from the blood into the spinal fluid in very small amounts.

Summary. After intravenous injection sucrose occurs in the spinal fluid in very low concentration. Even when hemorrhages into the central nervous system occur, the concentration does not approach that found in blood.

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Inhibition of Development of Sarcoma 180 by 4-Amino-N^{10}-Methyl Pteroylglutamic Acid.*

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In view of the extensive developments among the anti-metabolites it was a natural consequence that cancer chemotherapy studies should be initiated with analogs of folic acid. Interest in the folic acid antagonists has been stimulated by reports of the effectiveness of Aminopterin in the treatment of leukemia (Farber).[1] In an intensive screening program during the past year a large number of analogs of folic acid and various related simple compounds have been studied against mouse leukemia[2] and various mouse and rat tumors with particular emphasis on the Crocker mouse Sarcoma 180. Thus far, one folic acid antagonist,[3] 4-Amino-N^{10}-methyl pteroylglutamic acid (A-Methopterin),† has been outstanding in its ability to inhibit the growth of Sarcoma 180. The preliminary results of the study are presented.

Materials and methods. The material was tested for ability to inhibit the growth of the Sarcoma 180 and also for its ability to damage a well established tumor.

Carefully selected tumors were cut into 2 mm cubes and implanted subcutaneously into the axillary region of groups of 5 weighed and marked white mice. In the inhibition tests, intraperitoneal injections of 0.5 cc of different doses of A-Methopterin were begun 24 hours later. In the therapeutic test, injections were begun after 3 to 7 days of tumor growth. In all instances, injections were given twice daily at 6-hour intervals for 7 consecutive days. If the animals showed definite signs of a toxic effect of the drug, administration was omitted until recovery had taken place. At the higher dosage (2.5-2.0 mg/kg/day) 3 or 4 omissions were often necessary. Below this level the drug was well tolerated.

Following the period of injections the animals were weighed and the size of their tumors determined by measurement in 2 diameters with calipers. The size of the tumors was then compared with those of the untreated controls and tumor growth was graded according to the following scheme: 1) Inconclusive, in groups of animals where 3 or more died, 2) No effect, when the largest diameter was ¾ or more of the diameter of the control tumors, 3) Moderate effect, when the diameter was ¼ to ¾ of the diameter of the control tumors, 4) Marked inhibition when the diameter was ¼ or less of that of the control tumors; actually,

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1 Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., Jr., and Wolff, J. A., *New England J. Med.*, 1948, **238**, 787.

2 Burchenal, J. H., Burchenal, J. R., Kushida, M. N., Johnston, S. F., and Williams, B. S., *Cancer*, 1949, **2**, 113.

3 Franklin, A. L., Belt, M., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1949, **177**, 621.

† A-Methopterin is the name given to the compound by Lederle Laboratories, to whom we are indebted for supplies. The Calco Division, American Cyanamid Company also has provided a supply of this compound.

TABLE I.
Effect of Different Amounts of A-Methopterin on the Growth of the Sarcoma 180.

Dose in mg/kg/day	No. of groups of 5 mice	No. of mice	Deaths during inj. period		Effect on tumor*			
			No.	%	Inconclusive	Marked inhibition	Moderate effect	No effect
2.5	6	30	14	46.6	2*	4	0	0
2.0	15	75	14	18.6	2	9	4	0
1.5	31	155	11	7.1	0	16	11	4
1.0	8	40	2	5.0	0	1	4	3

* The tumor effects are expressed as the result obtained in each group of 5 mice. Thus, the numeral 2 indicates two groups of 5 mice each.

the volume of such a tumor would be 1/64 or less of the control if tumors may be considered spherical.

The animals were again weighed and their tumors measured at the end of the second week.

Results. Groups of 5 mice implanted with the Sarcoma 180 one day previously were injected intraperitoneally with daily doses of A-Methopterin at the following levels: 2.5 mg, 2.0 mg, 1.5 mg, and 1 mg/kg. Deaths due to the toxicity of the material were recorded during the 7-day period of injection. Tumor measurements were made at the end of the period and the effect of the substance on the tumor was evaluated. These data are summarized in Table I.

It is clear that A-Methopterin has a definite inhibitory effect on the growth of Sarcoma 180 at all the levels of drug above 1 mg/kg/day. The drug killed 46% and 18%, respectively, in the groups of mice receiving 2.5 and 2.0 mg/kg/day. At the lower dosages 7% and 5% of the mice receiving 1.5 and 1.0 mg died during the treatment. Male mice appeared to tolerate the drug better than female mice. The best results were obtained at the 1.5 mg dose where a definite effect was noted in almost all instances with little evidence of toxicity. The average weight loss of this group was 1.0 g as contrasted to a gain of 0.5 g for the controls. Fig. 1 shows the typical inhibition effect on the tumor of the injection of A-Methopterin at the level of 1.5 mg/kg/day.

The tumors have never been completely destroyed. When the injections are stopped, the small tumors grow normally.

When the tumors were allowed to grow for 3 to 7 days before the injections were begun, inhibition of growth was apparent in practically all instances. Of the 15 groups of mice receiving 2 mg/kg/day, inhibition was marked in 3 groups, moderate in 11 groups and absent in one group. Of the 24 groups receiving 1.5 mg/kg/day, inhibition was marked in 7, moderate in 16, and absent in one. The death rates for the 2 dosages were 12% and 16%, respectively. It is doubtful that these figures represent true toxicity deaths, because many of the tumors were so large at the time injections were initiated, that some of the mice probably died from the effects of tumor growth. The effect of A-Methopterin was definite although not as marked as in the groups of mice injected 24 hours after implantation.

In a few experiments a single injection of 50 mg/kg was administered when the tumor had grown one day. An inhibitory effect was evident when the tumors were measured a week later. In one group implanted 24 hours previously which received one injection of 50 mg/kg, small doses (0.5 mg/kg/day) were administered for 7 days. When the tumors were measured, they were very small but grew when the injections were stopped. The development of 7-day-old tumors was not affected by the injection of the single large dose.

Discussion. A-Methopterin has been shown to have a definite and consistent inhibitory effect on the growth of the transplantable mouse Sarcoma 180. In another report (Sugiura, Moore, Stock)[4] the folic acid antagonist, Aminopterin, was shown to have a

[4] Sugiura, K., Moore, A., and Stock, C. C., *Cancer*, in press.

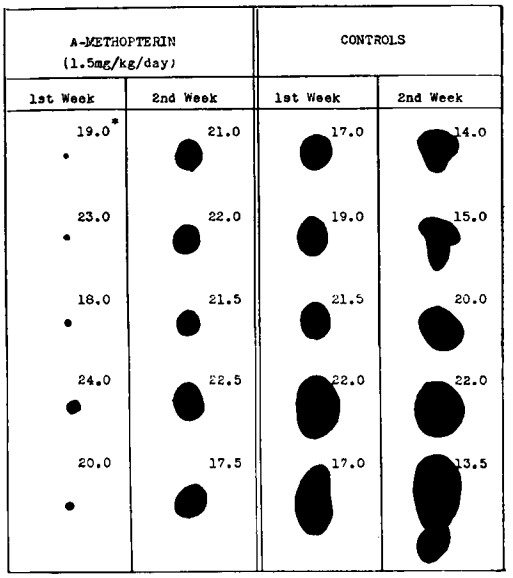

FIG. 1.
Area diagrams of tumors in mice treated with A-Methopterin compared with untreated controls.
* Weight of mouse in grams.

similar effect; however, marked inhibition could be demonstrated only when the drug was administered in amounts which killed a large per cent of the mice. A-Methopterin appears to exhibit a more favorable therapeutic index. It has been outstanding in its effect among numerous compounds which might interfere with the utilization of folic acid or possibly with the synthesis of nucleic acid. In fact, it has been the most effective compound of over 700 miscellaneous compounds that have been screened. It is therefore advanced as a compound worthy of further experimental study and for clinical trial in various types of cancer.

Summary. The compound 4-Amino-N^{10}-methyl pteroylglutamic acid, has shown the ability to inhibit the growth of the transplantable mouse Sarcoma 180 when administered intraperitoneally in concentrations of 1.5 mg/kg each day for 7 days. At this dosage very few mice died from drug toxicity. The inhibition is most apparent when the compound is administered early in the development of the tumor.

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Beneficial Effects of Liver on Growth and Survival in the Immature Hyperthyroid Mouse.*

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Available data indicate that desiccated whole liver will prolong survival and counteract the growth retardation of immature rats fed massive doses of thyroactive materials.[1-5] The protective factor is distinct from any of the known nutrients and is retained in the water-insoluble fraction of liver.[3-5] The present experiment was undertaken to determine the effects of liver and liver fractions on growth and survival in the immature hyperthyroid mouse.

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