aminosalicylic acid appear to exert inhibitory effects which are related to some degree to functions of folic acid; however, these data do not as yet demonstrate an enzymic interaction of the analog at the site of the *p*-aminobenzoic acid moiety.

References

Bichowsky-Slomnitzki, L. (1948), *J. Bacteriol. 55*, 33.

Fischer, F. G., and Bohn, H. (1957), *Z. Physiol. Chem. 308*, 108.

Hayashi, K., Skinner, C. G., and Shive, W. (1961), *Texas Reps. Biol. Med. 19*, 277.

Jensen, K. A., and Ingvorsen, H. (1952), *Acta Chem. Scand. 6*, 161.

Kay, D. (1959), *Biochem. J. 73*, 149.

Lansford, E. M., Jr., Harding, W. M., and Shive, W. (1958), *Arch. Biochem. Biophys. 73*, 180.

Massart, L. (1948), *Nature 162*, 779.

Miller, A. K., and Peters, L. (1945), *Arch. Biochem. 6*, 281.

Ravel, J. M., McCord, T. J., Skinner, C. G., and Shive, W. (1958), *J. Biol. Chem. 232*, 159.

Shive, W. (1961), The Robert A. Welch Foundation Conference on Molecular Structure and Biochemical Reactions, December, Houston, Texas.

Silverman, M., and Evans, E. A. (1944), *J. Biol. Chem. 154*, 521.

Skinner, C. G., McCord, T. J., Ravel, J. M., and Shive, W. (1956), *J. Am. Chem. Soc. 78*, 2412.

Skinner, C. G., McKenna, G. F., McCord, T. J., and Shive, W. (1958), *Texas Reps. Biol. Med. 16*, 439.

Skinner, C. G., McKenna, G. F., Ross, D. L., and Shive, W. (1961), *Texas Reps. Biol. Med. 19*, 860.

Snell, E. E. (1944), *J. Biol. Chem. 152*, 475.

Turner, R. B., Lansford, E. M., Jr., Ravel, J. M., and Shive, W. (1963), *Biochemistry 2*, 163 (this issue).

Williams, R. J., McAlister, E. D., and Roehm, R. R. (1929), *J. Biol. Chem. 83*, 315.

A Metabolic Relationship of Spermine to Folinic Acid and Thymidine*

Ralph B. Turner, Edwin M. Lansford, Jr., Joanne M. Ravel, and William Shive

From the Clayton Foundation Biochemical Institute and the Department of Chemistry, The University of Texas, Austin

Received July 25, 1962

Spermine reduces the amount of thymidine required for reversal of inhibition of growth of *Lactobacillus arabinosus* 17-5 caused by 2,4-diamino-6,7-diphenylpteridine, aminopterin, or sulfanilamide, under conditions of growth-limiting thymidine biosynthesis. Spermine also decreases the growth requirement of *Pediococcus cerevisiae* for folinic acid, and that of *L. leichmannii* 313 for folinic acid, thymidine, or thymine. Cells of *L. arabinosus* grown in the presence of diaminodiphenylpteridine show after approximately 20–24 hours' growth, if spermine is present in the growth medium, enhanced ability to incorporate formate into acid-insoluble material, and decreased utilization of exogenous thymidine. These and related results suggest that spermine is involved directly or indirectly within some area of single carbon unit metabolism.

The ability of spermine to prevent partially the toxicity of a benzyl thioester of *p*-aminobenzoic acid (Smith *et al.*, 1963) suggested the possibility of a biochemical relationship of spermine to coenzymes of the folic acid group, but the inability, as indicated in the same report, of *p*-aminobenzoic acid or its coenzymatic conjugates to affect the toxicity of the thioester analog precluded the use of that system for demonstration of a relationship of spermine to single carbon unit metabolism. Previously, a synergistic effect of certain natural extracts in combination with thymidine in preventing bacterial growth inhibition caused by diaminodiphenylpteridine was reported (Lansford *et al.*, 1958), and the chemical behavior of the active factors during concentration steps was found to be consistent with a polyamine structure. In the present study, spermine has been found to decrease several fold the amount of thymidine required for reversal of growth inhibition of *Lactobacillus arabinosus* in the presence of an inhibitory concentration of 2,4-diamino-6,7-diphenylpteridine, or of aminopterin. Spermine exerts a similar sparing effect upon the folinic acid (5-formyltetrahydrofolic acid) requirement of *Pediococcus cerevisiae*. These and related results involving a stimulation by spermine of endogenous thymidine biosynthesis and of formate utilization in *L. arabinosus* suggest a direct or indirect role of spermine within some area of single carbon unit metabolism.

* A preliminary report was presented at the Robert A. Welch Foundation Conference, "Molecular Structure and Biochemical Reactions," December, 1961, Houston, Texas.

Experimental Procedures

Culture Media for Bacterial Growth Assays.—A basal medium previously described (Lansford and Shive, 1952) was utilized for experiments with *Lactobacillus arabinosus* 17-5 (ATCC No. 8014) and *Pediococcus cerevisiae* (ATCC No. 8081) with the modification that the casein acid hydrolysate used was the salt-free grade supplied by the Nutritional Biochemicals Corporation (11 g/1000 ml double strength medium). For study of effects upon the growth response of *L. arabinosus* to thymidine (Fig. 1) and to folinic acid, the basal medium was supplemented with vitamins, purines, pyrimidines, formate, and glucose as described for thymidine assay using diaminodiphenylpteridine inhibitor (Lansford *et al.*, 1958) except that aminopterin replaced the pteridine inhibitor where indicated (Fig. 1). For *P. cerevisiae* no folic or folinic acid was included in the vitamin supplement, and to ensure removal of traces of these vitamins the medium was treated with activated charcoal (200 mg Darco G-60 per 100 ml double strength basal medium) before the addition of purine-pyrimidine and vitamin supplements. Growth medium for *Lactobacillus leichmannii* 313 (ATCC No. 7830) (U. S. Pharmacopeia, Vol. 16, Mack Publishing Co., Easton, Pa., p. 888) was supplemented with vitamin

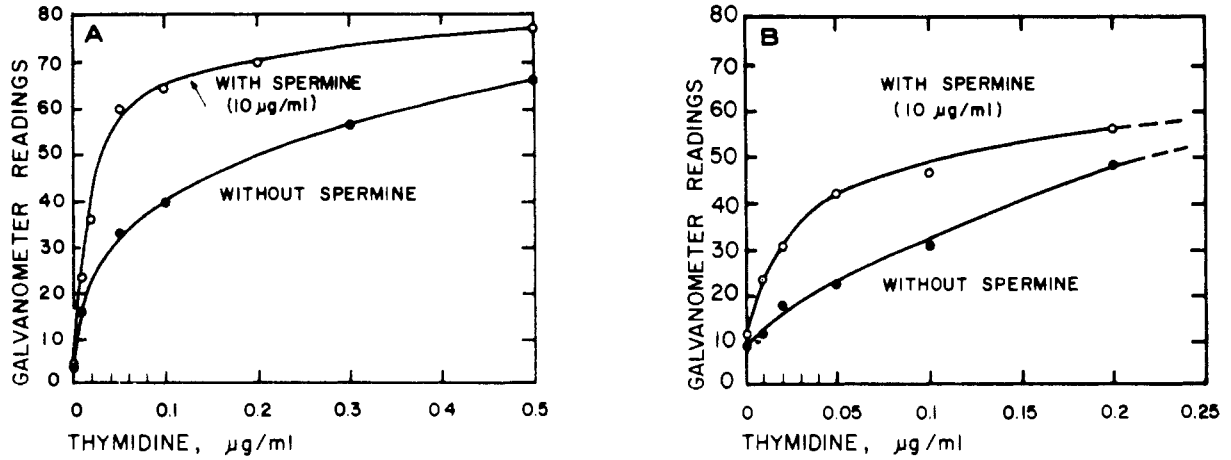

Fig. 1. Sparing effects of spermine upon thymidine requirement for growth of *L. arabinosus*. Incubation, 20 hours at 30°. Medium, as described in Experimental Procedures. *A*, with 2,4-diamino-6,7-diphenylpteridine, 3.0 μg/ml. *B*, with aminopterin, 0.004 μg/ml. Galvanometer readings: distilled water reads zero, an opaque object reads 100.

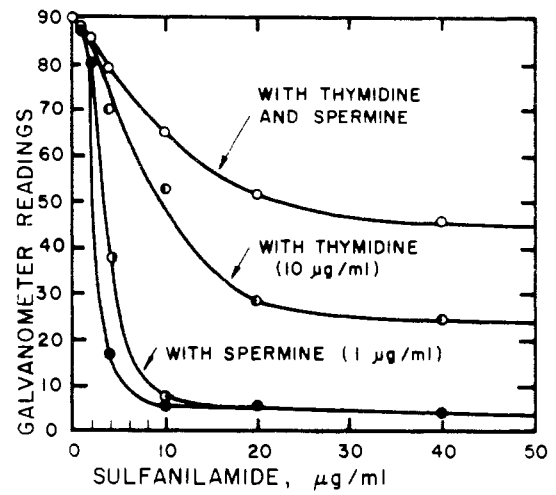

Fig. 2. Reversal by spermine and thymidine of growth inhibition of *L. arabinosus* caused by sulfanilamide. Incubation, 20 hours at 30°. Medium, same as described in Experimental Procedures for the experiment of Figure 1 except that purines and pyrimidines were included only as follows: uracil and adenine, 10 μg each per ml; and vitamins were modified as follows: *p*-aminobenzoic acid, 0.02 μg/ml, no folic or folinic acid; no inhibitor was included except sulfanilamide as indicated.

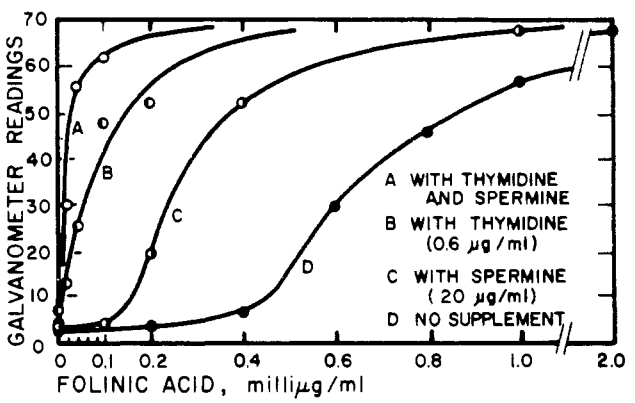

Fig. 3.—Sparing effects of spermine and thymidine upon folinic acid requirement for growth of *P. cerevisiae*. Incubation 19 hours at 37°. Medium, same as described in Experimental Procedures for the experiment of Figure 1 except that purines and pyrimidines were included only as follows: adenine, guanine, and uracil, 4 μg each per ml; and the vitamin supplement was modified to include 0.02 μg *p*-aminobenzoic acid per ml, and no folic or folinic acid.

B_{12} (0.01 μg/ml). For *Escherichia coli* growth experiments, the medium of Anderson (1946) was used.

Procedure for Bacterial Growth Assays.—Organisms were maintained in stock cultures by monthly transfers; standard bacteriological procedures were used. Inoculum cultures (10 ml) of *L. leichmannii* were prepared with the growth medium cited above for this organism, and of the other lactic acid bacteria with enriched medium (McMahan and Snell, 1944). Inoculum cultures of *E. coli* utilized the medium of Anderson (1946). The microbiological assay procedure for each organism was essentially as previously described for thymidine assay with *L. arabinosus* (Lansford *et al.*, 1958), except that for certain experiments (Fig. 2, 3) a subculture from the initial inoculum culture, in the same enriched medium and incubated for 8 hours, was used for inoculation of the assay tubes; in all experiments growth was measured turbidimetrically as described in the same reference.

Isotope Uptake Experiments.—Cultures of *L. arabinosus* were grown in medium as described for thymidine assay (Lansford *et al.*, 1958) containing diaminodiphenylpteridine, except where other supplements are indicated below. After 15–24 hours' growth, the cells were harvested and resuspended in cold medium of the composition desired during subsequent incubation with the isotopically labeled compound. Differences in cell population density in parallel cultures containing differing concentrations of spermine were equalized, after turbidimetric determination of cell weight, by appropriate dilutions so as to provide identical amounts of cells (2 mg dry weight per ml, twice the desired final cell density). For the subsequent incubation this 2 mg/ml cell suspension was then warmed to 27° and mixed rapidly with an equal volume of medium at 27° of the same composition except the C^{14} compound was included at double the desired final concentration. For each time point a 2.0-ml aliquot was removed from the incubating suspension and mixed with 2.0 ml of 10% trichloracetic acid. The precipitate was recovered by filtration through a membrane filter; the filters were dried and the incorporated isotope was then determined by counting with a Nuclear-Chicago thin-window detector and Model 192A scaler. Isotope incorporation into the cold trichloracetic acid precipitate was assumed to comprise incorporation into nucleic acid, since in one experiment using formate-C^{14}, an additional set of aliquots mixed with trichloracetic acid (5% final) and heated to 90°, before filtration and counting of the

precipitate, showed negligible incorporation into the "hot trichloracetic acid" precipitate.

Results

Spermine Effects Upon Bacterial Growth Under Conditions of Limited One-Carbon Transfer.—Under conditions previously reported (Lansford *et al.*, 1958), growth inhibition of *Lactobacillus arabinosus* 17-5 is caused by 2,4-diamino-6,7-diphenylpteridine (3 μg/ml) and is specifically reversed by low concentrations of thymidine. Spermine, as typical data in Figure 1 indicate, spares at least three-fold the amount of thymidine required to prevent this inhibition. Growth inhibition exerted by the same pteridine analog is reversed also by relatively much higher levels of folinic acid (0.2–1.0 μg/ml); spermine likewise spares the amount of folinic acid required for this reversal of inhibition. Spermine alone has little effect upon the inhibition exerted by the pteridine analog. In the absence of any folic acid antagonist, growth of *L. arabinosus* was not detectably stimulated by spermine (10 μg/ml) at any stage of incubation, as determined by turbidimetric measurements at 2-hour intervals during a total incubation period of 24 hours.

A very similar sparing effect is exerted by spermine in the presence of aminopterin (Fig. 1), another analog structurally related to folic acid which causes growth inhibition of *L. arabinosus* reversible by thymidine. Again, spermine at a concentration of 10 μg/ml shows little activity, when present without thymidine, in reversing the inhibition of growth.

In separate experiments, inhibition of growth of *L. arabinosus* was measured in the presence of varying concentrations of aminopterin. In the presence of thymidine, a supplement of spermine (1 μg/ml) increased almost two-fold the concentration of aminopterin necessary for a given degree of growth inhibition. Thymidine alone (10 μg/ml) increases approximately ten-fold the amount of aminopterin necessary for a given degree of inhibition; a supplement of spermine without thymidine only slightly increases the amount of inhibitor required for a given effect.

That the effect of spermine is closely related to *p*-aminobenzoic acid metabolism is indicated by results, with sulfanilamide as inhibitor, similar to the effects obtained with the pterin analog and with aminopterin. For *L. arabinosus* under the conditions of these experiments, sulfanilamide is specifically a competitive antagonist of *p*-aminobenzoic acid (Shive and Roberts, 1946). The amount of thymidine (in the concentration range 0.1–1.0 μg/ml) necessary for reversal of the growth inhibition of *L. arabinosus* in the presence of a constant inhibitory level of sulfanilamide (10 μg/ml) is decreased by a supplement of spermine (2 μg/ml). As in the case of aminopterin, in experiments in which the concentration of sulfanilamide was varied (Fig. 2), the amount of sulfanilamide required for inhibition is only slightly increased by a spermine supplement in the absence of thymidine, but is increased about two-fold in the presence of a thymidine supplement. This effect is not related to the time or stage of incubation of the cultures, since experiments parallel to that of Figure 2 but incubated 18, 21, and 24 hours each showed a similar result. In these experiments, at higher concentrations of sulfanilamide (*e.g.*, 1000 μg/ml) at which *p*-aminobenzoic fails to reverse the inhibition, spermine in combination with thymidine was also ineffective in revdrsing the inhibition.

Such sparing effects of spermine occur also with organisms having a natural requirement for a member of the folic acid vitamin group, rather than a limitation in one-carbon unit metabolism artificially induced by the presence of an antimetabolite. Thus, for *Pediococcus cerevisiae* 8081 (*Leuconostoc citrovorum*), which requires folinic acid for growth, spermine significantly decreases the amount of folinic acid required for growth (Fig. 3). As previously reported (Bardos *et al.*, 1949), thymidine spares several-fold the amount of folinic acid required for growth of this organism, and spermine in the presence of thymidine (Fig. 3) further decreases the requirement.

In separate experiments a supplement of spermine (20 μg/ml) decreased nearly to one-half the amount of folinic acid (in the concentration range 0.05–1.0 mμg/ml) or of thymidine (0.05–2.0 μg/ml) or thymine (0.05–2.0 μg/ml), required for growth of *Lactobacillus leichmannii* 313 in the presence of a supplement of vitamin B_{12}.

For comparison with the experiments involving limiting thymidine biosynthesis, it was desirable to investigate possible sparing effects exerted by spermine under conditions of limiting purine biosynthesis. In complete culture medium without inhibitor, growth of *L. arabinosus* is not dependent upon exogenous purines. In the presence of the diaminodiphenylpteridine inhibitor and exogenous thymidine, however, a purine compound is required for growth, indicating that the pteridine inhibitor prevents also single carbon unit incorporation into purines. Under the latter conditions, the amount of purine compounds required for growth is reduced by spermine supplements, but the reduction is somewhat variable, and smaller than in the case of the sparing effect upon the thymidine requirement. The sparing effect upon purine requirements was most marked in experiments involving relatively young inoculum cultures (*e.g.*, 7 hours' growth in enriched inoculum medium).

Growth inhibition of *L. arabinosus* exerted by 5-fluorouracil, an analog reported to inhibit the biogenesis of thymidylate (Hartman and Heidelberger, 1961), is not affected by spermine, nor does spermine exert a sparing effect upon the amount of thymidine (*e.g.*, 0.2 μg/ml in a typical experiment) required for reversal of the inhibition of growth caused by 5-fluorouracil (0.2 μg/ml).

The requirement for thymine of a mutant strain of *Escherichia coli* (strain 15T-) was not affected by supplements of spermine (in the concentration range 0.5–10.0 μg/ml).

Effect of Spermine on Utilization of Thymidine for DNA Synthesis.—In a series of cultures of *L. arabinosus* in growth media containing graded concentrations of thymidine, each level being duplicated with and without spermine (2 μg/ml), the amount of DNA synthesized by the time of harvesting of the cells was determined in cell suspension aliquots by the colorimetric method of Ceriotti (1952). The results summarized in Table I indicate that the final cellular DNA content was approximately the same, 38 μg DNA per mg cell dry weight, in the culture without spermine containing the higher thymidine level (0.1 μg ml), and in both cultures that included spermine plus 0.01 or 0.10 μg thymidine per ml respectively. The cellular DNA content was somewhat lower in the culture containing the severely growth-limiting thymidine level (0.01 μg/ml) without spermine. Calculations of the per cent contribution of exogenous thymidine (assuming that thymidine added to the medium was completely utilized) to the final yield of thymidine bound into cellular DNA are included in Table I. The results suggest a relatively greater contribution of endogenous (biosynthetic) thymidine to the total yield of DNA-bound thymidine in cultures supplemented with

TABLE I

DNA PRODUCTION BY *L. arabinosus* CELLS GROWN IN MEDIA OF DIFFERING SPERMINE AND THYMIDINE CONTENT

Growth Medium[a] Supplements		Terminal Cell Mass	Final DNA Content of Culture		Final Thymidine Content of Culture (Bound in DNA, calcd.[b])	Maximum Possible % Contribution by Exogenous Thymidine to Total DNA-Thymidine
Spermine (μg/ml)	Thymidine (μg/ml)	(mg dry wt./ml)	(μg/ml)	(μg/mg dry cells)	(μg/ml)	
None	0.010	0.059	1.28	21.8	0.24	4.2
None	0.10	0.125	4.80	38.5	0.89	11.3
2	0.010	0.112	4.20	37.5	0.78	1.3
2	0.10	0.23	8.76	38.1	1.62	6.2

[a] Medium for growth of cells was the same as for the experiment of Figure 1 (including 3 μg diaminodiphenylpteridine/ml) with supplements as tabulated. Each individual 125 ml culture was incubated 17 hours at 30°. [b] Calculated on the assumption that 18.5% by weight of *L. arabinosus* DNA consists of thymidine residues; this is equivalent to approximately 20.5 mole % thymine in relation to the other DNA bases.

spermine than in cultures without added spermine. Thus, considering the two cultures that produced approximately equal final yields of DNA (*i.e.*, the culture grown with high thymidine only, and that grown with low thymidine plus spermine), the data of Table I indicate that exogenous thymidine (assuming its essentially complete utilization from the medium) contributed a nine-fold greater fraction of the total DNA-bound thymidine in the culture to which spermine had not been added.

Effect of Spermine on Thymidine Incorporation into L. arabinosus Cells.—In exploratory experiments to determine possible effects of spermine on isotopic thymidine incorporation, cells cultured in the presence of diaminodiphenylpteridine (3 μg/ml) plus thymidine (2 μg/ml) in an otherwise complete medium, in parallel cultures with and without spermine (10 μg/ml), incubated 16 hours at 30°, were harvested by centrifugation and resuspended in fresh batches of the same respective media that had been present during the growth period except that C^{14}-thymidine was present. During a subsequent 40-minute incubation at 27°, the rate of incorporation by cells grown without spermine was 3.23×10^{-5} μmoles thymidine per minute per mg dry cell weight. In comparison, cells that had been grown in the presence of spermine and then incubated with C^{14}-thymidine in the presence of spermine showed a rate of incorporation reduced by approximately 23%. This reduction may possibly be the result of dilution of exogenous (labeled) thymidine, incorporated into the cells, with endogenous (non-labeled) thymidine that was synthesized by the cells at an enhanced rate in the presence of spermine.

In a separate experiment in which thymidine-C^{14} was added at the start of the 16-hour growth period, and at lower, suboptimal concentration (0.03 μg/ml), the incorporated isotope at the end of the 16-hour period accounted for approximately 92% of the starting amount of C^{14}-thymidine in the culture medium. This observation supports the assumption in the previous section that, in 17–24 hour cultures used for study of DNA production, essentially all the exogenous thymidine is utilized.

Effect of Spermine on Formate Incorporation into L. arabinosus Cells.—In the following exploratory observations of C^{14}-formate incorporation, cells from a series of cultures grown in media containing diaminodiphenylpteridine (3 μg/ml) plus thymidine (0.1 μg/ml), with and without spermine, were harvested after 16–24 hours' incubation at 30°. The cells were then resuspended in fresh media of the same respective composition except that C^{14}-formate was included, and the amount of incorporation of C^{14} into cold acid-insoluble material was determined.

When cultures of various ages were used for this determination, cells harvested after 20–24 hours' growth in the presence of spermine (10 μg/ml) incorporated formate at rates in the range (in various experiments) 1.5–3 mμmoles per hour per mg cells, during the 30-minute period of incubation with formate-C^{14} subsequent to the growth period. This rate is from 4 to 8-fold greater than the observed rates of incorporation (approximately 0.4 mμmole formate per hour per mg cells) into cells grown in parallel cultures (20–24 hours) without exogenous spermine. Cells harvested from younger cultures (18 hours' growth or less), otherwise identical with the 20-hour or older cultures, showed no such increases in rate of formate incorporation associated with the presence of a spermine supplement during growth of the culture.

A time-course experiment confirmed this dependence of the ability to incorporate formate upon the duration of the cell growth period in the presence of spermine. Comparison of parallel cultures, one including spermine (10 μg/ml) and the other without spermine, indicated that the cells grown in the presence of spermine underwent a rather rapid rise in their ability to incorporate C^{14}-formate; this incorporation rate increased from that of cells harvested after 18 hours' growth (approximately 0.3 mμmole formate incorporated per hour per mg cells) to that of cells incubated for 20 hours (approximately 1.7 mμmoles per hour per mg cells). In contrast, the cells from the parallel culture not supplemented with spermine showed a formate incorporation rate remaining in the range 0.3–0.5 mμmole per hour per mg cells, when measured for cells incubated 16, 18, 20, 21, 22, or 23 hours. Both in the culture including and that not including spermine, cell growth (as measured turbidimetrically) proceeded approximately linearly in the interval between 16 and 24 hours' incubation time, except that the increase of cell mass decelerated somewhat in the culture without spermine after the 22nd hour of incubation.

The effect of spermine in increasing the rate of formate incorporation into cells from diaminodiphenylpteridine-inhibited, relatively old cultures requires the presence of spermine during the growth period (of 20 hours or more), and does not occur when spermine is added only during the short period of incubation with formate-C^{14}. In experiments in which spermine was omitted completely during the incubation with formate-C^{14}, increases in rate of formate incorporation were observed with cells that had been grown in the presence of spermine prior to incubation with formate-C^{14}.

Cells grown in complete medium containing neither the pteridine inhibitor nor thymidine showed a much higher rate of formate-C^{14} incorporation, in the range 5–10 mμmoles formate per hour per mg cells, than cells grown in the presence of the pteridine inhibitor and thymidine either with or without spermine.

Discussion

Limitations in one-carbon transfer reactions imposed by the presence of a folic acid antagonist (*e.g.*, diaminodiphenylpteridine, aminopterin) are manifested, in the case of *L. arabinosus*, as a growth inhibition specifically reversible (*a*) by thymidine, provided purines and all other products of one-carbon transfer reactions are supplied in the medium, or (*b*) by purines, provided thymidine is supplied. The amount of thymidine required for such prevention of inhibition is significantly decreased by spermine. However, the amount of purines required is decreased to a lesser extent by spermine, which is somewhat variable in its effect on the purine requirement. These results suggest a closer biochemical relationship of spermine to thymidine than to purines, although an additional effect on folic acid coenzyme metabolism, other than in the biogenesis of the thymidine moiety, is suggested. Evidence of this additional effect is afforded by the experiments with *P. cerevisiae*, in which spermine decreases the requirement for folinic acid, either alone or in combination with thymidine.

Although the present observations are compatible with a direct effect of spermine upon single-carbon unit metabolism, an alternative possibility is that spermine may promote more rapid biogenesis of DNA, or enhance the biological quality and effectiveness, or conservation, of the polymerized product in relation to subsequent protein synthesis so as to overcome the effect of limiting one-carbon unit transfer reactions. The final net effect of such an indirect action of spermine would be an apparent enhancement of the biogenesis of the thymidine moiety. Such an indirect effect appears compatible with observations indicating that equal final concentrations of DNA were produced in cultures of cells that attained the same growth level whether growth was stimulated by thymidine or by a combination of thymidine with spermine, as well as with observations of enhancement by spermine of formate utilization after a relatively prolonged growth period, in the presence of a folic acid antagonist.

The inability of spermine to affect the thymidine requirement for reversal of inhibition of growth of *L. arabinosus* caused by 5-fluorouracil, an inhibitor of thymidylate synthetase activity, suggests that more than a simple sparing effect upon the thymidine requirement is involved. Spermine effects upon thymidine requirements are noted only under conditions of limiting folic acid coenzymes. Thus, a possible dual role of spermine is suggested, both in stimulating one-carbon unit incorporation reactions and also directly or indirectly in the biochemical functions of thymidine, to account for the sparing effect on thymidine observed under these specific conditions. Since the thymine requirement of a strain of *E. coli* (a Gram-negative organism) was not reduced by spermine, it is also interesting to note that polyamines have been found in highest concentration in Gram-negative species, in bacterial distribution studies (references in Tabor *et al.*, 1961); spermine effects in the present study have all involved lactic acid bacteria (Gram-positive organisms), which are relatively deficient as a class in polyamine content, and which may therefore more readily exhibit cellular responses to exogenous spermine.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Judy Cline, Judy Hamann, Barbara Mollenhauer, Lynette Taylor, Nan Clayton, and Carolyn Weathersbee.

References

Anderson, E. H. (1946), *Proc. Nat. Acad. Sci. U. S. 32*, 120.
Bardos, T. J., Bond, T. J., Humphreys, J., and Shive, W. (1949), *J. Am. Chem. Soc. 71*, 3852.
Ceriotti, G. (1952), *J. Biol. Chem. 198*, 297.
Hartman, K.-U., and Heidelberger, C. (1961), *J. Biol. Chem. 236*, 3006.
Lansford, E. M., Jr., and Shive, W. (1952), *J. Biol. Chem. 194*, 329.
Lansford, E. M., Jr., Harding, W. M., and Shive, W. (1958), *Arch. Biochem. Biophys. 73*, 180.
McMahan, J. R., and Snell, E. E. (1944), *J. Biol. Chem. 152*, 83.
Shive, W., and Roberts, E. C. (1946), *J. Biol. Chem. 162*, 463.
Smith, L. C., Hayashi, K., Ravel, J. M., Skinner, C. G., and Shive, W. (1963), *Biochemistry 2*, 159 (this issue).
Tabor, H., Tabor, C. W., and Rosenthal, S. M. (1961), *Ann. Rev. Biochem. 30*, 579.