5. Schubert, R., *Deutsche Med. Wochschr.*, 1951, v76, 1487.

6. Krech, U., *Z. f. Immunitatsforschung*, 1952, v109, 177.

7. Bickel, E., and Dieckhoff, J., *Z. ges. inn. Med. u. Grenzgeb.*, 1955, v3, 116.

8. Bickel, E., and Dieckhoff, J., *Z. f. Kinderheilkunde*, 1951, v2, 177.

9. Schubert, R., *Deutsche Med. Wochschr.*, 1954, v5, 179.

10. Verse, H., *Z. f. Kinderheilkunde*, 1954, v74, 559.

11. Hummel, K., *Z. f. Immunitatsforschung u. exp. Ther.*, 1955, v4, 309.

12. Bick, H., Halberg, F., and Spink, W. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 7.

13. Dove, B. T., Borkar, S. A., Joag, G. G., Lewis, R. A., and Patel, J. C., *Exp. Med. Surg.*, 1955, v13, 293.

Received December 26, 1956. P.S.E.B.M, 1957, v94.

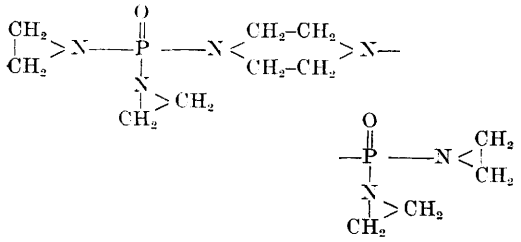
IV. Chemotherapy of Tumors in Rats with Piperazine-1,4-*bis* (N,N'-diethylenephosphondiamide).* (22956)

M. L. CROSSLEY, J. B. ALLISON, D. R. SEEGER AND E. KUH

Bureau of Biological Research, Rutgers University, New Brunswick, N. J., and Calco Chemical Division, Am. Cyanamid Co., Bound Brook, N. J.

This investigation is a continuation of the study of ethylenephosphoramides in the treatment of transplantable tumors in rats (1).

Materials and methods. Piperazine-1,4-*bis*(N,N' - diethylenephosphondiamide), (PDA) is a white crystalline compound; m.p. 185-186°C; very soluble in water and alcohol but almost insoluble in acetone and ether. It is slightly hygroscopic. Its structural formula is:



It has been reported to be active against a granulocytic chloroleukemia of the rat.† Adult, male Sprague-Dawley and Wistar rats, averaging about 150 g were used. In each experiment there were, as a rule, normal control (NC), tumor control (TC), drug control (DC), and tumor treated (TT) groups consisting of 8-10 animals each. The number of groups varied with dose levels of the drug chosen for comparison in any one experiment. An isotonic saline solution of the drug prepared weekly and kept at about 5°C, was given in one daily dose by intraperitoneal injection, the volume being that required to provide the desired dose in mg/kg of body weight of each rat at time of treatment. The Flexner-Jobling carcinoma and the Walker 256 carcinosarcoma were studied in Sprague-Dawley rats, the sarcoma Rl in Wistar rats. Methods of implantation and measurement of growth were essentially the same as those previously described(2). The animals were weighed periodically, length and width of the tumors measured in millimeters and size expressed as mm^2. Therapeutic effectiveness was determined by comparing average size of tumors of treated groups with that of untreated controls. When possible tumor weights were also compared. Regression was considered complete when the tumor was no longer palpable, no regrowth occurred in about 2 weeks, and gross examination at autopsy showed no evidence of tumors. Treatment of rats with Flexner-Jobling and Rl tumors began 5 days after implantation and with Walker Carcinosarcoma 256 3 days. The rats were group pair-fed, using 18%

* This investigation was supported in part by an Institutional Grant from Am. Cancer Soc.

† Sparks, S. *et al.* abstract published.

TABLE I. Effect of PDA on Sprague-Dawley Rats, with and without Flexner-Jobling Tumor.

Rat group*	Dose, mg/kg/D	Days after implantation	Days treated	% carcass gain	g tumor/100 g carcass wt	% regressions	% survival	g organs/100 g body wt	
								Spleen	Testes
NC		22		54			100		1.32
TC		26		48	14.0	0	90	1.20	1.07
DC	.2		22	50			100		1.37
TT	.2	40	35	72	12.5	30	100	.40	.77
NC		58		105			100	.19	.95
TC		34		66	20.5	0	90	.97	1.00
DC	.5		53	104			100	.31	.74
DC	1.0	58	53	105			80	.16	.54
TT	.5	58	53	109	3.0	30	85	.32	.79
TT	1.0	58	53	107	0.7	30	90	.34	.58
NC		29		31			100	.26	
TC		29		39	12.0	0	80	.80	
TT	3.0	29	24	25	0.3	90	100		
TT	3.0	43	38	56	0.2	90	90	.33	.56
NC		36		86			100	.21	1.23
TC		36		50	12.0	0	70	.67	1.17
DC	3.5	36	31	76			80	.18	.59
TT	3.5	36	31	61	1.0	85	70	.35	

* NC, normal controls; TC, tumor controls; DC, drug controls; TT, tumor treated.

casein diet(3). The duration of the experiments was usually from 4-6 weeks. Blood counts were taken for certain groups, particularly the normal and the drug controls, before treatment began and again just before it ended. This and other indications of toxicity, such as carcass weight change, development of anemia, and general well being of the animals were recorded. Organ weights were expressed in g/100 g of gross body weight. Weight of tumor is given in g/100 g of carcass weight. All the data are from group averages.

Results. Sprague-Dawley rats given doses of 0.2 to 1.0 mg/kg/D showed no significant leukopenia or other toxic symptoms after 20-30 days of continuous treatment. After 53 days treatment, there was a significant drop in testes weight of 30-40%. With doses of 2.0-4.5 mg/kg/D there was a drop in white blood cell counts after 30-40 days of about 40% but it was no greater for the larger dose than for the smaller.

Animals bearing the Flexner-Jobling carcinoma were treated with doses of .2, .5, 1.0, 3. and 3.5 mg/kg/D for periods of 24-53 days. The tumor regressed completely in about 30% of the animals receiving the 0.2 mg dose after about 6 weeks. At this time another 20% had very small tumors, averaging less than 0.5 g/100 g carcass weight. In the remaining 50%, tumor growth seemed to be unrestrained by the drug. The tumor averaged 25 g/100 g carcass weight after 35 days of treatment (DT) or 40 days after implantation (DaI). The tumors were affected in 100% of the animals treated with 0.5 and 1.0 mg/kg/D for 53 days; 30% regressed completely; the remaining 70% regressed to a very small size averaging 3 g and 0.7 g/100 g carcass weight respectively. Occasionally this tumor in the Sprague-Dawley rat will regress spontaneously in a limited number of cases. Under the conditions of this experiment there were no regressions among the tumor controls in 34 days when the tumors were large, averaging 21 g/100 g carcass weight. With a dose of 3 mg/kg/D the tumors regressed completely in about 90% of the animals, the remainder diminishing to very small size. There was little difference in the results when treatment was prolonged from 24-38 days. Similar results were obtained with 3.5 mg/kg/D for 31 days. This piperazine compound has a wide therapeutic range which makes it interesting for further investigation.

The Rl sarcoma was retarded in growth by doses of 1.5 and 3.0 mg/kg/D but none of the tumors regressed. Treated animals lived longer and their carcass weight gain was greater than that of the tumor controls. In

2 weeks the results of treatment were:

Group	Dose, mg/kg/D	g tumor per 100 g carcass wt	% carcass gain
TC		20	3
TT	1.5	14	15
TT	3.0	9	27

A dose of 0.5 mg/kg/D did not affect growth of the Walker carcinosarcoma and 1 mg/kg D caused only slight retardation in tumor growth. With a dose of 3 mg/kg D, tumor growth was considerably retarded and survival time of the experimental animals lengthened. In 24 days of treatment, the tumor averaged 5 g/100 g carcass weight, in comparison with 42 g for the TC group 15 days after implantation. Treatment with a dose of 4.5 mg/kg/D inhibited growth of tumors for a time, but after 2-3 weeks growth resumed and continued at a rapid rate in spite of daily therapy. Ultimately tumors in treated rats grew to be as large as those in the controls, which, however, died in much shorter time. The tumor became resistant to the drug. In becoming resistant it seemed to have changed its character permanently and its growth was not thereafter inhibited by the same dose, even after 5 serial transplantations into new hosts. The tumor which had become resistant to the 4.5 mg/kg/D dose was taken for serial transplantation just after growth resumed and when it was still small. The treatment each time was with a daily dose of 4.5 mg/kg. Treated animals were the donors of the tissue for the next transplant. Growth of the tumor in treated rats was retarded each time in comparison with that in untreated, but not inhibited. It has been previously reported that the Walker carcinosarcoma developed resistance to TEPA and MEPA(4).

In these experiments the treatment had been continuous, *i.e.*, daily and with the same dose of drug throughout. It was desirable to know if this was necessary and if there was any difference in resistance to the drug when it was administered for a shorter time. It was also of interest to know if regression of growth was influenced by following the early treatment of a large dose, with a small maintenance dose of either the same drug or another of the same series. Forty Sprague-Dawley rats were implanted with the Walker tumor and divided into 4 groups of 10 rats each. Group I was treated with 4.5 mg/kg/D of PDA for 4 weeks. Group II received treatment for 2 weeks. Group III was treated with the same dose for 2 weeks, then the drug was changed to TSPA, in doses of .4 mg/kg/D for 2 weeks. Group IV was untreated. The effect on growth of the tumor was as shown in Table II.

TABLE II. Tumor Size, mm^2 (W-256).

Days treated	I	II	III	IV
3	142	182	107	193
5	187	148	110	551
7	191	144	129	1480
12	273	252	170	2853
17	316	334	192	5368
21	342	427	247	
24	354	469	331	
27	785	987	456	

The period of growth inhibition was about the same in the 3 cases. The tumors became resistant in all 3 treated groups. It was possible that the processes of resistance had already set in during the 2 weeks treatment with the 4.5 mg/kg/D dose. Treatment with 4.5 mg/kg/D for 1 week, followed by maintenance dose of 1 mg/kg/D for 3 weeks did not prove to be adequate for growth inhibition.

With a dose large enough, growth of this tumor was inhibited when treatment was started 3 days after implantation. An important question was the behavior of the tumor when treatment was delayed. With tumors permitted to grow to a size about 500 mm^2, before treatment began, a daily dose of 4.5 mg/kg/D, inhibited growth for a few days but later resistance developed.

Summary. 1. Piperazine-1, 4-*bis*(N,N'-diethylenephosphondiamide) (PDA) in doses up to 1 mg/kg/D for 4-6 weeks produced no significant toxic reaction in rats: at higher doses there was some leukopenia but it was not serious, even with doses of 3.0-4.5 mg/kg/D administered for 4-6 weeks. 2. Flexner-Jobling carcinoma regressed completely in 30% of rats treated with .2 mg/kg/D of PDA and in about 90% of those with 3.5 mg/

kg/D. There were no regressions among the controls 34 days after implantation. 3. Growth of sarcoma Rl in Wistar rats was retarded about 50% by the product at 3 mg/kg/D. 4. Walker 256 carcinosarcoma was retarded in growth by doses of 1-3 mg/kg/D and inhibited for about 2-3 weeks with a dose of 4.5 mg/kg/D. 5. After initial period of growth inhibition the Walker tumor became resistant to the drug and then grew rapidly in spite of continued therapy.

We acknowledge with deep gratitude our indebtedness to Frances S. Jencks for technical assistance with the biological experiments.

1. Crossley, M. L., Allison, J. B., Parker, R. P., Kuh, E., and Seeger, D. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 438.

2. Crossley, M. L., Allison, J. B., Wainio, W. W., and Muenzen, J. B., *J. Nat. Cancer Inst.*, 1951, v12, 305.

3. Wase, A. W., and Allison, J. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 147.

4. Jackson, H., *Brit. J. Cancer,* 1954, v8, 336.

Received June 15, 1956. P.S.E.B.M., 1957, v94.

Effect of Preweanling Administration of Sucrose on Subsequent Caries.* (22957)

RALPH R. STEINMAN AND MELVIN I. HALEY. (Introduced by U. D. Register)
School of Dentistry and Department of Biochemistry, College of Medical Evangelists, Loma Linda, Calif.

Sognnaes(1,2) indicated that ingestion of sucrose by dams of rats and monkeys during the calcification of teeth of the young may affect their subsequent caries experience. In another study(3) ashed laboratory chow, fed to pregnant rats, decreased the amount of decay in the offspring. This observation might suggest that the increased caries, using the refined diet alone, was due to lack of some important constituent in the diet. The technic developed by the authors† in which various food components were given directly to the young during the suckling period was very useful in studying the systemic effect of diet in caries. When using this technic various carbohydrates given to the suckling young in doses of approximately 1 to 1000 by body weight produced a marked increase in decay of teeth undergoing calcification. Of the carbohydrates used from the 2nd day following birth to weaning, sucrose produced the most consistent increase in caries. The present study was designed to show the time during suckling period that these changes take place.

Method. Thirty-six new born caries-susceptible rats from 6 litters were divided into 4 equal groups. Twenty percent sucrose solutions were administered orally, using amounts not greater than 1 to 1000 of body weight. One-fourth of this group received drops 3 times a day from the day following birth through the 6th day. One-fourth received drops from the 7th through the 13th day. The next group received drops 3 times a day from 14 through the 21st day. The last 4th received only maternal milk throughout the suckling period. All animals received mothers milk *ad libitum* during the suckling period. When weaned at 21 days of age all animals were placed on the cariogenic diet of the following composition: Casein 24, sucrose 65, salts U.S.P. XIV 4, corn oil 5, dehydrated whole liver 4, vitamin mix(4) 2, choline 0.2 parts.

Animals were sacrificed at the end of 13 weeks and the molars examined by grinding successive planes parallel to the occlusal surface. Individual charts which showed the relationship of the enamel, dentin, and the pulp

* This investigation was supported by a USPHS grant.

† Reported at Internat. Assn. for Dental Research, 1956 meeting.