Studies in a Tumor Spectrum

III. The Effect of Phosphoramides on the Growth of a Variety of Mouse and Rat Tumors*

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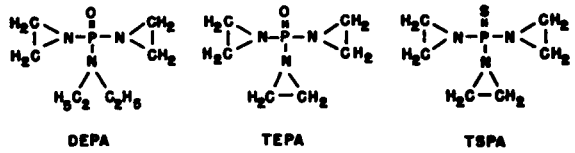
Previous investigations with a spectrum of tumors demonstrated that nitrogen mustards (17) and triethylene melamine (19) had a definite inhibitory or destructive action on certain types of tumors. In view of the fact that the biological activity of these compounds is believed to be due to the formation of ethylenimmonium ions in the case of the former (7) and the presence of ethylenimine groups in the case of the latter, the study was extended with compounds closely related to these substances on a wide spectrum of tumors.

Results in experimental animal tumors reported from other laboratories (1–3, 6, 9–11, 20, 21) and in human cancer (4, 8, 20) have indicated that triethylene phosphoramide and triethylene thiophosphoramide merited intensive study. Our observations on the effect of these compounds on a variety of transplantable mouse and rat tumors have been briefly reported (14, 18) and are now presented in more detail. The structural formulae of these compounds have in common the cyclic structure, ethylenimine:



DEPA TEPA TSPA

DEPA = N,N-Diethyl-N′,N″-diethylene phosphoramide

TEPA = N,N′,N″-Triethylene phosphoramide

TSPA = N,N′,N″-Triethylene thiophosphoramide

MATERIALS AND METHODS

The tumors used in the present study are as follows: Sarcoma 180 (solid and ascitic forms), Bashford carcinoma 63, Ehrlich carcinoma (solid and ascitic forms), Krebs 2 ascites carcinoma and Harding-Passey melanoma in Rockland Swiss albino mice; Sarcoma T 241, Adenocarcinoma E 0771, Lewis bladder carcinoma and Lewis lung carcinoma in C57BL mice; Sarcoma MA 387, Miyono adenocarcinoma, Carcinoma 1025, Wagner osteogenic sarcoma, Ridgway osteogenic sarcoma, Patterson lymphosarcoma and Mecca lymphosarcoma in AKR mice; Grand epidermoid carcinoma in CFW mice; Gardner lymphosarcoma in C3H mice; Andervont hepatoma in C mice; Flexner-Jobling carcinoma, Walker carcinosarcoma 256, and Sarcoma R 39 in Sherman rats; Jensen sarcoma in Sprague-Dawley rats; and Murphy-Sturm lymphosarcoma in Wistar rats.

The history, biological properties, and cytological description of most of these tumors were presented previously (17, 19). Similar descriptions of other tumors used in this paper are given below.[1]

Grand mouse epidermoid carcinoma.—This tumor was discovered September 7, 1951, by C. G. Grand at New York University. It originated spontaneously in the skin of a CFW mouse. It was first cultivated in tissue culture and retransplanted into CFW mice. The histological picture of the original tumor was that of a squamous-cell carcinoma (Fig. 1). The tumor shows occasional squamous pearl formation. The tumor grows rapidly, and 14 days after transplantation into CFW mice it reaches a size of approximately 15 × 12 × 8 mm. This tumor becomes extensively necrotic and cystic in the center after 14 days. The tumor transplants take in 100 per cent of cases, and about 30 per cent of them regress. The 76th transplantation generation was reached on June 10, 1954.

Lewis mouse bladder carcinoma.[2]—This transplantable

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[2] Subcutaneous or intraperitoneal injection of fresh blood of mice bearing the Lewis bladder carcinoma or Lewis lung carcinoma into mice produced identical tumors. Dr. Margaret R. Lewis, personal communication, 1954.

tumor was discovered in a male C57BL mouse in December, 1950. The tumor is a very malignant type of epidermoid carcinoma (Fig. 2). It grows rapidly and becomes very hemorrhagic; 14 days after transplantation into C57BL mice, it reaches a size of approximately 19 × 13 × 10 mm. The transplants take in 100 per cent of cases, and about 2 per cent of them regress. In our laboratory the 16th transplantation generation was reached on July 5, 1954.

Lewis mouse lung carcinoma.[2]—This tumor originated spontaneously as a carcinoma of the lung of a C57BL mouse in 1951. It is a rapidly growing tumor, reaching a size of approximately 18 × 12 × 9 mm. 14 days after transplantation. The transplants take in 100 per cent of cases, and about 4 per cent of them regress. The tumor is a very malignant type of epidermoid carcinoma (anaplastic carcinoma) and becomes extremely hemorrhagic (Fig. 3). In our laboratory the 16th transplantation generation was reached on July 6, 1954.

The methods employed in the chemotherapy studies of the tumors have been described before (16). In general, subcutaneous implantations of tumor material (small pieces of tumor, each measuring about 1.5 mm. in any dimension and weighing approximately 6 mg.) into healthy young animals (18–22-gm. mice; 100–125-gm. rats) were carried out by the usual trocar method (a single implant into the right axillary region).

In every set of experiments, tumor-bearing animals were divided into two groups, one to be treated with compounds and the other to be used as controls. The progress of the tumors in the animals was recorded graphically by measuring them in two diameters with calipers at the end of 1, 2, 3, and 4 weeks after tumor transplantation. The final measurements of the tumors were made at the end of the 6th week. In the case of ascites tumors (13), intraperitoneal injection of 0.1 cc. of the fluid containing about 1 million cancer cells into mice was made in the inguinal region with a ½-cc. syringe having a 23-gauge needle.

The first intraperitoneal injection of the compounds was given either 1 or 7 days after tumor transplantation. Fresh saline solutions of DEPA, TEPA, and TSPA were prepared daily, and 0.5 cc. was injected once daily for 7 days. The animals were given food (Purina Laboratory Chow) and water ad libitum.

The degree of inhibition of tumor growth was graded according to the following scheme:

- — No effect; tumor growth to three-quarters or more of the diameter of the controls.
- ± Slight inhibition; tumor growth from one-half to three-quarters of the diameter of the controls.
- \+ Moderate inhibition; tumor growth from one-fourth to one-half of the diameter of the controls.
- ++ Marked inhibition; failure to grow or growth to approximately one-fourth of the average diameter of the controls.
- +++ Complete destruction of tumors (Complete regression of a higher percentage of tumors in the treated animals than in the controls which show no more than 10 per cent spontaneous regressions with the exception of the Grand epidermoid carcinoma, which has 30 per cent).

The degree of inhibition of ascites tumor growth was graded according to the following scheme:

No effect (−) indicates marked abdominal distention, or 4 times the normal gain in body weight or more.

Slight inhibition (±) indicates moderate abdominal distention.

Moderate inhibition (+) indicates slight abdominal distention, or 2 times the normal gain in body weight.

Marked inhibition (++) indicates no abdominal distention, or normal gains in body weight of animal—about 0.5 gm/day.[3]

In all the tables one column gives average weight change in grams of treated animals over control animals after 1 week of treatment. All the results with Sarcoma 180 are evaluated only on the basis of 1st-week results.

RESULTS

General findings.—Tables 1–6 summarize the effects of DEPA, TEPA, and TSPA on the growth of eighteen tumors of the mouse and five tumors of the rat. With the exception of the Andervont hepatoma, controls of these experiments showed 100 per cent takes and, except for the Grand epidermoid carcinoma, there was a low rate of regressions. The average percentages of tumor regressions in control animals were as follows: Sarcoma 180, 2; Sarcoma T 241, 0; Sarcoma MA 387, 6; adenocarcinoma E 0771, 2; Bashford carcinoma 63, 6; Miyono adenocarcinoma, 6; Ehrlich carcinoma, 0; Carcinoma 1025, 4; Grand epidermoid carcinoma, 30; Wagner osteogenic sarcoma, 7; Ridgway osteogenic sarcoma, 7; Patterson lymphosarcoma, 3; Mecca lymphosarcoma, 4; Gardner lymphosarcoma, 4: Harding-Passey melanoma, 6; Andervont hepatoma, 0; Lewis bladder carcinoma, 0; Lewis lung carcinoma, 4; Flexner-Jobling carcinoma, 10; Walker carcinosarcoma 256, 3; Sarcoma R 39, 7; Jensen sarcoma, 6; and Murphy-Sturm lymphosarcoma, 5 per cent.

The compounds have been studied at a number of dose levels in an attempt to obtain data at maximum tolerated doses or at comparable degrees of toxicity. It is apparent from the data that both TEPA and TSPA at their maximum tolerated doses have shown a similar degree of definite inhibitory or destructive actions upon certain of the transplantable tumors. The mouse tumor most affected by TSPA was Carcinoma 1025. Complete destruction of 1-day-old tumors resulted from daily doses of 4 mg/kg or 2 mg/kg in 3 weeks (Chart 1). DEPA was considerably less effective, though it did cause a marked retardation in the growth of Carcinoma 1025 and the Ridgway osteogenic sarcoma. The decreased effectiveness of DEPA was also apparent in tests with the ascites forms of the tumors.

It is extremely interesting that repeated injections of TSPA had a marked inhibitory effect on Harding-Passey melanoma. We have tested over 500 other compounds with this tumor, and they caused only slight or no inhibitory effect.

Results with spontaneous mammary cancers.—Since TEPA and TSPA had a distinct inhibitory effect on the growth of four transplantable mam-

[3] In some animals the daily weight increase may be much less due to toxic action of compounds. Survival of more than 50 per cent of treated an mals beyond 3 weeks was considered as a marked effect. Control animals die in 2–3 weeks.

TABLE 1.—EFFECT OF DIETHYLENE PHOSPHORAMIDE ON VARIOUS 1-DAY-OLD MOUSE TUMORS

(dose: 4 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Sarcoma 180 (solid)	1/20	6/20	0/+2	±	−	
Sarcoma 180 (ascitic)	0/20	10/20	+5/+8	−	−	
Sarcoma T 241	0/15	1/15	−1/+1	−	−	
Sarcoma MA 387	0/20	1/20	−1/+2	±	±	
Adenocarcinoma E 0771	0/20	0/20	−0.5/+1	−	−	
Bashford carcinoma 63	0/25	2/25	+0.5/+2	−	±	
Miyono adenocarcinoma	0/30	1/30	−1/+1.5	±	±	
Ehrlich carcinoma (solid)	0/15	0/15	+1/+3	−	−	
Ehrlich carcinoma (ascitic)	0/30	10/30	0/+4.5	+	±	
Krebs 2 carcinoma (ascitic)	0/30	17/30	+7/+8.5	±	−	
Carcinoma 1025	0/20	0/20	−1.5/+1.5	+	++	Normal growth thereafter
Grand epidermoid carcinoma	0/15	0/15	0/+1.5	±	−	
Wagner osteogenic sarcoma	2/10	6/10	−2/+1.5	−	−	
Ridgway osteogenic sarcoma	0/40	1/40	−0.5/+1	±	++	Normal growth thereafter
Patterson lymphosarcoma	0/25	6/25	−2/+1	±	−	
Mecca lymphosarcoma	0/20	0/20	−1/+2	±	−	
Gardner lymphosarcoma	0/20	1/20	−0.5/+1.5	+	−	
Harding-Passey melanoma	0/25	0/25	+1.5/+4	−	±	
Andervont hepatoma	0/10	0/10	−4/+1	?	?	90 per cent inhibition at 19th week
Lewis bladder carcinoma	0/10	0/10	−1/+1	−	−	
Lewis lung carcinoma	0/10	2/10	−1.5/+1.5	±	±	

* Five or ten animals in each test group. The results presented are the averages of combined groups.

TABLE 2.—EFFECT OF DIETHYLENE PHOSPHORAMIDE ON VARIOUS 1-DAY-OLD RAT TUMORS

(dose: 1 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Flexner-Jobling carcinoma	0/25	1/25	+7/+25	+	+++	47 per cent inhibition at 2d week; 75 per cent at 3d week
Walker carcinosarcoma 256	0/35	2/35	+9/+26	+	++	15 per cent inhibition at 2d week; 29 per cent at 3d week
Sarcoma R 39	2/25	6/25	+17/+23	++	+++	97 per cent inhibition at 2d week; 100 per cent at 3d week
Jensen sarcoma	0/20	3/20	+14/+25	++	+++	100 per cent inhibition at 2d and 3d week
Murphy-Sturm lymphosarcoma	0/20	4/20	+15/+27	−	−	

* Five or ten animals in each test group. The results presented are the averages of combined groups.

mary carcinomas, TSPA was therefore tested in a total of 80 Rockland Farms Swiss albino mice with recently developed spontaneous mammary adenocarcinomas. In various groups of these mice injections of TSPA (2 or 4 mg/kg/day for 10–18 days) stopped the growth of the majority of spontaneous breast tumors, but there was no significant increase in tumor regression over the 7 per cent observed in the controls. This suggests that the regressions observed with well developed tumors after treatment with TEPA or TSPA may result from extensive initial damage from the drug followed by

TABLE 3

EFFECT OF TRIETHYLENE PHOSPHORAMIDE ON VARIOUS 1-DAY-OLD MOUSE TUMORS

(dose: 6 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Sarcoma 180 (solid)	0/25	5/25	$\frac{-1}{+2}$	++	+	Normal growth thereafter
Sarcoma 180 (ascites)	0/20	5/20	$\frac{+1.5}{+9}$	+	±	
Sarcoma T 241	0/20	5/20	$\frac{-1.5}{+1}$	+	−	
Sarcoma MA 387	0/20	6/20	$\frac{-2}{+1.5}$	±	±	
Adenocarcinoma E 0771	0/20	2/20	$\frac{-2.5}{+1}$	±	+	Normal growth thereafter
Bashford carcinoma 63	0/15	1/15	$\frac{-1.5}{+2.5}$	−	±	
Miyono adenocarcinoma	0/20	4/20	$\frac{-3.5}{+1}$	++	++	Normal growth thereafter
Ehrlich carcinoma (solid)	0/20	4/20	$\frac{-1.5}{+4}$	+	+	Normal growth thereafter
Ehrlich carcinoma (ascites)	0/40	9/40	$\frac{-1}{+6.5}$	++	++	
Krebs 2 carcinoma (ascites)	0/20	2/20	$\frac{-1.5}{+8}$	++	++	100 per cent inhibition in group at 2d week
Krebs 2 carcinoma (ascites)	0/10	2/10	$\frac{0\dagger}{+5}$	++	++	
Carcinoma 1025	0/40	7/40	$\frac{-3}{+1.5}$	++	+++	42 per cent inhibition at 2d week; 100 per cent at 3d week
Grand epidermoid carcinoma	2/20	2/20	$\frac{-1.5}{+1.5}$	+	+	Normal growth thereafter
Wagner osteogenic sarcoma	2/30	9/30	$\frac{-3}{+1}$	+	+	Normal growth thereafter
Ridgway osteogenic sarcoma	0/30	5/30	$\frac{-3.5}{+1.5}$	++	+++	70 per cent inhibition at 2d week; 85 per cent at 3d week
Patterson lymphosarcoma	0/25	6/25	$\frac{-2.5}{+1.5}$	+	+	Normal growth thereafter
Mecca lymphosarcoma	0/30	9/30	$\frac{-2.5}{+1.5}$	++	+	Normal growth thereafter
Gardner lymphosarcoma	2/45	18/45	$\frac{-3}{+1}$	++	++	25 per cent inhibition at 3d week
Gardner lymphosarcoma	0/10	0/10	$\frac{-1\dagger}{+2}$	++	++	Normal growth thereafter
Harding-Passey melanoma	0/20	1/20	$\frac{+1}{+5}$	−	±	
Andervont hepatoma	0/20	0/20	$\frac{-3}{+0.5}$	?	?	65 per cent inhibition at 19th week
Lewis bladder carcinoma	1/20	4/20	$\frac{-1.5}{+1.5}$	±	+	Normal growth thereafter
Lewis lung carcinoma	0/20	2/20	$\frac{-2.5}{+1}$	±	+	Normal growth thereafter

* Five or ten animals in each test group. The results presented are the averages of combined groups.
† 4 mg/kg/day.

immunological reactions of the host. The latter presumably would not come into play after damage to spontaneous tumors, even though that damage be extensive enough to stop growth of the established tumor during treatment.

Toxicity.—Extensive studies on the pharmacology of DEPA and TEPA in normal animals have been reported (20). The following summarizes the toxicity data of TEPA at different dosages: at 10, 6, and 4 mg/kg/day for 7 days, 25, 1.5, and 0 per cent, respectively, of tumor-bearing mice died during treatment. At 6 mg/kg/day, the animals lost body weight; the weight loss was extensive at higher levels. At 4 mg/kg/day for 7 days, there were no deaths, and the mice maintained body weight or gained weight. Rats tolerated the compound less well than mice, 16 per cent dying from 5 mg/kg/day for 7 days. At 1 mg/kg/day only about 2 per cent of the rats died. Although there was no gain in body weight for 1 week, there was normal growth thereafter.

The following summarizes the toxicity data of TSPA at different dosages: at 8, 4, 2, and 1 mg/kg/day for 7 days, 50, 1, 0, and 0 per cent, respectively, of tumor-bearing mice died during treatment. At 4 mg/kg/day the animals lost weight for 2 weeks, and those without tumors grew normally thereafter. At 2 mg/kg/day the animals maintained body weight for 7 days; there was normal growth thereafter. At 1 mg/kg/day for 7 days there was normal gain in body weight. Rats tolerated TSPA less than mice, 50 per cent dying from 4 mg/kg/day for 7 days. At 2 mg/kg/day only about 1 per cent of rats died and, while there was no gain in body weight for 1 week, there was normal growth thereafter. Histological examination of the spleen, bone marrow, and organs of animals showed no abnormality. At daily doses of 1 mg/kg/day there was gain in body weight, but it was below normal. At 0.5 mg/kg/day or less all rats grew normally.

Results with rat tumors.—The greater toxicity of DEPA, TEPA, and TSPA for rats was paralleled by their greater capacity to produce damage in some of the rat tumors, as has been previously reported (16, 17, 19) for other compounds. The phosphoramides were most effective in Sarcoma R 39 and Jensen sarcoma and decreasingly effective in the Flexner-Jobling carcinoma, Walker carcinosarcoma 256, and the Murphy-Sturm lymphosarcoma. At a dose of 1 mg/kg/day of TEPA for 7 days, for example, the growth of 1-day-old implants of tumors in that order was prevented as follows: 100, 90, 64, 83, and 0 per cent. At a dose of 1 mg/kg/day of DEPA, the following figures were obtained: 100, 100, 75, 29, and 0 per cent. At a dose of 2 mg/kg/day of TSPA, the following figures were obtained: 100 for Jensen sarcoma, 100 for Flexner-Jobling carcinoma, 67 for Walker carcinosarcoma 256, and 0 per cent for Murphy-Sturm lymphosarcoma. No figures were available for Sarcoma R 39, because the tumor was contaminated and lost. Chart 2 illustrates the effect of TSPA on the Jensen sarcoma. Following seven daily doses of 2 mg/kg of TSPA, all original 1-day-old tumors were destroyed by the end of 2 weeks. TSPA at a dose of 0.5 mg/kg/day or one-fourth of the maximum tolerated dose had complete destructive effects, but at a dose of 0.25 mg/kg or one-eight of a maximum tolerated dose, the compound had only a moderate inhibitory effect on this tumor. It is interesting to note that seven additional injections at a dose of 0.25 mg/kg resulted in marked inhibition of growth, but there was no significant amount of tumor destruction.

Results with well established mouse and rat tumors.—The success of these phosphoramides in producing damage in 1-day-old tumors of the

TABLE 4

EFFECT OF TRIETHYLENE PHOSPHORAMIDE ON VARIOUS 1-DAY-OLD RAT TUMORS

(dose: 1 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Flexner-Jobling carcinoma	1/30	5/30	+5 / +26	+	+++	27 per cent inhibition at 2d week; 64 per cent at 3d week
Walker carcinosarcoma 256	0/30	3/30	+1 / +23	+	+++	76 per cent inhibition at 2d week; 83 per cent at 3d week
Sarcoma R 39	1/20	1/20	+9 / +19	++	+++	80 per cent inhibition at 2d week; 100 per cent at 3d week
Jensen sarcoma	0/30	0/30	+15 / +27	++	+++	80 per cent inhibition at 2d week; 90 per cent at 3d week
Murphy-Sturm lymphosarcoma	1/30	4/30	+8 / +32	−	−	

* Ten animals in each test group. The results presented are the average of combined groups.

TABLE 5

Effect of Triethylene Thiophosphoramide on Various 1-Day-old Mouse Tumors (dose: 4 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Sarcoma 180 (solid)	0/20	0/20	$\frac{-1.5}{+3}$	+	±	
Sarcoma 180 (ascites)	0/20	20/20	$\frac{+1.5}{+3.5}$	+	±	
Sarcoma T 241	1/20	5/20	$\frac{-2}{+1.5}$	±	±	
Sarcoma MA 387	0/20	3/20	$\frac{-1.5}{+2.5}$	±	±	
Adenocarcinoma E 0771	3/40	6/40	$\frac{-2}{+1}$	+	++	Normal growth thereafter
Bashford carcinoma 63	0/30	1/30	$\frac{+0.5}{+5}$	±	++	Normal growth thereafter
Bashford carcinoma 63	0/20	0/20	$\frac{+1\dagger}{+2.5}$	±	+	Normal growth thereafter
Miyono adenocarcinoma	0/30	5/30	$\frac{-1.5}{+1.5}$	+	+	Normal growth thereafter
Ehrlich carcinoma (solid)	0/20	3/20	$\frac{-2.5}{+2.5}$	±	+	Normal growth thereafter
Ehrlich carcinoma (ascitic)	0/30	9/30	$\frac{+2}{+7.5}$	++	++	
Krebs 2 carcinoma (ascitic)	0/20	10/20	$\frac{+1}{+8}$	+	+	
Carcinoma 1025	0/40	7/40	$\frac{-2.5}{+1.5}$	++	+++	65 per cent inhibition at 2d week; 100 per cent at 3d week
Carcinoma 1025	0/20	2/20	$\frac{-1\dagger}{+2.5}$	++	+++	35 per cent inhibition at 2d week; 85 per cent at 3d week
Grand epidermoid carcinoma	0/20	0/20	$\frac{-1}{+3}$	±	+	Normal growth thereafter
Wagner osteogenic sarcoma	1/40	3/40	$\frac{-2}{+1.5}$	±	+	Normal growth thereafter
Ridgway osteogenic sarcoma	0/40	4/40	$\frac{-2}{+1}$	±	+++	79 per cent inhibition at 2d week; 87 per cent at 3d week
Ridgway osteogenic sarcoma	0/10	0/10	$\frac{0\dagger}{+1.5}$	±	++	++ at 3d week
Patterson lymphosarcoma	0/20	1/20	$\frac{-2.5}{+1.5}$	+	±	
Mecca lymphosarcoma	1/40	15/40	$\frac{-2}{+1}$	+	±	
Gardner lymphosarcoma	0/40	2/40	$\frac{-2.5}{+1}$	++	+++	55 per cent inhibition at 2d week; 60 per cent at 3d week
Gardner lymphosarcoma	0/40	0/40	$\frac{-1\dagger}{+1}$	+	+	Normal growth thereafter
Harding-Passey melanoma	2/50	3/50	$\frac{+0.5}{+4.5}$	−	+	++ at 3d week; retarded growth thereafter
Harding-Passey melanoma	0/20	0/20	$\frac{+0.5\dagger}{+5}$	−	+	++ at 3d week; retarded growth thereafter
Andervont hepatoma	0/20	6/20	$\frac{-3}{+1}$	?	?	90 per cent inhibition at 8th week
Lewis bladder carcinoma	0/30	3/30	$\frac{-2}{+1}$	±	+	Normal growth thereafter
Lewis lung carcinoma	1/20	2/20	$\frac{-2.5}{+1}$	±	+	Normal growth thereafter

* Ten animals in each test group. The results presented are the averages of combined groups.
† 2 mg/kg/day.

mouse and of the rat led to the more rigorous test on well established tumors. The results obtained from these experiments are summarized in Table 7 and presented in detail in Tables 8–10. Treatment of 7-day-old carcinoma (C1025), osteogenic sarcomas (Ridgway), and lymphosarcomas (Gardner) with 6 mg/kg/day of TEPA or 4 mg/kg/day of TSPA for 7 consecutive days caused growth to stop for a few days; then the tumors regressed completely (about 60–90 per cent of cases) in 2–3 weeks. There was no reappearance of tumors during experimental periods of 2–3 months, and on autopsy no material was found at the former site of the tumor. Such cases are recorded as "cures."

In contrast to the findings with untreated tumors (Fig. 4), the histological examination of a number of the tumors (Jensen sarcomas) removed from the host after seven injections of 2 mg/kg of TSPA showed almost complete necrosis of the tumor cells as seen in the lower right half of Figure 5. The surrounding tissue (upper left half) is made up of granulation tissue, with a few lympho-

TABLE 6

EFFECT OF TRIETHYLENE THIOPHOSPHORAMIDE ON VARIOUS 1-DAY-OLD RAT TUMORS (dose: 2 mg/kg/day)*

Tumor	No. deaths 1st week	No. deaths 2d week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Remarks
Flexner-Jobling carcinoma	1/30	3/30	$\frac{-8}{+20}$	++	+++	90 per cent inhibition at 2d week; 100 per cent at 3d week
Flexner-Jobling carcinoma	0/10	1/10	$\frac{+5\dagger}{+15}$	++	+++	100 per cent inhibition at 2d and 3d weeks
Walker carcinosarcoma 256	1/30	1/30	$\frac{+5}{+25}$	+	++	50 per cent inhibition at 3d week; 67 per cent at 4th week
Walker carcinosarcoma 256	0/10	0/10	$\frac{+10\dagger}{+26}$	+	++	50 per cent inhibition at 3d week
Jensen sarcoma	0/40	0/40	$\frac{+0.5}{+23}$	++	+++	100 per cent inhibition at 2d and 3d weeks
Jensen sarcoma	0/20	0/20	$\frac{+1\dagger}{+27}$	++	+++	100 per cent inhibition at 2d and 3d weeks
Jensen sarcoma	0/20	0/20	$\frac{+22\ddagger}{+26}$	++	+++	100 per cent inhibition at 2d and 3d weeks
Jensen sarcoma	0/20	1/20	$\frac{+18\S}{+25}$	+	++	25 per cent inhibition at 3d week
Jensen sarcoma	0/20	0/20	$\frac{+26\#}{+24}$	−	−	
Murphy-Sturm lymphosarcoma	0/30	2/30	$\frac{-3}{+18}$	+	±	
Murphy-Sturm lymphosarcoma	0/20	0/20	$\frac{+9\dagger}{+20}$	−	−	

* Ten animals in each test group. The results presented are the averages of combined groups.
† 1 mg/kg/day.
‡ 0.5 mg/kg/day.
§ 0.25 mg/kg/day.
# 0.1 mg/kg/day.

ing experimental periods of 2–3 months, indicating the possibility of permanent regression.

At the lower doses of 2 and 1 mg/kg/day, the TSPA was less effective. Thus, seven treatments with this compound resulted in only a slight to moderate inhibition of tumor growth but with no complete destruction of 7-day-old tumors.

Treatment of 7-day-old carcinomas (Flexner-Jobling) and sarcomas (R 39 and Jensen) with 1.0 mg/kg of TEPA, 1.0–2.5 mg/kg of DEPA, and 2.0 mg/kg of TSPA caused growth to stop for a few days and then complete regressions of the tumors (about 70–90 per cent of cases) in 2–3 weeks. There was no reappearance of tumors during excytes, macrophages, and plasma cells. Tumors with this histological appearance when transplanted into normal rats do not grow.

The Walker carcinosarcoma 256 did not respond as well to the antitumor activity of TEPA and DEPA as did the Flexner-Jobling carcinoma, Sarcoma R 39, and Jensen sarcoma in the rat. At a dose of 1.0 mg/kg/day of TEPA and 1.0–2.5 mg/kg/day of DEPA for 7 days, the growth of 7-day-old tumors was slightly or moderately inhibited but there was no complete tumor regression. TSPA at a level of 2 mg/kg/day had a definite destructive effect on well established 7-day-old Walker carcinosarcoma 256. As indicated in

Tables 8–10, none of the three phosphoramides had an effect on 7-day-old Murphy-Sturm lymphosarcoma. All tumors grew normally.

The study was continued with lower doses of TSPA on Flexner-Jobling carcinoma and Jensen sarcoma in rats. The results of the experiments showed that daily doses of 1 mg/kg of TSPA resulted in complete destruction of 7-day-old tumors in about 70 per cent, whereas injections of 0.5 and 0.25 mg/kg for 7 consecutive days caused much less complete destruction of 7-day-old carcinomas and sarcomas. The average diameters of the Flexner-Jobling carcinoma and Jensen sarcoma, measured 7 days after implantation, were 8.5 × 5.5 × 4 mm. and 20 × 9 × 7 mm., respectively.

During the course of the study we determined the effect of TSPA on the growth of two different tumors growing simultaneously in the same rat. In this case we selected first Jensen sarcoma and Murphy-Sturm lymphosarcoma. The former is sensitive, while the latter is resistant to the compound.

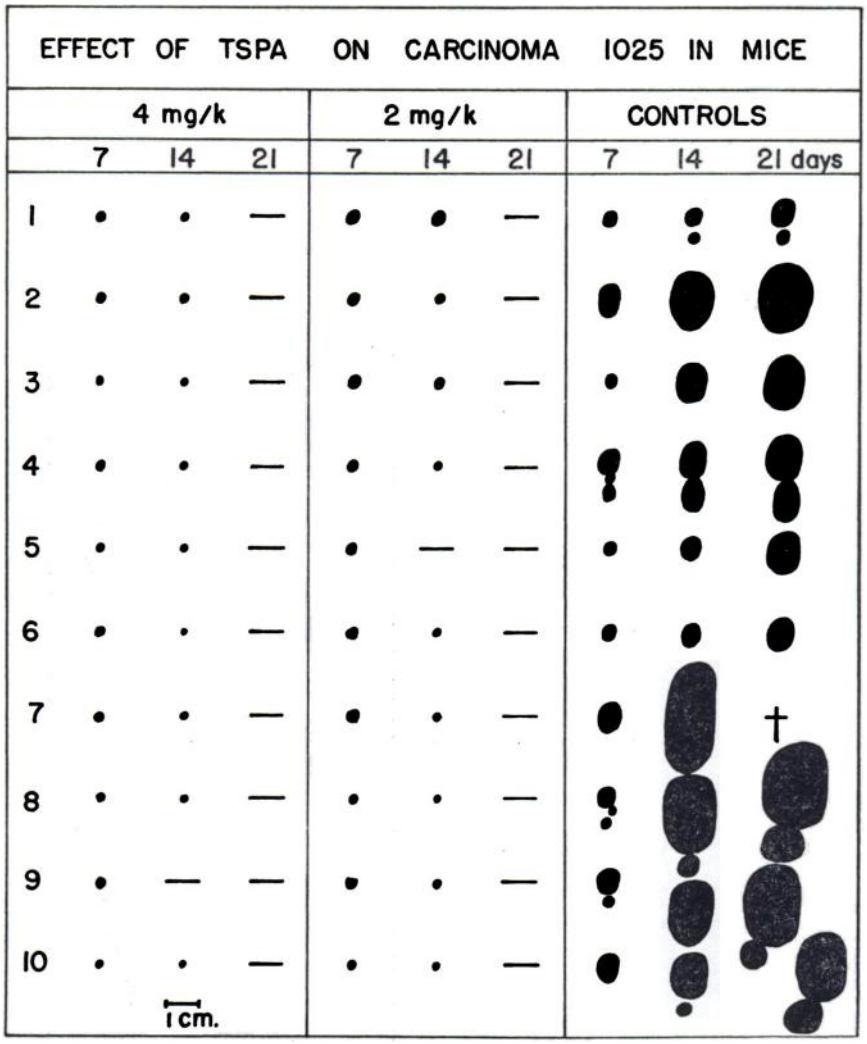

CHART 1.—Carcinoma 1025 in mice after seven daily doses of 4 mg/kg of TSPA, showing complete destruction of tumors. Similar destruction of the tumor resulted from half that dose. Controls, no tumor regression.

Intraperitoneal injections of TSPA at a dose of 2 mg/kg/day were begun when the tumors were 1 day or 7 days old, and injections were continued for 7 days. The experiments showed that TSPA caused complete inhibition of 1-day-old sarcomas in all cases, while all lymphosarcomas continued to grow rapidly. Under similar treatment, more than 80 per cent of 7-day-old Jensen sarcomas regressed

completely, while nearly all of the 7-day-old Murphy-Sturm lymphosarcomas in the same host grew normally.

The above experiments were repeated with Carcinoma 1025 and Sarcoma MA 387, growing simultaneously in the same mouse. Carcinoma 1025 is sensitive, while Sarcoma MA 387 is resistant to TSPA. Repeated intraperitoneal injections of 4 mg/kg of TSPA caused complete destruction of 1- or 7-day-old carcinomas, but the compound had no effect on the sarcomas, all of which were growing rapidly. In both experiments, then, the responses of these tumors to the chemical agent were the same whether they were growing alone in different animals or side by side in the same animal. These findings emphasize the specificity of reaction of these tumors to this chemical agent.

DISCUSSION

It is well known that the response of various tumors to a given agent may be strikingly different (5, 12, 15, 17–19). The present results show that

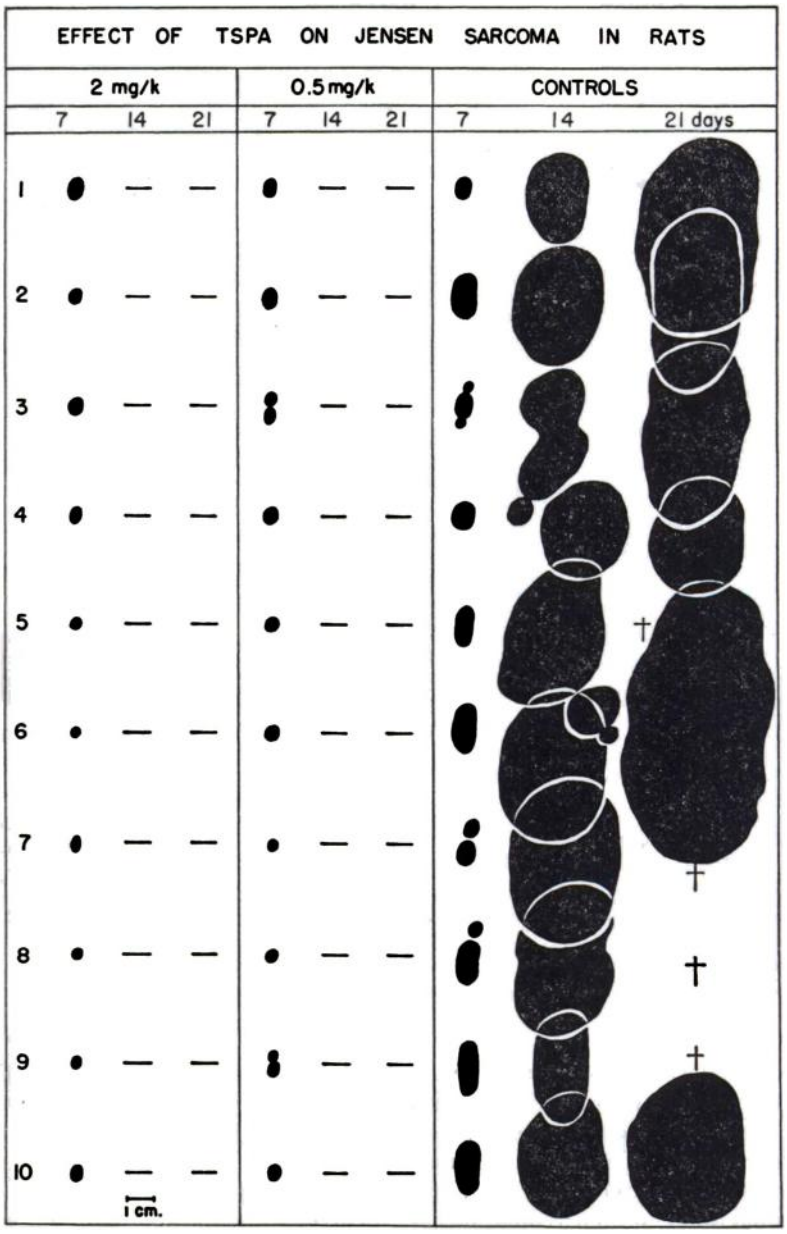

CHART 2.—Jensen sarcoma in rats after seven daily doses of 2 mg/kg of TSPA, showing complete destruction of tumors. Similar destruction of the tumors resulted from ½ that dose. Controls, rapid growth and no tumor regression.

TABLE 7

APPROXIMATE PER CENT OF 7-DAY-OLD MOUSE AND RAT TUMORS CURED BY VARIOUS PHOSPHORAMIDES*

Compound administered	Dose (mg/kg/day)	Tumor	No. treated	No. cured	Per Cent cures
Diethylene phosphoramide	1–2.5	Flexner-Jobling carcinoma	70	56	80
" "	1–2.5	Walker carcinosarcoma 256	50	0	0
" "	1–2.5	Sarcoma R 39	40	26	65
" "	1	Jensen sarcoma	30	24	80
Triethylene phosphoramide	6	Carcinoma 1025	50	41	82
" "	6	Ridgway osteogenic sarcoma	30	25	83
" "	6	Gardner lymphosarcoma	40	32	80
" "	1	Flexner-Jobling carcinoma	70	51	73
" "	1	Walker carcinosarcoma 256	60	0	0
" "	1	Sarcoma R 39	30	28	93
" "	1	Jensen sarcoma	60	45	75
Triethylene thiophosphoramide	4	Carcinoma 1025	60	50	83
" "	4	Ridgway osteogenic sarcoma	70	55	78
" "	4	Gardner lymphosarcoma	70	49	70
" "	2	Flexner-Jobling carcinoma	50	40	80
" "	2	Walker carcinosarcoma 256	50	30	60
" "	1–2	Jensen sarcoma	130	112	86

* The approximate percentage of spontaneous tumor regressions for controls for the experiments in this table are: 2 per cent for Carcinoma 1025, 5 per cent for Ridgway osteogenic sarcoma, 5 per cent for Gardner lymphosarcoma, 15 per cent for Flexner-Jobling carcinoma, 2 per cent for Walker carcinosarcoma 256, 5 per cent for Sarcoma R 39, and 5 per cent for Jensen sarcoma.

TABLE 8

EFFECT OF DIETHYLENE PHOSPHORAMIDE ON WELL ESTABLISHED 7-DAY-OLD RAT TUMORS*

Tumors	Dose (mg/kg/day)	No. deaths 1st week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Tumor regression
Flexner-Jobling carcinoma	2.5	0/40	$\frac{+4}{+24}$	+	+++	75 per cent at 2d week; 90 per cent at 3d week
Flexner-Jobling carcinoma	1.0	0/30	$\frac{+14}{+28}$	+	++	67 per cent at 4th week
Walker carcinosarcoma 256	2.5	0/30	$\frac{+7}{+21}$	±	+	Normal growth thereafter
Walker carcinosarcoma 256	1.0	0/20	$\frac{+15}{+35}$	±	+	Normal growth thereafter
Sarcoma R 39	2.5	0/20	$\frac{+7}{+25}$	+	+++	60 per cent at 2d week; 100 per cent at 3d week
Sarcoma R 39	1.0	2/20	$\frac{+10}{+24}$	+	+++	35 per cent at 2d week; 50 per cent at 3d week
Jensen sarcoma	1.0	2/30	$\frac{+7}{+28}$	++	+++	50 per cent at 2d week; 87 per cent at 3d week
Murphy-Sturm lymphosarcoma	1.0	0/20	$\frac{+17}{+22}$	−	−	

* Ten animals in each group. The results presented are the average of combined groups.

TABLE 9

EFFECT OF TRIETHYLENE PHOSPHORAMIDE ON WELL ESTABLISHED 7-DAY-OLD MOUSE AND RAT TUMORS*

Tumor	Dose (mg/kg/day)	No. deaths 1st week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Tumor regressions
Carcinoma 1025	6.0	0/50	$\frac{-3}{+1.5}$	++	+++	30 per cent at 2d week; 85 per cent at 3d week
Ridgway osteogenic sarcoma	6.0	2/30	$\frac{-3.5}{+1.5}$	++	+++	50 per cent at 2d week; 87 per cent at 3d week
Gardner lymphosarcoma	6.0	2/40	$\frac{+0.5}{+3}$	+++	+++	65 per cent at 1st week; 85 per cent at 2d week
Gardner lymphosarcoma	4.0	0/10	$\frac{+1}{+5}$	+++	+++	40 per cent at 1st week and 2d week
Flexner-Jobling carcinoma	1.0	1/70	$\frac{+7}{+27}$	++	+++	40 per cent at 2d week; 77 per cent at 3d week
Walker carcinosarcoma 256	1.0	0/60	$\frac{+8}{+27}$	+	+	Normal growth thereafter
Sarcoma R 39	1.0	0/30	$\frac{+17}{+30}$	++	+++	70 per cent at 2d week; 93 per cent at 3d week
Jensen sarcoma	1.0	2/60	$\frac{+11}{+24}$	++	+++	42 per cent at 2d week; 74 per cent at 3d week
Murphy-Sturm lymphosarcoma	1.0	0/20	$\frac{+8}{+16}$	−	−	

* Ten animals in each group. The results presented are the average of combined groups.

this holds true for diethylene phosphoramide, triethylene phosphoramide, and triethylene thiophosphoramide. These compounds produced complete and permanent regression of two mouse tumors, Ridgway osteogenic sarcoma and Gardner lymphosarcoma (6C3HED), which hitherto have not been amenable to chemical cure. Previously, it has been found in our laboratories that Sarcoma 180 has been cured by 6-mercaptopurine and that Carcinoma 1025 has been cured by 3-bis(β-chloroethyl)aminomethyl-4-methoxymethyl-5-hydroxy-6-methyl pyridine and by triethylene melamine. These results encourage the hope that more chemicals may be found which have a destructive action specific for different tumors.

The differences in the effectiveness of DEPA,

TABLE 10

EFFECT OF TRIETHYLENE THIOPHOSPHORAMIDE ON WELL ESTABLISHED 7-DAY-OLD MOUSE AND RAT TUMORS*

Tumor	Dose (mg/kg/day)	No. deaths 1st week	Av. wt. change treated/controls (gm.)	Results of treatment 1st week	Results of treatment 2d week	Tumor regression
Sarcoma 180	4.0	0/20	$\frac{-1.5}{+1.5}$	−	−	
Bashford carcinoma	4.0	0/20	$\frac{-0.5}{+1}$	−	−	
Carcinoma 1025	4.0	1/60	$\frac{-2.5}{+1.5}$	++	+++	44 per cent at 2d week; 87 per cent at 3d week
Carcinoma 1025	2.0	0/10	$\frac{-1.5}{+1}$	++	++	25 per cent at 3d week
Carcinoma 1025	1.0	0/10	$\frac{0}{+1}$	++	+	Normal growth thereafter
Ridgway osteogenic sarcoma	4.0	1/70	$\frac{-2.5}{+1.5}$	++	+++	60 per cent at 2d week; 80 per cent at 3d week
Ridgway osteogenic sarcoma	2.0	0/20	$\frac{0}{+1.5}$	+	+	Normal growth thereafter
Ridgway osteogenic sarcoma	1.0	0/20	$\frac{+1}{+1}$	±	+	Normal growth thereafter
Gardner lymphosarcoma	4.0	1/70	$\frac{-1}{+4}$	++	+++	50 per cent at 2d week; 70 per cent at 3d week
Gardner lymphosarcoma	2.0	0/20	$\frac{+2}{+4}$	±	±	
Gardner lymphosarcoma	1.0	0/10	$\frac{+5}{+7}$	−	−	
Flexner-Jobling carcinoma	2.0	0/50	$\frac{-2}{+26}$	++	+++	70 per cent at 2d week; 80 per cent at 3d week
Flexner-Jobling carcinoma	1.0	0/20	$\frac{+13}{+18}$	++	++	75 per cent at 3d week
Flexner-Jobling carcinoma	0.5	0/20	$\frac{+22}{+27}$	±	++	40 per cent at 3d week
Walker carcinosarcoma 256	2.0	2/50	$\frac{0}{+35}$	+	+++	38 per cent at 2d week; 60 per cent at 3d week
Walker carcinosarcoma 256	1.0	0/20	$\frac{+8}{+28}$	+	++	30 per cent at 3d week
Walker carcinosarcoma 256	0.5	0/20	$\frac{+23}{+31}$	+	+	Normal growth thereafter
Jensen sarcoma	2.0	15/90	$\frac{-1}{+20}$	++	+++	85 per cent at 2d week; 95 per cent at 3d week
Jensen sarcoma	1.0	2/40	$\frac{0}{+26}$	++	+++	67 per cent at 2d week; 80 per cent at 3d week
Jensen sarcoma	0.5	0/20	$\frac{+7}{+26}$	++	+++	40 per cent at 2d week; 65 per cent at 3d week
Jensen sarcoma	0.25	0/20	$\frac{+23}{+31}$	+	++	40 per cent at 3d week
Murphy-Sturm lymphosarcoma	2.0	0/30	$\frac{+4}{+22}$	−	−	
Murphy-Sturm lymphosarcoma	1.0	0/10	$\frac{+8}{+28}$	−	−	

* Ten animals in each group. The results presented are the average of combined groups.

TEPA, and TSPA against the various experimental tumors merit discussion. Thus, both TEPA and TSPA are more effective against all four of the mouse mammary carcinomas tested than against two of the three sarcomas. Similarly, these compounds showed a greater inhibitory effect on the development of Ehrlich ascites carcinoma and Krebs 2 ascites carcinoma (both mammary cancer origin) than on Sarcoma 180 ascites tumor. Other epithelial tumors which responded well to treatment with TEPA and TSPA were Carcinoma 1025, Grand epidermoid carcinoma, Lewis bladder carcinoma, and Lewis lung carcinoma.

It is interesting to note that TEPA and TSPA had a destructive effect on the well established Ridgway osteogenic sarcoma but only a slight inhibitory effect on the Wagner osteogenic sarcoma. Both tumors arose spontaneously in AKR mice (17). Transplants of these tumors grow rapidly in AKR mice and kill animals in 3–4 weeks. Morphologically these tumors are similar. However, the alkaline phosphatase content of Ridgway osteogenic sarcoma is less than that of Wagner osteogenic sarcoma—about 20 units of alkaline glycerophosphatase/gm against about 50 units.

There is a striking difference in the response of different mouse lymphosarcomas toward the action of TEPA and TSPA. Although these compounds had a destructive effect on 7-day-old Gardner lymphosarcoma (about 60 per cent), they had relatively no effect on the growth of the Patterson and Mecca lymphosarcomas. Therefore, we attempted to discover any difference in biological behavior or in histological picture among them which might be responsible for the different sensitivity to antitumor action of TEPA and TSPA. Gardner lymphosarcoma arose in a C3H mouse (19), while the Patterson and Mecca lymphosarcomas arose in AKR mice (17). Histological examinations of these tumors revealed that Gardner lymphosarcoma is composed almost exclusively of large immature lymphocytes, while the Patterson and Mecca lymphosarcomas are composed almost exclusively of large lymphocytes. The Gardner lymphosarcoma does not metastasize into the liver, spleen, or mesentery, although it metastasizes into the lymph nodes. On the other hand, the Patterson and Mecca lymphosarcomas metastasize into lymph nodes, spleen, liver, kidney, and mesentery, indicating that these lymphosarcomas are more malignant than the Gardner lymphosarcoma. The radiosensitivity of Gardner lymphosarcoma and Mecca lymphosarcoma *in vivo* appeared to be the same. It was found that a high percentage of 1-day-old tumors irradiated with 3,000 r failed to grow, whereas tumors irradiated at a dose of 2,000 r grew in almost all cases. Thus, it is possible that the natural histories of the tumors contributed to the differences in their response to these agents.

In general, the rat tumors were more susceptible to the phosphoramides than the mouse tumors. This is similar to our findings with aminopterin (16), A-methopterin (unpublished data), HN2 (17), 3-bis(β-chloroethyl)aminomethyl-4-methoxymethyl-5-hydroxy-6-methylpyridine (17), triethylene melamine (19), and 1,9-dimethanesulfonyloxynonane (12). Complete regression was obtained in 30–100 per cent of well established 7-day-old tumors, namely, Flexner-Jobling carcinoma, Walker carcinosarcoma 256, Sarcoma R 39, and Jensen sarcoma, by administration of the above-mentioned nine compounds. The Murphy-Sturm rat lymphosarcoma appeared insensitive to the phosphoramides, as it did in all comparable studies thus far (12, 14, 18, 19). Particularly interesting is the fact that triethylene thiophosphoramide resulted in 48 per cent cures of Walker carcinosarcoma 256, which was completely resistant to TEPA and DEPA, related chemicals. Replacement of the oxygen in the TEPA molecule by sulfur resulted in an increase in antitumor activity.

SUMMARY

1. The effects of diethylene phosphoramide (DEPA), triethylene phosphoramide (TEPA), and triethylene thiophosphoramide (TSPA) have been tested against a spectrum of eighteen tumors of the mouse, five tumors of the rat, and three ascites tumors of the mouse.

2. Daily maximum tolerated doses of 6 mg/kg of TEPA in mice had a destructive effect on Carcinoma 1025, Ridgway osteogenic sarcoma, and Andervont hepatoma; a marked inhibitory effect on Miyono adenocarcinoma and Gardner lymphosarcoma; a moderate inhibitory effect on Sarcoma 180, Adenocarcinoma E 0771, Ehrlich carcinoma, Grand epidermoid carcinoma, Wagner osteogenic sarcoma, Lewis bladder carcinoma, and Lewis lung carcinoma; a slight inhibitory effect on Sarcoma MA 387, Bashford carcinoma 63, and Harding-Passey melanoma, but no effect on Sarcoma T 241.

3. Daily maximum tolerated doses of 4 mg/kg of TSPA in mice had a destructive effect on Carcinoma 1025, Ridgway osteogenic sarcoma, Gardner lymphosarcoma, and Andervont hepatoma; a marked inhibitory effect on Adenocarcinoma E 0771 and Bashford carcinoma 63; a moderate inhibitory effect on Sarcoma 180, Miyono adenocarcinoma, Ehrlich carcinoma, Grand epidermoid carcinoma, Wagner osteogenic sarcoma, Harding-Passey melanoma, Lewis bladder carcinoma, and

Lewis lung carcinoma, and a slight inhibitory effect on Sarcoma T 241, Sarcoma MA 387, Patterson lymphosarcoma, and Mecca lymphosarcoma.

4. The effects of TEPA and TSPA on these eighteen mouse tumors were generally similar, and these compounds were far more effective than DEPA.

5. Both TEPA and TSPA had a marked inhibitory effect upon the development of Ehrlich ascites carcinoma and Krebs 2 ascites carcinoma, but only a slight inhibitory effect on Sarcoma 180 ascites tumor. DEPA had relatively no effect on these three ascites tumors.

6. The growth of spontaneous mammary adenocarcinomas in Swiss albino mice was stopped by repeated injections of TSPA, but there was no significant increase in complete tumor regression over the controls.

7. Daily doses of 1 mg/kg of DEPA, 1 mg/kg of TEPA, and 2 mg/kg of TSPA in rats had a destructive effect on 1-day-old implants of Flexner-Jobling carcinoma, Walker carcinosarcoma 256, Sarcoma R 39, and Jensen sarcoma, but no effect on Murphy-Sturm lymphosarcoma.

8. Complete regression was obtained in large numbers of certain well established 7-day-old mouse and rat tumors by administration of seven daily doses of DEPA, TEPA, and TSPA. For rats, daily doses of 1–2.5 mg/kg of DEPA gave 80 per cent regressions for Flexner-Jobling carcinoma, 65 per cent for Sarcoma R 39, and 80 per cent for Jensen sarcoma. For mice, daily doses of 6 mg/kg of TEPA gave 82 per cent regressions for Carcinoma 1025, 83 per cent for Ridgway osteogenic sarcoma, and 80 per cent for Gardner lymphosarcoma. Similarly, for rats, daily doses of 1 mg/kg of TEPA gave 73 per cent regressions for Flexner-Jobling carcinoma, 93 per cent for Sarcoma R 39, and 75 per cent for Jensen sarcoma. Similarly, for mice, daily doses of 4 mg/kg of TSPA gave 88 per cent regressions for Carcinoma 1025, 78 per cent for Ridgway osteogenic sarcoma, and 70 per cent for Gardner lymphosarcoma. For rats, daily doses of 2 mg/kg gave 80 per cent regressions for Flexner-Jobling carcinoma, 60 per cent for Walker carcinosarcoma 256, and 86 per cent for Jensen sarcoma.

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Fig. 1.—Section of Grand mouse epidermoid carcinoma, showing early pearl formation. (H & E ×200.)

Fig. 2.—A section of the Lewis mouse bladder carcinoma, showing a compact sheet of cells having vesicular nuclei with irregularity in size and shape, and numerous mitoses. (×200.)

Fig. 3.—A section of the Lewis mouse lung carcinoma, showing a sheet of poorly differentiated epidermoid carcinoma. The tumor cells have large nuclei of irregular shape and varying chromosia. (×200.)

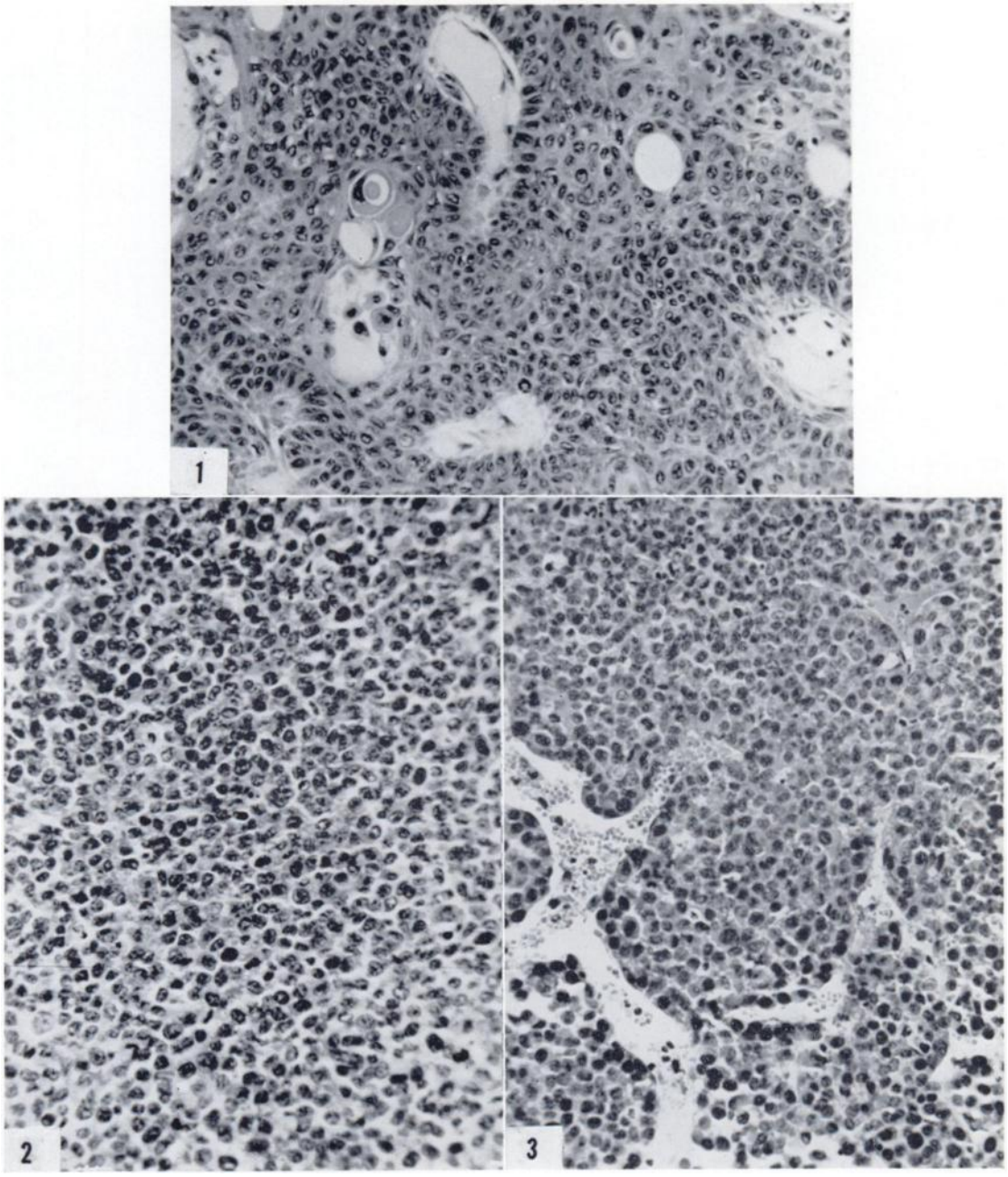
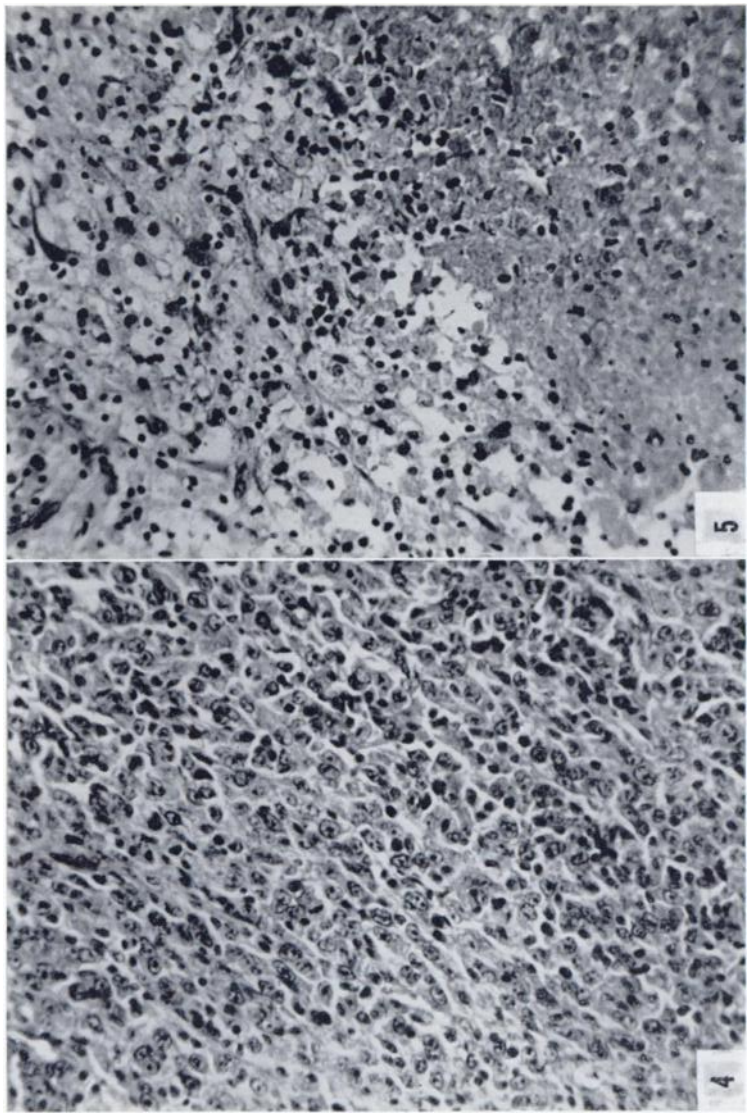


FIG. 4.—Section of untreated Jensen rat sarcoma, showing numerous spindle cells with voluminous cytoplasm. (×200.)

FIG. 5.—Jensen sarcoma after treatment with TSPA (2 mg/kg for 7 days). Shows complete necrosis of the tumor cells (as seen in the lower right half of the picture). The surrounding tissue (upper left half) is made up of granulation tissue with a few lymphocytes, macrophages, and plasma cells. (×200.)



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