5. Details of the biological phase of the research are in preparation.
6. The generous supply of the tetracycline antibiotics provided by Lederle Laboratories Division of the American Cyanamid Co., and Chas. Pfizer and Co., is hereby gratefully acknowledged.
7. Model SC-5041, Hanovia ultraviolet light, Hanovia Chemical and Manufacturing Co., Newark, N.J. (366 mμ).
8. Visking Corp., Chicago, Ill.
9. The completion of dialysis at this time was shown by the fact that further dialysis yielded no more precipitate of the fluorophore when the dialyzate was made alkaline.
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Prevention of Toxicity of Amethopterin for Sarcoma-180 Cells in Tissue Culture

The present paper is a preliminary report on the finding that sarcoma-180 (S-180) cells grow normally when the function of folic acid is prevented by amethopterin (Methotrexate), if the medium is supplemented with products of the biosynthetic reactions dependent on folic acid cofactors. It is known that certain microorganisms which require folic acid for growth—for example, *Streptococcus faecalis* 8043—can grow in the absence of this vitamin in a medium containing thymine and adenine or guanine (*1*). That folic acid is one of the nutritional requirements for the growth of mammalian cells in tissue culture was shown by Eagle (*2*). The inhibition of the growth of sarcoma-180 cells in such a medium by amethopterin has been demonstrated (*3*).

The techniques of Eagle (*2, 4*) were used. The medium contained 5 percent thoroughly dialyzed horse serum. The cells were grown in the experimental media for 7 days. The growth of the cells in the presence of amethopterin in a medium containing hypoxanthine, thymidine, and glycine is shown in Table 1. Disintegration of the cells occurred if hypoxanthine or thymidine was omitted from the mixture. In the absence of glycine, some growth was observed, indicating either a small amount derived from the dialyzed horse serum or some formation of glycine, possibly from the exogenous L-threonine (*5*). When glycine was added to this medium, the growth was comparable to that of the control. The complete mixture fully supported the growth of sarcoma-180 cells even in the presence of amethopterin at concentrations 10,000 times that ordinarily required for complete inhibition (Table 1). In body fluids, the concentrations of such compounds could be critical to the effectiveness of amethopterin on neoplastic cells *in vivo*. Similar compounds in the crude medium (chicken plasma clot) might also explain the failure of amethopterin to inhibit sarcoma-180 cells, as reported by Biesele (*6*).

When the function of folic acid was prevented by amethopterin, it was found that sarcoma-180 cells were able to utilize adenine, adenosine, deoxyadenosine, hypoxanthine, and inosine equally well; guanosine supported slower growth of sarcoma-180 cells under these conditions; that is, there was only a threefold increase in 7 days, whereas xanthine and xanthosine were inactive. These results indicate that some "adenine" was derived from guanosine, but the extent to which "guanine" or "adenine," or both, were derived from xanthine and xanthosine was insignificant.

In the presence of amethopterin, thymidine, and glycine, the cells disintegrate if purines are not supplied in the medium (Table 1). Under such conditions it is unlikely that purine synthesis *de novo* occurs. Thus, the single purine in the medium must serve as the sole source, not only of adenine and guanine of nucleic acids, but also of all the coenzymes containing purines as structural constituents. Accordingly, this technique appears to be useful for the study of the pathways of purine metabolism. The present work demonstrates for the first time that mammalian cells (sarcoma-180) are fully capable of using exogenous purines for growth and multiplication.

Further work demonstrated that thymidine could be replaced by thymidylic acid for the growth of sarcoma-180 cells in the presence of amethopterin, glycine, and a purine, but thymine and thymine-riboside had no activity in this respect. A mixture of thymine and deoxyadenosine did not replace a mixture of thymidine and adenine, indicating that this type of transdeoxyribosidation did not occur.

It is seen that the presence of amethopterin creates new requirements for the growth of the tumor cells *in vitro*. When these requirements are met by preformed purines, thymidine, and glycine, inhibition of growth might still be achieved if the utilization of even one of these compounds were prevented. Logical combinations for chemotherapy are thus suggested. The new requirements created by amethopterin probably differ in different species and tissues. It is already known that differences exist in the abilities of various tissues and species to utilize preformed purines *in vivo* (*7*). In addition, rabbit fibroblasts, unlike sarcoma-180 cells, require exogenous L-serine for growth in tissue culture (*8*).

Table 1. Growth of sarcoma-180 cells in tissue culture in the presence of amethopterin. Folic acid (pteroylglutamic acid) was present at a concentration of $2 \times 10^{-7} M$.

Varied supplements in the medium				Degree of cellular multiplication†
Amethopterin* (M)	Hypoxanthine $3 \times 10^{-5} M$	Thymidine $3 \times 10^{-5} M$	Glycine $1 \times 10^{-4} M$	
0				5.7‡
3×10^{-8}				0.70
3×10^{-7}				0.34
3×10^{-7}	+	+	+	5.7§
3×10^{-4}	+	+	+	6.1
3×10^{-7}		+	+	0.60
3×10^{-7}	+		+	0.43
3×10^{-7}	+	+		1.8

* 4-Amino-10-methyl-pteroylglutamic acid.
† Referred to inoculum as 1; determined by the method of Oyama and Eagle (*11*); correlation of the protein determinations with cell counts is discussed by Oyama and Eagle (*11*).
‡ Control.
§ The culture has been carried for 3 weeks under these conditions and is being maintained.

Thymine or thymidine increased the rate of the development of amethopterin resistance in *Streptococcus faecalis* 8043 (*9*). The effect of similar factors on the development of amethopterin resistance in mammalian cells is under study (*10*).

MAIRE T. HAKALA
Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York

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Gastric Secretagogue Effect of Lysine Monohydrochloride

There have been recent references in medical literature to the use of lysine monohydrochloride as a nutritional supplement (*1*). Certain clinical responses, such as increase of appetite, weight gain, and rapid restoration of hemoglobin values, have been reported. Such effects might be produced by either or both of these mechanisms: (i) correction of pre-