the question whether this difference from the Lansing strain is due to the fewer number of mouse passages which the Y-SK has had, or to an inherent difference between the two strains. It is known that many laboratory virus variants have appeared as a result of continuous passage in a new animal host. Therefore the possibility exists that both viruses initially had the same cytopathogenic ability, and that for the Lansing strain, this ability was lost by continuous mouse passage. In this connection, Theiler(10) has found that continuous mouse passage of the Lansing strain resulted in a marked drop in its capacity to infect monkeys.

Summary. The Y-SK strain of poliomyelitis virus may be titrated in roller tube cultures by observation of the degeneration of monkey testicular fibroblasts within 4 days following the addition of virus to the tissue culture. The extent of fibroblastic degeneration is inversely related to the metabolic activity of the tissue, as measured by the amount of acid production. The 2 immunologically related Y-SK and Lansing strains of poliomyelitis virus differ in the degree to which they cause fibroblastic degeneration, and alter the amount of acid production by the tissue. In contrast to the Lansing strain, the Y-SK strain produces more extensive degeneration in fibroblasts of monkey testicles and also in those of human embryonic skin-muscle.

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10. Theiler, M., *Medicine,* 1941, v20, 443.

Growth Promotion in Tetrahymena by Folic Acid Analogs.* (18983)

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The most widely used of the substituted folic acids in inhibition studies is 4-aminopteroylglutamic acid (Aminopterin). Early studies have shown it to be inhibitory to all organisms tested,† with the single exception of the rabbit, which had not been previously rendered folic acid deficient(1). It has been shown(2,3) that inhibition by low concentrations of inhibitor can be reversed by large doses of folic acid but reversal fails when the inhibitor concentration is high. More recently(4,5) reversal of inhibition has been obtained over wide ranges with citrovorum factor.

It is of considerable interest, therefore, to find that the animal microorganism, *Tetrahymena,* is not only uninhibited by Aminopterin but that this compound will replace folic acid in its diet. The same is true of N^{10}-methylfolic acid (Methopterin), although in this case the growth promoting power is lower and mild inhibition results at very high concentrations.

This report deals with Aminopterin, Methopterin, pteroylaspartic acid, 4-aminopteroylaspartic acid and citrovorum factor.

Experimental. The organism used was *Tetrahymena geleii* W, grown in bacteria-free culture. The medium used was Medium

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† A note by G. E. Foley reports that the partial utilization (probably by deamination) of 4-aminopteroylglutamic acid by *Streptococcus faecalis* 8043 in appropriate pteroylglutamic acid-free substrate has been demonstrated (personal communication).

1. Dinning, J. S., Keith, C. K., Davis, P. L., and Day, P. L., *Arch. Biochem.,* 1950, v27, 89.

2. Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 398.

3. Franklin, A. L., Stokstad, E. L. R., Hoffmann, C. E., Belt, M., and Jukes, T. H., *J. Am. Chem. Soc.,* 1949, v71, 3549.

4. Sauberlich, H. E., *Arch. Biochem.,* 1949, v24, 224.

5. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.,* 1950, v185, 399.

TABLE I. Comparison of the Activities of Folic Acid, 4-Aminofolic Acid and N^{10}-Methylfolic Acid for *Tetrahymena*.

Compound	Amt (mγ/ml) required for half max growth		% activity	
	No thymine	40 γ/ml thymine	No thymine	Thymine
Folic acid	.131	.022	100	100
4-aminofolic acid	.76	.13	17.2	16.9
N^{10}-methylfolic acid	7	1.2	1.9	1.8

A(6) in which Tween 80 at 10 mg/ml was substituted for the Tween 85, and folic acid was omitted in appropriate experiments. All experiments were run in triplicate and the various tests were repeated a number of times. The procedure has been described elsewhere(7,8). Dose response tubes were inoculated with organisms which had been grown in the presence of only half maximal concentrations of folic acid for 7-8 days. The medium was autoclaved together with the folic acid or the derivatives (the Tween, dextrose and phosphate were autoclaved in a separate solution), after adjustment to pH 7.0 with the Beckman pH meter. All procedures were carried out in the virtual absence of white light, which is absolutely necessary when working with high dilutions of these light sensitive compounds. In the replacement experiments the results were checked with multiple serial transplants to insure against folic acid carryover either in the medium or in the protoplasm of the organisms.

Results. It had earlier been shown that growth of *Tetrahymena* fails in the absence of folic acid(9-11) provided at least two serial transplants are made. Indefinitely transplantable growth occurs, however, in the presence of Aminopterin or Methopterin. The activity of Aminopterin is about 17%, while that of Methopterin is about 2% that of folic acid. The addition of thymine to the medium spares folic acid(12) and this compound spares both Aminopterin and Methopterin (Table I).

Aminopterin is capable of reversnig the inhibition caused by 4-amino-9-methyl-pteroylglutamic acid, 4-amino-N^{10}-methylpteroylglutamic acid and 4-amino-9,10-dimethylpteroylglutamic acid(13) when amounts possessing activity equivalent to folic acid are added.

The above results could be explained on the basis of folic acid contamination of the folic acid analogs. This is unlikely, however, because of the methods of synthesis.‡ Moreover, when the activities of commercial samples of both analogs were compared to the activities of highly purified samples§ they were found to be in good agreement. The likelihood of such close correspondence in degree of contamination between diverse samples seems remote.

It is impossible to say at the present time whether Aminopterin is deaminated and Methopterin demethylated to folic acid, or whether these compounds can function unchanged in place of folic acid. Some deamination of Aminopterin during heat sterilization appears to take place, as filter-sterilized material is somewhat less active. Autoclaving Methopterin, however, does not change its activity. It seems probable that *Tetrahy-*

6. Dewey, V. C., Parks, R. E., Jr., and Kidder, G. W., *Arch. Biochem.*, 1950, v29, 281.

7. Kidder, G. W., and Dewey, V. C., in Biochemistry of Protozoa, A. Lwoff, ed., Academic Press, 1951.

8. Kidder, G. W., Dewey, V. C., and Parks, R. E., Jr., *Physiol. Zool.*, 1951, v24, 69.

9. Kidder, G. W., and Fuller, R. C., III, *Science*, 1946, v104, 60.

10. Kidder, G. W., *Arch. Biochem.*, 1946, v9, 51.

11. Kidder, G. W., and Dewey, V. C., *Arch. Biochem.*, 1949, v21, 66.

12. Kidder, G. W., Dewey, V. C., Parks, R. E., Jr., and Heinrich, M. R., *Proc. Nat. Acad. Sci.*, 1950, v36, 431.

13. Dewey, V. C., Kidder, G. W., and Parks, R. E., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, —.

‡ Smith, J. M., Jr., private communication.

§ Obtained from Calco Chemical Division of American Cyanamid Company through the courtesy of Dr. J. M. Smith, Jr. The control number for the Aminopterin is R-2795-25-A and for Methopterin, R-2629-80.

mena possesses enzymes capable of changing both analogs to folic acid. Deamination, if it occurs, is not enhanced by increasing the concentration of pyridoxal in the medium, but this may mean that this specific deamination reaction is not mediated by way of a pyridoxal coenzyme. Moreover, the very low folic acid activity (less than 1%) of 4-dimethylaminopteroylgutamic acid‖ might indicate that deamination is blocked by the methyl substitutions, and therefore, deamination is a step necessary for activity.

When equivalent activities of folic acid, Aminopterin and Methopterin are compared for their effect on the growth rate of *Tetrahymena* no differences are found. Slightly suboptimal amounts of any of these compounds causes growth rate depression, but maximum yields are finally attained.

Pteroylaspartic acid¶ does not produce inhibition of the growth of *Tetrahymena,* but, unlike the other 2 analogs, it will not replace folic acid in the diet. Some folic acid sparing action occurs when this compound is added to medium containing suboptimal folic acid concentrations. Amino-an-fol¶ (4-amino-pteroylaspartic acid) behaves similarly.

The first few samples of citrovorum factor (CF) which we obtained‖ appeared to be more active in promoting growth of *Tetrahymena* than folic acid. This was due to the large amount of free folic acid present as unreacted starting material. Later samples of CF and of folinic acid** which were more highly purified were never greater in activity and usually less active than folic acid.

Discussion. Tetrahymena possesses an enzyme pattern unlike most other organisms in at least three distinct, though not necessarily unrelated, respects. First, it appears to be the only organism studied which has a requirement for folic acid together with an absolute requirement for purine(12,14). Second, the citrovorum factor is no more active than folic acid. Third, it appears unique in its ability ot utilize Aminopterin and Methopterin for growth.† According to Nichol and Welch(15) Aminopterin produces its inhibition in rats by interference with the conversion of folic acid to citrovorum factor. As CF does not appear to be the active form in *Tetrahymena* one should perhaps expect no inhibition. On the other hand, Dinning *et al.* (1) suggest that the site of action of Aminopterin is in its interference with choline oxidase activity. The recent report by Williams(16) that CF may be the prosthetic group of choline oxidase indicates that the previous observations may not be unrelated. It is possible that in *Tetrahymena,* as in the rabbit(1), choline oxidase is absent. Either of these hypotheses would explain the fact that Aminopterin is not inhibitory but would not explain its growth promoting activity. It would be interesting and important to determine whether or not Aminopterin would promote growth in a folic acid deficient rabbit. Law and Boyle(17) and Burchenal *et al.*(18) have shown that resistance to 4-amino derivatives of folic acid (including Aminopterin) develops in certain strains (L1210 and AK4) of mouse leukemia. It seems possible that these cells become resistant following the acquisition of enzymes capable of deaminating the 4-position, thereby reverting to a condition similar to what appears to be the case in *Tetrahymena.*

Summary. Tetrahymena geleii W is not inhibited by either 4-amino-pteroylglutamic acid or N^{10}-methylpteroylglutamic acid. Moreover, both of these analogs promote growth in the absence of folic acid. It is suggested that the organism possesses enzymes which deaminate the 4-position and enzymes

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¶ Supplied by the Lederle Laboratories through the courtesy of Dr. T. H. Jukes.

** Furnished through the courtesy of Dr. F. R. Van Abeele of the Lilly Research Laboratories.

14. Kidder, G. W., and Dewey, V. C., *Proc. Nat. Acad. Sci.,* 1948, v34, 566.

15. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 403.

16. Williams, J. N., Jr., *J. Biol. Chem.,* 1951, v191, 123.

17. Law, L. W., and Boyle, P. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 599.

18. Burchenal, J. H., Robinson, E., Johnston, S. F., and Kushida, M. N., *Science,* 1950, v111, 116.

which demethylate the 10-position. Pteroylaspartic acid and 4-amino-pteroylaspartic acid spare, but do not replace, folic acid. Citrovorum factor is never more active, and usually less active, than folic acid.

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Growth Inhibition in Tetrahymena by Folic Acid Analogs.* (18984)

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Tetrahymena geleii is at present the only organism known, with the exception of *Streptococcus faecalis* 8043(1), which can utilize Aminopterin, 4-amino-pteroylglutamic acid, in place of folic acid(1). This compound has proved to be an effective inhibitor of a variety of other organisms(2-5), but complete reversal of the effects of high concentrations can be obtained in such cases only by citrovorum factor(2,6-9), by folic acid and/or desoxyribosides(2,5,10,11). Methopterin, N^{10}-methylpteroylglutamic acid, was also found(1) to have some activity for *Tetrahymena* when used in place of folic acid. However, this compound proved to be slightly inhibitory when tested at sufficiently high concentrations.

The effect on the growth of *T. geleii* of other antifolics was, therefore, of interest. The present paper deals with the inhibition obtained with 4-amino-9-methylpteroylglutamic acid (A-ninopterin), 4-amino-10-methylpteroylglutamic acid (A-methopterin) and 4-amino-9,10-dimethylpteroylglutamic acid (A-denopterin).† These compounds have been shown to have an effect similar to that of Aminopterin in inhibiting mouse leukemias (10,11). A-methopterin reversibly inhibits the growth of *Streptococcus faecalis* R and irreversibly affects the growth of rats(12).

Material and methods. *Tetrahymena geleii* W was grown in Medium A(13) in which Tween 80 (10 mg/ml) was substituted for Tween 85. Preparation of the inoculum and all other conditions of growth have been previously described(1).

Results. Results with folic acid analogs were extremely variable quantitatively, although they were in good agreement qualitatively. Attempts to eliminate this variation by standardization of technics were made. Slight variations in the age and size of the inoculum made appreciable differences in quantitative responses. It was necessary, therefore, to repeat the experiments a large

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1. Kidder, G. W., Dewey, V. C., and Parks, R. E., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 88.
2. Sauberlich, H. E., *Arch. Biochem.*, 1949, v24, 244.
3. Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 398.
4. Oleson, J. J., Hutchings, B. L., and SubbaRow, Y., *J. Biol. Chem.*, 1948, v175, 359.
5. Snell, E. E., and Cravens, W. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 87.
6. Cravens, W. W., and Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 43.
7. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 403.
8. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1950, v185, 399.
9. Franklin, A. L., Stokstad, E. L. R., Hoffmann, C. E., Belt, M., and Jukes, T. H., *J. Am. Chem. Soc.*, 1949, v71, 3549.
10. Burchenal, J. H., Babcock, G. M., Broquist, H. P., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 735.
11. Skipper, H. E., Bell, M., and Chapman, J. B., *Cancer*, 1951, v4, 357.

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12. Franklin, A. L., Belt, M., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1949, v177, 621.
13. Dewey, V. C., Parks, R. E., Jr., Kidder, G. W., *Arch. Biochem.*, 1950, v29, 281.