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Effect of a Series of Ethylenimine Derivatives against Metastasizing Mammary Adenocarcinoma of the Rat. (19961)

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Two N-ethylene substituted phosphoramides were reported by Buckley *et al.*(1), Burchenal *et al.*(2) and Lewis *et al.*(3) to be as effective as Triethylene melamine in retarding the growth of a number of experimental tumors. The inhibitory effect of a large series of ethylenimine derivatives against a wide tumor spectrum has been reported independently by this laboratory(4,5).

The purpose of the studies reported here was to select ethylenimine derivatives* which markedly inhibit the metastasizing tendency of the mammary adenocarcinoma of the Hooded Brown Rat.

Materials and methods. Approximately 2 x 2 mm fragments of mammary adenocarcinoma, R2426, were implanted aseptically by trocar into the subcutaneous tissue of the right side of inbred Hooded rats weighing 100-120 g. The rats were weighed, housed individually and arranged 7 females and 8 males per group. Treatments were started 48 hours after implantation. Included in each experiment were one saline-treated group that served as a negative control, and one Triethylene-melamine-treated group that served as a positive control. A synthetic diet(5) and water were given *ad libitum*. The compounds were prepared in buffered saline and the concentrations used as indicated in Table I. Injections were administered intraperitoneally daily or every other day in a volume of 0.5-1.0 ml. The period of treatment was approximately 42 days. At the end of this time all animals were sacrificed and the lungs excised. Observations were made on the gross metastatic lesions of the lungs and a section† was fixed for histological study. The inhibitory effects on the primary tumor have been reported previously(5).

Results. The pooled results of several experiments in Table I indicate that the 5 compounds at the levels tested markedly reduce the incidence of metastases to the lungs. Fig. 1 illustrates the effect of these compounds on the gross metastatic tumors of the lung. The control lungs on the left show the typical metastatic growths. On the right is shown typical treated lungs with no visible metastatic lesions. Histologically the control lungs show tumor masses so extensive that the normal architecture of the lungs has been nearly destroyed. The lungs of the treated animals

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TABLE I. Effect of Phosphoramide Series on Lung Metastases.

Chemical No.	Chemical name	Treatment, mg/kg	No. T c M / No. T. rats	No. K c M / No. K rats	% T c M / % K c M
3155C	*N,N',N"*-Triethylenephosphoramide	.25– .5	17/37	25/36	46/70
3172C	*N,N',N"*-Tris(1-methylethylene)phosphoramide	2.5 –10	2/17	12/24	10/50
3173C	*N*-(3-Oxapentamethylene)-*N'*, N"-diethylenephosphoramide	1.75– 2.5	10/24	14/21	38/67
3193C	*N,N',N"*-Triethylenethiophosphoramide	.5 – 1	6/24	17/23	25/74
3066C	Triethylene melamine	.1	11/49	35/57	18/58

K = Control. M = Metastases. T = Treated.

have only a few sparsely scattered viable tumor cells in close proximity to the larger blood vessels.

Fifty to 74% of the negative control rats developed gross metastases in the lungs 6.5 weeks after the implantation of the primary tumor. In the case of the rats treated with the ethylenimine derivatives, only 10-46% had small gross metastatic lesions.

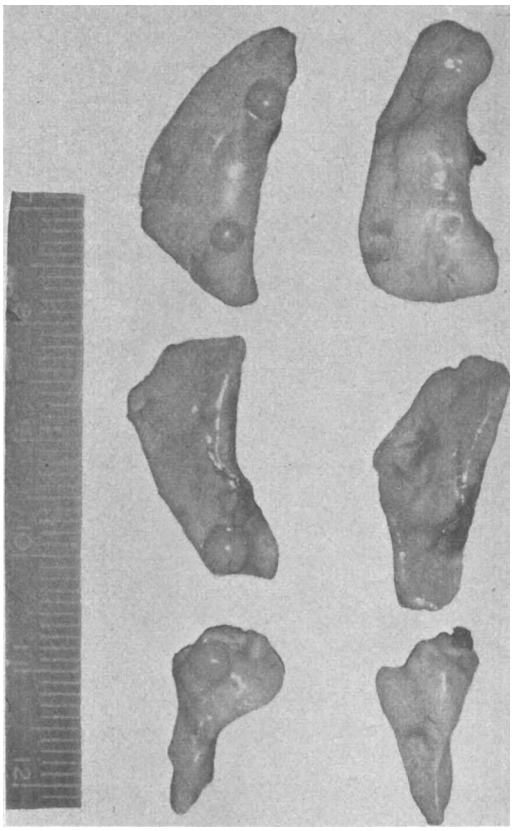

FIG. 1. Excised lung. Left row, untreated; right row, treated.

The chemicals greatly reduced the size of the primary tumors.

Discussion. All of the compounds of this series significantly affected the development of the metastatic foci in the lungs. The most potent one, unfortunately, is unstable. However two of the chemicals, *N*-(3-Oxapentamethylene)*N',N"* - diethylenephosphoramide (3173C) and *N,N',N"*-Triethylenethiophosphoramide (3193C), are stable and compare favorably with compounds now showing clinical promise(6,7).

Summary. 1. A method has been developed for testing chemicals against the secondary metastatic foci of the mammary tumor. 2. Five compounds were found to be active in inhibiting the development of metastatic foci. *N,N',N"*-Tris(1-methylethylene) phosphoramide and *N,N',N"*-Triethylenethiophosphoramide were most active whereas the others are only moderately so.

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Combination Chemotherapy of Cancer: Potentiation of Carcinostatic Activity of 8-Azaguanine by 6-Formylpteridine.* (19962)

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In a previous publication(1), it was reported that combination therapy with folic acid and 8-azaguanine produced greater carcinostasis than did therapy using 8-azaguanine alone. Folic acid itself caused no inhibition of the growth of the 755 mouse breast carcinoma used in these experiments. Insight into the mechanism of action whereby the carcinostatic activity of 8-azaguanine is augmented by folic acid was obtained through the observation that mammalian tissues contain enzymes capable of deaminating 8-azaguanine to 8-azaxanthine(2-4). Unpublished experiments evaluating the latter compound against tumor growth revealed this deaminated product of 8-azaguanine to be inactive as a carcinostatic agent. These initial observations have recently been confirmed and extended (4).

It was therefore hypothesized that folic acid or its degradation product 2-amino-4-hydroxy-6-formylpteridine (6-formylpteridine) inhibited the enzymatic deamination of 8-azaguanine *in vivo*, preventing the conversion of this carcinostatic agent into the non-carcinostatic compound, 8-azaxanthine(1). *In vitro* demonstration of the inhibitory effect of folic acid upon the enzymatic deamination of guanine and 8-azaguanine has been obtained (2). Evidence is presented in this report that (a) 6-formylpteridine augments the carcinostatic activity of 8-azaguanine, and (b) that this compound inhibits the enzymatic deamination of 8-azaguanine by tumor extracts.

Experimental. In vivo studies. The 755 tumor, a mammary adenocarcinoma, was subcutaneously transplanted to the axillary region of C_{57} black male mice by the usual trocar technic. The mice, weighing 18-25 g, and from 2 to 3 months of age received an *ad libitum* diet of Rockland pellets and water. The preparation of the 8-azaguanine for administration, its consistent carcinostatic effect upon the 755 tumor, the details of tumor growth measurement, and the method of statistical analysis have been previously described(1,5). 6-Formylpteridine, in weak alkaline solution, was injected intraperitoneally at various time intervals in relation to 8-azaguanine.

In vitro studies. The transplanted tumors (755) grown in C_{57} black mice, were removed immediately after killing the animals with ether, and stored for 24 or 48 hours in the frozen state until used. Pooled tumor tissue was homogenized for 3-5 minutes in a glass homogenizer of the Potter type with ice cold 0.1 M borate buffer at pH 8.4. The homogenates were then centrifuged at 22,000 g for 20 minutes in the cold. The resulting clear, pink, supernatant fluid at a concentration equivalent to 30% fresh tissue was used as the enzyme source. All operations were carried out at 4°C. 8-Azaguanine solutions were prepared as described previously(2) except that the

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