TABLE I.
The Renal Clearance of Allantoin in Human Subjects.

Patient	Age, yr	Avg urine flow (cc/min)	Plasma allantoin conc. (mg/100 cc)			Renal allantoin clearance (cc/min)		
			10:00	10:30	11:00	Per. I	Per. II	Avg
W.J.	45	13.45	7.4	7.0	7.5	101	113	107
H.B.	22	3.70	6.1	7.1	6.8	124	136	130
G.C.*	43	10.00	5.5	5.1	4.3	134	140	137
H.S.	43	5.70	6.9	7.7	8.8	132	114	123
R.S.	42	5.20	5.3	4.4	5.1	115	114	127
D.C.	32	5.90	4.3	4.3	4.8	131	123	115
Avg		7.33	5.9	5.9	6.2	123	123	123

* Female.

cates that the clearance of allantoin probably measures the rate of glomerular filtration in man. Thus, the average allantoin clearance obtained in the 6 subjects (123 cc per minute) is approximately the same as the value found for the inulin clearance (123 cc per minute) in man by Smith, Goldring and Chasis[4] and by one of us (M.F.) in a previous study.[5]

Conclusion. The average renal clearance of allantoin in 6 normal human subjects was found to be 123 cc per minute. The similarity of this value to that now accepted for the renal clearance of inulin (a glomerular filtrate without tubular reabsorption) strongly suggests that the renal clearance of allantoin is at the level of glomerular filtration.

4 Smith, H. W., Goldring, W., and Chasis, H., *J. Clin. Invest.*, 1938, **17**, 263.

5 Friedman, M., Selzer, A., and Rosenblum, H., *J. A. M. A.*, 1941, **117**, 92.

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Effect of Folic Acid* and Bis (β-Chloroethyl) Sulfide (Mustard Gas)† on Transplanted Mouse Lymphosarcoma.‡

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Leuchtenberger *et al.* [1,2] have shown that folic acid is a tumor growth inhibitor for sarcoma 180 and for spontaneous mammary tumors in mice. Bass and Freeman[3] were not able to demonstrate a similar regression in transplanted lymphoma $6C_3HED$ in mice. The present investigation was designed to further study the effect of folic acid on the $6C_3HED$ tumors, when administered alone or in combination with bis (β-chloroethyl) sulfide. It was hoped that the folic acid might reduce the severity of the toxic side effects of bis (β-chloroethyl) sulfide. Although this did not prove to be the case, certain interesting observations were made which are reported in this paper.

* The "synthetic folic acid," "Folvite," and "Teropterin" used were supplied by Lederle Laboratories, Pearl River, N.Y.

† The bis (β-chloroethyl) sulfide was supplied by Edgewood Arsenal, Md.

‡ These studies have been made with the assistance of a grant-in-aid from the National Cancer Institute.

1 Leuchtenberger, C., Lewisohn, R., Laszlo, D., and Leuchtenberger, R., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 204.

2 Leuchtenberger, R., Leuchtenberger, C., Laszlo, D., and Lewisohn, R., *Science*, 1945, **101**, 46.

3 Bass, Allan D., and Freeman, Marion L. H., *J. Nat. Cancer Inst.*, 1946, **7**, 171.

Methods. Adult C_3H mice of both sexes obtained from Jackson Memorial Laboratories at Bar Harbor, Maine and Lymphosarcoma $6C_3HED$ were used. Small fragments of tumor were implanted subcutaneously into the right axillary region with a 16-gauge needle. When the tumors were large enough to measure, treatment was started. Ninety animals were used for this study; 30 receiving bis (β-chloroethyl) sulfide alone, 30 synthetic folic acid alone, and 30 both the bis (β-chloroethyl) sulfide and folic acid.§ Folic acid was administered in daily doses of 0.1 mg per mouse. The bis (β-chloroethyl) sulfide was given in doses of 3 mg per kg at 48-hour intervals unless the animals developed toxic symptoms in which case the interval was prolonged or the dose reduced to fit the individual case. Diarrhea and/or a weight loss of 1 g or more in 24 hours were used as criteria for varying the procedure until cessation of such symptoms. Both folic acid and bis (β-chloroethyl) sulfide were administered intraperitoneally; folic acid in a phosphate buffer solution of pH 7.1 and bis (β-chloroethyl) sulfide in 0.076% by volume solution in propylene glycol. All animals were fed Purina dog chow *ad libitum.*

Results. Fig. 1 summarizes the results obtained. Since bis (β-chloroethyl) sulfide is lethal for some animals at the dosage used, the points plotted on the curve represent the average tumor size of surviving animals. Tumor size is indicated as one half the length plus the width expressed in centimeters. Tumors of animals receiving bis (β-chloroethyl) sulfide showed a diminished rate of growth with a definite period of regression on the sixth day after the treatment was begun. Tumors of animals receiving both folic acid and bis (β-chloroethyl) sulfide clearly showed less effect of the therapy than when bis (β-chloroethyl) sulfide alone was administered. Although less regression or growth inhibition was evident in the animals receiving both bis (β-chloroethyl) sulfide and folic acid the survival time was not significantly altered. The rate of growth of the tumors in control animals receiving phosphate buffer or propylene glycol was approximately the same as the tumor growth rate of mice receiving folic acid (Fig. 2).

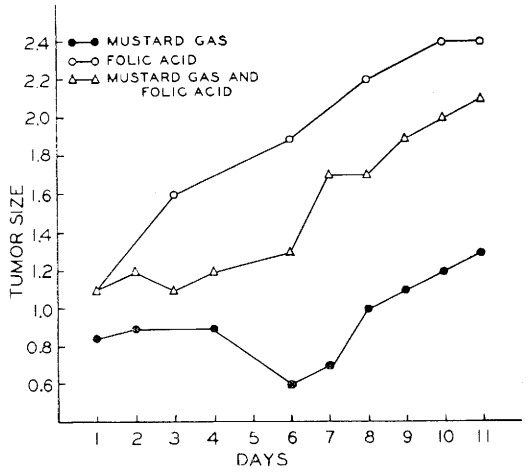

FIG. 1.

The indicated treatment was begun on the first day shown in graph. This is approximately 7 days after transplantation.

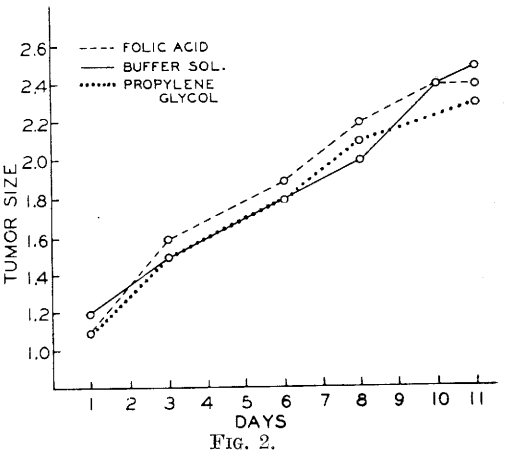

FIG. 2.

Graph to show the growth rate of $6C_3HED$ tumors in control animals as compared with animals receiving "synthetic folic acid." Propylene glycol and phosphate buffer were given intraperitoneally to 10 animals each. Thirty animals received folic acid.

To determine whether there was any difference in growth rate of lymphoma $6C_3HED$ when tumor-bearing mice were given pteroyl mono-glutamic acid or pteroyl tri-glutamic acid, we gave 15 C_3H mice 0.1 mg of "Folvite" daily in phosphate buffer and another 15 mice 0.1 mg of Teropterin (pteroyl tri-

§ Unless otherwise stated, folic acid in this paper will refer to "synthetic folic acid" (Lederle).

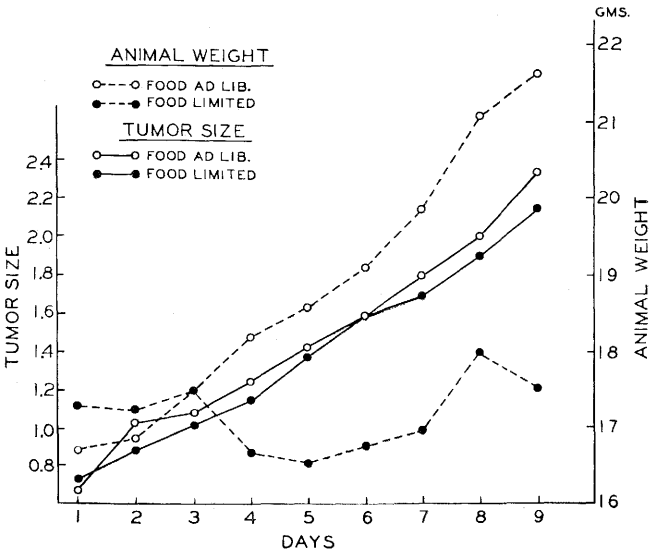

FIG. 3.
Food limitation was begun on the second day.

glutamic acid) daily for 5 days. The growth curve of the tumor in these two groups was not significantly different from controls treated with phosphate buffer alone, or from the growth curves when "synthetic folic acid" was used.

The weights of the animals receiving both bis (β-chloroethyl) sulfide and folic acid were maintained better than those receiving bis (β-chloroethyl) sulfide alone. To exclude the possibility of weight loss associated with drug administration as the cause for the differences in tumor regression a paired feeding experiment was carried out, the results of which are shown in Fig. 3. Here it is shown that limitation of food to 60% of that eaten by the control mice has no measurable effect upon the tumor growth. The weight loss produced by the food limitation is comparable to that which is frequently encountered after bis (β-chloroethyl) sulfide administration. Additional studies are necessary to elucidate the mechanism of folic acid antagonism to bis (β-chloroethyl) sulfide.

Summary. "Synthetic folic acid", "Folvite," or "Teropterin" in the dosage employed do not produce regression in transplanted $6C_3HED$ tumors in C_3H mice. Administration of synthetic folic acid partially inhibits the effect of bis (β-chloroethyl) sulfide on lymphosarcoma $6C_3HED$.

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Activation of Staphylocoagulase.*

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Smith and Hale[1] have shown that the inability of staphylocoagulase to clot citrated plasmas is, in most cases, due to absence of an "activator substance" for the staphylo-

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