. Total variation in this new metric of response was analysed into 4 parts: one part due to the effect of glucose, another to PT, a third to interaction of glucose and PT, and a residual due to uncontrolled sources of variation. There was found a probability of about 7 in 8 that injections of glucose and peanut oil *per se* had an effect on tumor weight. Inhibition of another tumor by glucose has been observed by Bass and Feigelson(7). PT as expected had a great effect. Alteration of the effect of PT by glucose was slight, with a probability of about 1 in 5 that the observed alteration was due to chance alone.

Microscopic examination of neoplasms from mice injected with PT revealed little difference in cellular structure of those from mice which had also received glucose, as compared with those from mice that had not; the latter showed more necrosis and less differentiation of cellular elements. Neoplasms from mice not given PT exhibited greater pleomorphism and mitotic activity, together with more frequent areas of necrosis. No appreciable effects of injections of glucose and oil without PT were seen.

Discussion. That the effectiveness of glucose in altering the action of PT was not greater might be explained in many ways. For example, perhaps glucose injections produced only slight increase in tumor acidity. This could have been because the tumors were somewhat acid initially, or alternatively because acid failed to accumulate in them.

An initially acidic condition could have been caused by prolonged hyperglycemia due to PT. The only other triazene tested for effect on blood sugar, N-p-chlorophenyldiazothiourea, has been shown to produce prolonged hyperglycemia in rats(8). Hyperglycemia was looked for following injection of PT into each of 6 mice with tumors and 4 mice without. On the day following tumor implantation, tail blood was taken, either PT in peanut oil or the oil alone was injected intraperitoneally, and blood was again taken at 1 and at 2 hours after injection. This procedure was repeated with the same mice on the 2 following days; mice without tumors were tested only once. Samples of 0.020 ml of blood were obtained, and sugar was measured by Nelson's method(9). Analysis of total variation in these data into 18 parts, each associated with the various conditions of the experiment, revealed no source of variation with as much as a 10 to 1 probability of being the result of anything but chance. Almost inappreciable alteration of blood sugar by PT, about 2 mg/100 ml of blood, was found.

Another explanation of the ineffectiveness of glucose might be that absorption of PT was too slow to permit its reaching the tumor at

TABLE II. Recovery of PT and of Peanut Oil from Abdominal Cavities of Mice following Intraperitoneal Injection, with and without Injection of Glucose, One Hour Prior to Injection of PT in Oil.

Time from inj. of PT to killing (min.)	% of inj. PT recovered	% of inj. oil recovered
Without inj. of glucose		
1	61	94
2	68	93
6	50	79
60	4	63
60	6	77
60	15	68
60	19	31
61	2	56
With subcut. inj. of glucose		
35	24	75
38	26	30
77	4	62
92	3	70
120	2	44
120	5	12
With intraper. inj. of glucose		
59	71	101
60	30	88
60	72	100
61	12	91

the time tumor acidity was increased. Because no facts concerning the fate of PT in the body were known, a simple analytical technic based upon ultraviolet spectra was originated. With it, rate of disappearance of the 50 mg/kg dose of PT in peanut oil from the abdominal cavity was measured after its intraperitoneal injection. The mice were killed by withdrawal of blood from the heart, or by evacuating the air from a chamber in which they were placed. The abdominal cavity was rinsed with 40 ml of iso-octane followed by 2 ml of 1.2% aqueous sodium bicarbonate solution. The washings were shaken in a bottle for 10 minutes in darkness to insure extraction. The optical densities of the iso-octane solutions at 302 and at 240 mμ were measured. From these measurements the recovery of PT and of peanut oil, respectively, were calculated. Similar extracts from uninjected mice showed negligible optical densities (less than 0.03) at these wave lengths.

The data (Table II) indicate rapid disappearance of PT and slow disappearance of oil (the estimated half time was 60 minutes) from the abdominal cavities of mice other than those injected intraperitoneally with glucose. As to the latter, the disappearance of both PT and oil was slow and variable. Because of both these findings, intraperitoneal glucose injections were not used in the main experiment. Blood samples of 1 ml contained too little PT for measurement by the technic noted in the preceding paragraph.

Microscopic observation, using a simple, possibly novel, histochemical technic, confirmed the supposition that intact PT may reach tumor cells. Appearance of PT in appreciable concentrations in liver and, a little later, in tumors, was demonstrated within 15 to 30 minutes after intraperitoneal injection of doses of 200 to 500 mg PT in oil/kg. This was done by treating frozen sections with 1% aqueous N-(1-naphthyl)-ethylenediamine dihydrochloride and *N* hydrochloric acid. Triazene promptly hydrolyzed and the diazonium ion then coupled to yield dyes. These stained the nuclei purple and the cytoplasm orange with cherry-red granules. Squamous epithelium of skin treated in the same way stained similarly. These experiments are the only known demonstration of the catalysis of triazene decomposition by acid in tissues.

Summary. The effects of intraperitoneal injections of 3,3-dimethyl-1-phenyltriazene dissolved in peanut oil and of subcutaneous injections of glucose, separately and in combination, upon growth rate of Sarcoma 180 in mice were measured. The triazene markedly depressed tumor growth. This effect was only slightly altered by injections of glucose. Glucose itself had a slight effect upon tumor growth. The triazene disappeared rapidly from the abdominal cavity. By acidification and coupling with N-(1-naphthyl)-ethylenediamine, it could be demonstrated in tissue. Triazene injections were without effect on blood sugar.

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Copper Metabolism. XXVIII. Influence of Biliary Duct Ligation on Serum and Tissue Copper.* (24249)

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In both man(1,2) and dogs(3), the most important excretory route for copper is through the biliary system. However, no detailed studies have been performed in animals to determine the influence of ligation of bile ducts on tissue copper. The purpose of this paper is to present the results of studies on tissue and serum copper in rats with ligated bile ducts. Although normal serum copper values have been observed in swine and dogs with obstruction to outflow of bile(3), hypercupremia has been observed in patients with biliary cirrhosis(4). Copper accumulates in livers of patients with cirrhosis of the liver and associated cholangiolitis, but not in livers of most patients with uncomplicated cirrhosis (5). In one patient with primary biliary cirrhosis, an amount of copper (426 mg) was found in the liver, which was in excess even of that usually observed in livers of patients with Wilson's disease(5). Several investigators(2,6) have observed that a smaller proportion of intravenously administered radioactive copper is excreted in stools of patients with Wilson's disease than in stools of normal subjects. It has been suggested that the excessive deposition of copper in tissues may be due to impaired biliary excretion of copper (6). Therefore, it seemed of interest to determine if impairment of the outflow of bile in animals would be accompanied by excessive deposition of copper in the liver, brain and kidneys.

Methods. Adult male rats of Sprague-Dawley strain, 150 to 200 g in weight, were used. The animals were grouped into units of 3 of the same weight. One animal in each unit, designated as "bile duct-ligated," was anesthetized with sodium "Nembutal" solution given intraperitoneally. The common bile duct was located through a paramedian incision, ligated in 2 places, and sectioned between the ligatures. A careful search was made for accessory ducts. When these were found, they were also ligated and sectioned. The second animal in each unit, designated as "sham-operated," was treated in a similar manner except that the bile duct was not ligated or sectioned. The third animal in each unit, designated as "control," served as a dietary control. Bile duct ligation impaired the appetite of rats. To maintain dietary intake of copper at as constant a level as possible, the "bile duct-ligated" animal in each unit was offered 25 g of diet daily. On the following day, the uneaten portion of the diet was weighed and an amount equal to that consumed by the rat was fed to the "sham-operated" and "control" animal of that unit. Composition of diet was as follows (g/kg): casein, 200; sucrose, 640; lard, 110; sodium chloride, 6.75; magnesium carbonate, 4.25; monopotassium phosphate, 2.85; calcium phosphate, 23.15; potassium chloride, 3.35; potassium iodide, 0.1135 calcium carbonate, 8.45; ferric pyrophosphate, 1.05; copper sulfate, 0.011; manganese chloride, 0.01; zinc oxide, 0.0008; cobalt chloride, 0.008; and choline

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