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EFFECT OF A NUMBER OF N-PYRIMIDYL AMINO ACIDS AND OF SOME OF THEIR 5-ARYLAZO DERIVATIVES ON THE GROWTH OF CERTAIN MICROORGANISMS

P. ROY-BURMAN* and D. SEN

Department of Applied Chemistry, University Colleges of Science and Technology, Calcutta, India

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Abstract—Sixteen new N-pyrimidyl amino acids were obtained by substitution at the C-2, C-4, or C-6 position of 2,4-diamino-6-methylpyrimidine by 'aromatic amino acids' or different carboxylalkylamino groups (i.e. aliphatic amino acid moiety). These compounds were tested for their effect on the growth of *Streptococcus faecalis*, *Lactobacillus arabinosus*, and *Escherichia coli*. The new compounds, 2,4-diamino-5-arylazo-6-methylpyrimidine and six N-(5-arylazo-4-pyrimidyl) amino acids, were also studied. It was found that 2,4-diamino-6-methylpyrimidine inhibited growth of all three microorganisms tested, but substitution at the C-2, C-4, or C-6 position by carboxyalkylamino groups produced compounds with little or no inhibitory effects. The three N-pyrimidyl compounds substituted with aromatic amino acids inhibited growth but were less effective than the parent compound. All the 5-arylazopyrimidines significantly inhibited growth of *S. faecalis* and, to a lesser extent, *L. arabinosus*. It was observed that among these compounds the inhibitory activity decreased with increase in the bulk of the amino acid moiety. Investigation of the mechanism of the inhibitory action of these compounds in *S. faecalis* revealed that they acted primarily as folic acid antagonists in a manner consistent with an assumption that they interfere with the enzymatic conversion of folic acid to ^{5}N-formyl tetrahydrofolic acid in *S. faecalis*.

EXTENSIVE work has been done with structural analogues of pyrimidine metabolites and many exhibited interesting biological and chemotherapeutic properties. 2,4-Diaminopyrimidines, containing bulky substituents at the C-5 position[1–6] and 6-substituted pyrimidines having substituted amino groups at C-2 and/or C-4 positions,[7–12] possess growth-inhibitory properties affecting various living systems. In our search for compounds of possible chemotherapeutic value, a number of pyrimidine derivatives having carboxylalkylamino groups (i.e. amino acid moiety) at the C-2, C-4, or C-6 position (compounds I–XIII; Table 1) were synthesized,[13–15] and the effects of these compounds on the growth of *Streptococcus faecalis*, *Lactobacillus arabinosus*, and *Escherichia coli* were investigated. Compounds with 'aromatic amino acid' moieties (derived from *p*-aminobenzoic acid and sulfanilic acid) at C-4 or C-2 position (compounds XV–XVII) and the hydrazide derivative (compound XIV) of N-(2-pyrimidyl)-glycine were also synthesized[15] for similar investigations.

The work was extended by the preparation of 5-arylazo derivatives of some of the N-pyrimidyl amino acids in Fig. 1. It is known that the 5-arylazopyrimidines are folic acid antagonists in bacterial systems.[16–20] Some of them have been found to have

* Present address: Department of Biochemistry, University of Southern California, School of Medicine, Los Angeles, Calif. 90033, U.S.A.

antitumor activity against transplanted tumors in experimental animals.[18, 19] Therefore it was of interest to investigate the biological properties of pyrimidines with 5-arylazo and amino acid substituents. It is possible that the amino acid side chain of the proposed compounds may increase cell penetration and thereby maintain a

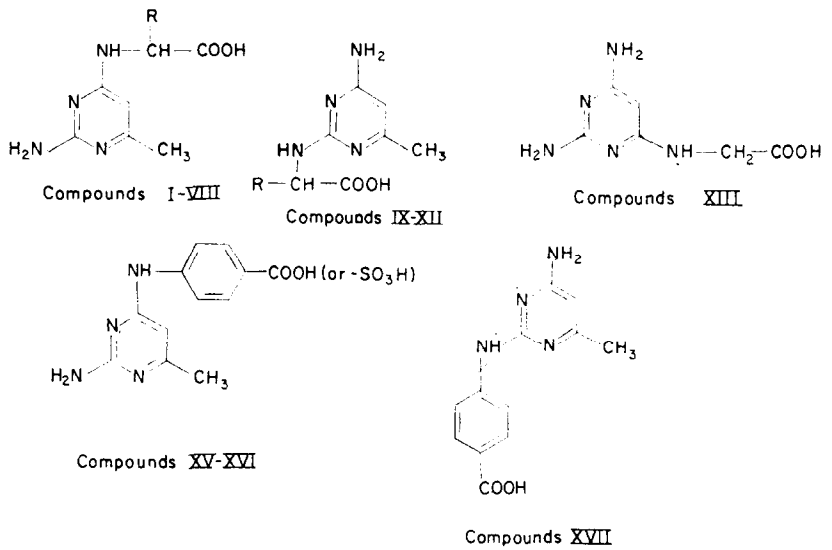


FIG. 1. Structures of N-pyrimidyl amino acids.

higher cell level. The *p*-carboxybenzeneazo moiety was chosen as a substituent because some pyrimidine derivatives having this group in the 5-position show marked antitumor activity.[18, 19] Thus several N-(arylazo-4-pyrimidyl) amino acids (compounds XIX–XXIV; Fig. 2) and the parent compound of the series (compound XVIII) were synthesized and their effect on growth of the three microorganisms studied.

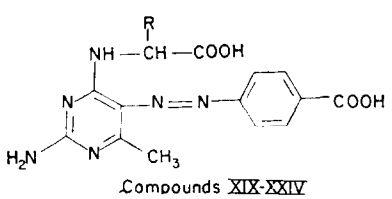


FIG. 2. Structure of N-(5-arylazo-4-pyrimidyl) amino acids.

The results of the microbiological investigations on the synthetic pyrimidine compounds are presented in this paper. A preliminary note of the results has been published.[21]

MATERIALS AND METHODS

Assay organisms

Streptococcus faecalis (ATCC 8043) and *Lactobacillus arabinosus* 17-5 (ATCC 8014) were maintained in stab cultures as recommended by Jones and Morris.[22] *Escherichia*

coli was similarly maintained on nutrient agar slants.[23] These bacteria were grown at 37° for 20–24 hr and then preserved at 2–4°.

Media

The basal medium of *S. faecalis* was the same as the standard folic acid assay medium[24] except that purines and pyrimidines were omitted. The basal medium of *L. arabinosus* was the same as described in *Methods of Vitamin Assay*.[24] The basal medium of *E. coli* was the same as described by Roy *et al.*[12] The incoculum medium of *E. coli* had the same composition as the basal medium except that glucose was added at a concentration of 0·25 g/100 ml.

Preparation of inocula

Eighteen-hour cultures of *S. faecalis* and *L. arabinosus* were added to 10 ml enriched culture medium[24] and incubated for 18 hr at 37°. Cells were harvested by centrifugation and resuspended in 10 ml normal saline. 0·1 ml of the suspension was added to another 10 ml normal saline and thoroughly mixed. This suspension was used to inoculate each assay tube. Preparation of *E. coli* inoculum was that described by Beltz and Visser.[25]

Assay methods

S. faecalis. To a series of culture tubes containing different amounts of each inhibitor, basal medium (5 ml) and other addenda were added to a final volume of 10 ml. After sterilization at 10-lb pressure (116°) for 10 min, inoculation, and incubation at 37° for 18 hr without shaking, growth was measured as turbidity with a Klett–Summerson photoelectric colorimeter (filter 66). A tube containing complete medium minus metabolite served as blank. From the growth curve (Klett reading versus inhibitor concentration), 'half inhibitory concentration' was determined by noting the inhibitor concentration at 50% inhibition of standard growth (Fig. 3). All determinations were run in duplicate and all experiments repeated at least once.

L. arabinosus and *E. coli*. The method was the same as for *S. faecalis* except that 2·5 ml basal medium was used for each culture tube, and the final volume was made up to 5 ml. In each series of experiments an uninoculated tube served as blank.

Preparation of the solutions of the compounds for testing

The compounds used were of analytical purity as previously indicated.[13–15] All the compounds except 2,4-diamino-6-methylpryimidine and the hydrazide of N-(2-pyrimidyl)-glycine (XIV), were dissolved in water containing a few drops of 30% sodium hydroxide solution. It was found that the small amount of alkali used did not change significantly the pH of the culture media. However, the same amount of alkali introduced into the assay tubes along with the inhibitor was added to the control culture, containing no inhibitor.

Reversal studies

To a series of tubes containing a given amount of the inhibitor, increasing amounts of the metabolite were added. The amount of growth at different concentrations of metabolite indicates the reversal pattern (Fig. 4).

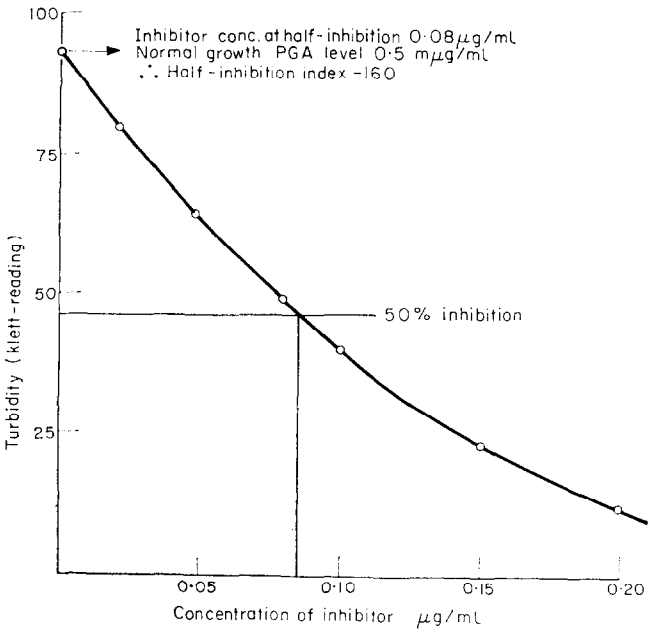


FIG. 3. Determination of half-inhibitory concentration of 2,4-diamino-5-*p*-carboxybenzeneazo-6-methylpyrimidine (XVIII) in presence of PGA (0·5 mμg/ml) with regard to the growth of *S. faecalis*.

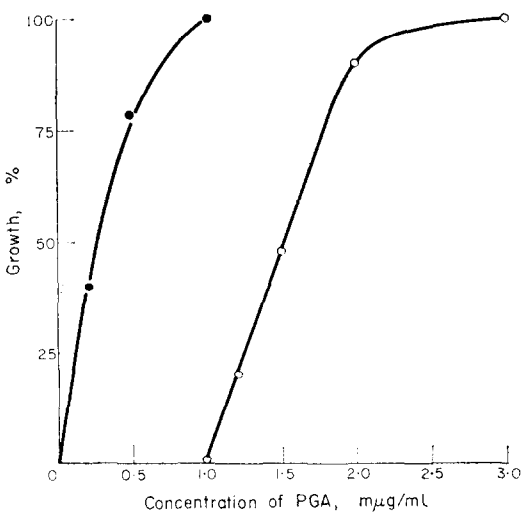


FIG. 4. Inhibition of growth of *S. faecalis* by 2,4-diamino-5-*p*-carboxybenzeneazo-6-methylpyrimidine (XVIII) and its reversal by increasing concentrations of PGA.
——●——●——, Growth curve in absence of inhibitor.
——○——○——, Growth curve in presence of compound XVIII (0·4/μg/ml; cf. Table 4).

RESULTS AND DISCUSSION

Table 1 shows the effects of the synthetic pyrimidines (I–XXIV) on growth of *S. faecalis*, *L. arabinosus*, and *E. coli*. The inhibitory effects were measured in terms of half-inhibitory concentration (Fig. 3). It is evident from Table 1 that the parent member of the present series of pyrimidines, i.e. 2,4-diamino-6-methylpyrimidine, is

strong inhibitor of the growth of *S. faecalis*. It also inhibits the growth of *L. arabinosus* and *E. coli*, but to a much lesser extent. Substitution of carboxyalkylamino groups at C-4 (compounds I–VIII) or at C-2 (compounds IX–XII) destroys almost all the inhibitory activity in all three organisms. Replacement of the methyl group at C-6 by carboxymethylamino group (compound XIII) also inactivates the compound against all three microorganisms, although the 2,4-diaminopyrimidine moiety remains intact. These observations may be correlated with those of McNair Scott *et al.*[26] and Falco *et al.*,[5] who found that substitution of the amino group of some 2,4-diaminopyrimidines by groups like cyanamino, morpholino, etc. destroys the antibacterial or antimalarial activity. The presence of a 2,4-diaminopyrimidine moiety does not always lead to antimicrobial activity. Falco and co-workers have recently observed that, unlike the closely related 5-phenoxy and 5-benzylpyrimidines, 2,4-diamino-5- phenylmercaptopyrimidines were practically devoid of activity against *Plasmodium gallinaceum* infections in chicks, *P. berghei* in mice, and a variety of bacteria.[27]

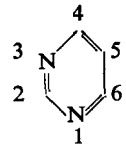
Since the biological activity of amino acid hydrazides as antimetabolites of amino acids was reported by Suzuki and Tanaka,[28] one of the N- pyrimidyl amino acids was converted to its hydrazide derivative (compound XIV). This compound does not inhibit the growth of *S. faecalis* and *L. arabinosus* but inhibits growth of *E. coli* at high concentration. This inhibition cannot be reversed by PGA (folic acid) or ^{5}N-formyl tetrahydrofolic acid (^{5}N-formyl THF) or any of the nucleic acid bases, nucleosides, or amino acids.

Roy and co-workers[12] observed that substitution of the amino groups of 2,4-diamino-6-hydroxypyrimidine by *p*-substituted phenylamino groups increased the inhibitory activity of the secompounds. The *p*-substituted phenylamino compounds were powerful inhibitors of the growth of *S. faecalis*, *L. arabinosus*, and *E. coli*. The substituents in the *para* position of the benzene ring were chloro-, nitro-, methyl-, and methoxyl- in the compounds studied by Roy *et al.* Our observations show, however, that the substitution of the amino groups of 2,4-diamino-6-methylpyrimidine by a *p*-carboxy (or *p*-oxysulphonyl) phenylamino group decreases the activity of the parent compound. However, the low solubility of compound XVI (Table 1) does not permit precise conclusions.

It can be seen (Tables 1 and 2) that 2,4-diamino-6-methyl-5-arylazopyrimidine (compound XVIII) has significant inhibitory activity. The inhibitory effect of this series of compounds is most pronounced with *S. faecalis*, is less with *L. arabinosus*, and is practically absent with *E. coli*. Relative growth-inhibitory activities of these compounds on *S. faecalis* and *L. arabinosus* are given in Tables 2 and 6. It is observed that the 2,4-diamino derivative (XVIII) shows maximal effect on *S. facealis* and *L. arabinosus*. Among the N-pyrimidyl amino acid derivatives (XIX-XXIV) ,the inhibitory activity decreases with the increase of the bulk of amino acid moiety. It is possible that the increase of the bulk of amino acid side chain decreases the cell-permeability of the compound and thus reduces its inhibitory effect.

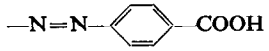
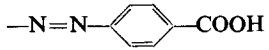
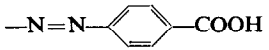
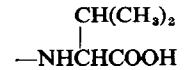
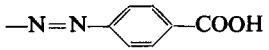
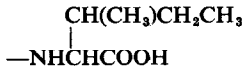
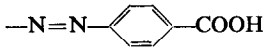
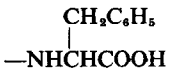
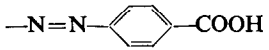
Various experiments were carried out to determine the mechanism of inhibitory action of the 5-arylazopyrimidines on *S. faecalis*. These include (a) determination of half-inhibition indices of the compounds at various levels of pteroylglutamic acid (PGA) and ^{5}N-formyl THF, and (b) reversal studies with excess of PGA and other purines and pyrimidines. It can be observed from Table 2 that for each of these arylazo compounds, the values of the half-inhibition index at different levels of PGA

TABLE 1. RELATIVE GROWTH-INHIBITORY EFFECTS OF SUBSTITUTED PYRIMIDINES



Among the compounds listed below, compound VI is the L-isomer; compounds II–V, VII, VIII, X–XII, and XX–XXIV are DL mixtures.

Compound no.	Position of substituent				*S. faecalis* (PGA medium[a])	*L. arabinosus*	*E. coli*
	2	4	5	6			
Nil	$-NH_2$	$-NH_2$		$-CH_3$	+++	+	+
I–VIII[b]	$-NH_2$	—NHCH(R)COOH		$-CH_3$	−	−	−
IX–XII[c]	—NHCH(R)COOH	$-NH_2$		$-CH_3$	−	−	−
XIII	$-NH_2$	$-NH_2$		$-NHCH_2COOH$	−	−	−
XIV	$-NHCH_2CONHNH_2$	$-NH_2$		$-CH_3$	−	−	+
XV	$-NH_2$	—NH—C$_6$H$_4$—COOH		$-CH_3$	±	±	±
XVI	$-NH_2$	—NH—C$_6$H$_4$—SO_3H		$-CH_3$	⊕	⊕	⊕
XVII	—NH—C$_6$H$_4$—COOH	$-NH_2$		$-CH_3$	±	±	±
XVIII	$-NH_2$	$-NH_2$	—N=N—C$_6$H$_4$—COOH	$-CH_3$	++++	+++[d]	*

XIX	$-NH_2$	$-NHCH_2COOH$		$-CH_3$	+++	+[d]	*
XX	$-NH_2$	$-NHCH(CH_3)COOH$		$-CH_3$	+++	+[d]	*
XXI	$-NH_2$	$-NHCH(CH_2CH_3)COOH$		$-CH_3$	+++	+[d]	*
XXII	$-NH_2$			$-CH_3$	+++	±[d]	*
XXIII	$-NH_2$			$-CH_3$	+++	±[d]	*
XXIV	$-NH_2$			$-CH_3$	++	±[d]	*

a Contains PGA (1 mμg/ml), allowing half-maximal growth.
b Compound I: R = —H; II: R = $-CH_3$; III: R = $-CH_2OH$; IV: R = $-CH_2CH_3$; V: R = $-CH(CH_3)_2$; VI: R = $-CH_2CH(CH_3)_2$; VII: R = $-CH(CH_3)CH_2CH_3$; VIII: R = $-CH_2C_6H_5$.
c Compound IX: R = —H; X: R = $-CH_3$; XI: R = $-CH_2OH$; XII: R = $-CH_2C_6H_5$.
d See Table 6.
— No inhibition up to 1,000 μg/ml.
* No inhibition up to 500 μg/ml. Higher concentrations could not be used, owing to low solubility of the compound.
± Inhibitory at 500 μg/ml. Higher concentration could not be used, owing to low solubility of the compound. Half-inhibition concentrations could not be determined.
⊕ Inhibitory at 100 μg/ml. Higher concentration could not be used, owing to low solubility of the compound. Half-inhibition concentration could not be determined.
+ Inhibition 50% at 101–1,000 μg/ml.
++ Inhibition 50% at 11–100 μg/ml.
+++ Inhibition 50% at 1–10 μg/ml.
++++ Inhibition 50% at 0·1–1 μg/ml.

(or ^{5}N-formyl THF) are of the same order of magnitude over a 20-fold range of concentration of PGA (or ^{5}N-formyl THF). A greater constancy of the values of inhibition index is observed when PGA or ^{5}N-formyl THF is present at a concentration higher than that required for maximal growth in the absence of inhibitors. This indicates that the arylazo compounds act as competitive antagonists of PGA or ^{5}N-formyl THF.

TABLE 2. HALF-INHIBITION INDICES OF 5-ARYLAZOPYRIMIDINES AT DIFFERENT LEVELS OF PGA AND ^{5}N-FORMYL THF IN *S. faecalis*

Compounds	Metabolite level (mμg/ml)	Half-inhibition index[12]	
		PGA	N^5-formyl THF*
2,4-Diamino-6-methyl-5-(*p*-carboxybenzeneazo)-pyrimidine (XVIII)	0·5	160	3,340
	1·0	105	2,170
	5·0	84	1,548
	10·0	88	1,225
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-glycine (XIX)	0·5	1,520	78,680
	1·0	1,070	50,000
	5·0	880	[a]
	10·0	1,040	[a]
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-DL-alanine (XX)	0·5	2,080	215,960
	1·0	2,250	178,000
	5·0	2,300	[a]
	10·0	2,480	[a]
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-DL-α-aminobutyric acid (XXI)	0·5	3,640	550,000
	1·0	2,700	380,000
	5·0	4,000	[a]
	10·0	4,000	[a]
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-DL-valine (XXII)	0·5	8,000	[a]
	1·0	7,200	[a]
	5·0	6,000	[a]
	10·0	7,300	[a]
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-DL-isoleucine (XXIII)	0·5	8,500	[a]
	1·0	7,800	[a]
	5·0	6,600	[a]
	10·0	8,400	[a]
N-(2-Amino-6-methyl-5-*p*-carboxybenzeneazo-4-pyrimidyl)-DL-phenylalanine (XXIV)	0·5	16,000	[a]
	1·0	22,800	[a]
	5·0	15,000	[a]
	10·0	12,400	[a]

* Since synthetic ^{5}N-formyl THF is half as active as naturally occurring ^{5}N-formyl THF because of the presence of the inactive isomer, all concentrations of ^{5}N-formyl THF in *this and subsequent* tables refer to 50% of the weight of ^{5}N-formyl THF used.

[a] Concentration of the compound required for 50% inhibition could not be reached, owing to low solubility of the compound.

This is further corroborated by the results of the reversal experiments (Tables 3 and 4 and Fig. 4) which show that excess of PGA and also of ^{5}N-formyl THF can completely reverse the growth-inhibitory effects of high concentrations of the arylazopyrimidines in *S. faecalis*.

It is known that PGA and ^{5}N-formyl THF are equally effective as a growth-requirement of *S. faecalis*[29] and that *S. faecalis* cells synthesize ^{5}N-formyl THF (or its intracellular form) from PGA.[1] The arylazo compounds are strong inhibitors of *S. faecalis* when grown in the presence of PGA but, when ^{5}N-formyl THF is used in the

TABLE 3. EFFECTS OF INCREASING DOSES OF PGA AND ^{5}N-FORMYL THF ON GROWTH OF *S. faecalis* IN MEDIA* CONTAINING INHIBITORS (XVIII–XXIV)

Inhibitor	Concentration of inhibitor (μg/ml)	Concentration of PGA or ^{5}N-formyl THF† (mμg/ml)	Per cent growth‡	
			PGA	^{5}N-formyl THF
Nil	0	0	100	100
XVIII	0·2	0	31	31
		0·25	37	41
		0·5	51	67
		1·0	100	100
XIX	2·0	0	22	22
		0·25	32	50
		0·5	64	78
		1·0	100	100
XX	4·0	0	40	40
		0·25	46	57
		0·5	64	86
		1·0	100	100
XXI	8·0	0	48	48
		0·25	58	75
		0·5	86	100
		1·0	100	100
XXII	16·0	0	43	43
		0·25	50	66
		0·5	74	97
		1·0	100	100
XXIII	20·0	0	24	24
		0·25	36	53
		0·5	74	92
		1·0	100	100
XXIV	60·0	0	24	24
		0·25	36	53
		0·5	74	92
		1·0	100	100

* The medium contains PGA (1 mμg/ml) in all cases.

† This does not include 1 mμg of PGA/ml present in the medium in each case.

‡ Standard growth (without inhibitor) of *S. faecalis* with 1 mμg of PGA/ml has been taken as 100.

growth medium instead of PGA, inhibitory effects of these compounds are low (Table 2). This finding indicates that these compounds may act as antagonists of PGA and that they may interfere with the enzymatic conversion of PGA to ^{5}N-formyl THF, a view that is supported by the fact that the growth inhibition caused by these aryl-azopyrimidines can be more efficiently reversed by ^{5}N-formyl THF than by PGA (Tables 3 and 4). However, the low inhibitory effects of arylazopyrimidines in

presence of ^{5}N-formyl THF (Table 2) indicates that some other unknown inhibitory effect, unrelated to ^{5}N-formyl THF synthesis, is exerted by the analogues.

Evidence that the arylazo compounds interfere with folic acid metabolism in *S. faecalis* is supported by the results of reversal studies with purines, pyrimidines, and amino acids. Metabolites used were adenine, guanine, xanthine, orotic acid, uracil,

TABLE 4. EFFECTS OF INCREASING DOSES OF PGA AND ^{5}N-FORMYL THF ON GROWTH OF *S. faecalis* IN MEDIA* CONTAINING HIGH CONCENTRATIONS OF INHIBITORS XVIII AND XIX

Inhibitor	Concentration of inhibitor (μg/ml)	Concentration of PGA or N^5-formyl THF† (mμg/ml)	Per cent growth‡	
			PGA	N^5-formyl THF
Nil	0	0	100	100
XVIII	0·4	0	0	0
		0·2	20	25
		0·5	48	62
		1·0	90	100
		2·0	100	100
XIX	5·0	0	0	0
		0·2	10	15
		0·5	23	35
		1·0	54	70
		2·0	100	100

* † ‡ Same as in Table 3.

TABLE 5. EFFECTS OF INCREASING CONCENTRATIONS OF THYMINE AND THYMIDINE ON THE INHIBITORY ACTION OF ARYLAZOPYRIMIDINE DERIVATIVES (XVIII–XXIII) IN REGARD TO *S. faecalis* IN MEDIA CONTAINING PGA*

Inhibitors	Concentrations of the inhibitors (μg/ml)	Per cent relative growth†						
		Concentration of metabolite (μg/ml)						
		0	Thymine			Thymidine		
			0·5	1·0	10·0	0·5	1·0	10·0
Nil	0	66	77	95	100	72	82	100
XVIII	0·2	11	40	64	95	34	61	93
XIX	1·0	27	47	69	84	34	50	80
XX	2·0	36	69	83	94	46	68	87
XXI	3·0	45	51	75	86	50	63	93
XXII	5·0	50	75	82	94	53	69	92
XXIII	10·0	41	53	86	92	53	71	92

* The medium contains 1 mμg of PGA/ml.

† Growth (without inhibitor) of *S. faecalis* with 10μg of thymine or thymidine/ml has been taken as 100.

thymine, thymidine, cytidine, serine, and methionine. Among these metabolites only thymine and thymidine are capable of reversing the growth inhibition produced by the arylazopyrimidines (Table 5)—others being incapable of doing so. The reversal by thymine and thymidine indicates that the compounds interfere with the endogenous

synthesis of these metabolites which require ^{5}N, ^{10}N-methylenetetrahydrofolate[30] for their biosynthesis.

The requirement for folic acid in *S. faecalis* can be satisfied by a combination of thymine and a purine derivative.[31, 32] For this reason, the effect of the arylazopyrimidines on *S. faecalis* in a medium containing adenine and thymine but no PGA was studied. The results show that the inhibitory activity of the compounds in adenine–thymine medium is much less than in PGA medium (Table 6). This indicates that

TABLE 6. CONCENTRATIONS OF THE ARYLAZOPYRIMIDINES REQUIRED FOR 50% GROWTH INHIBITION OF *S. faecalis* AND *L. arabinosus*

Compound	Half-inhibitory concentration (μg/ml)		
	S. faecalis		*L. arabinosus*
	Adenine and thymine medium*	PGA medium†	
XVIII	17·74	0·42	7·20
XIX	45·00	4·40	120·00
XX	142·00	11·50	160·00
XXI	320·00	20·00	200·00
XXII	[a]	30·00	[a]
XXIII	[a]	33·00	[a]
XXIV	[a]	75·00	[a]

* Medium contains 3 μg of thymine/ml and 30 μg of adenine/ml. Approximately 2·5 μg of thymine/ml is required for maximal growth.

† Medium contains 5 mμg of PGA/ml (3 mμg is required for maximal growth). The corresponding inhibitor concentrations in the presence of 3 mμg PGA/ml would be lower than the values given here.

[a] Compounds are inhibitory at 500 μg/ml. Higher concentrations were not used, owing to low solubility. Half-inhibitory concentrations of these compounds could not be determined.

when these products of the folic acid requirement are supplied, the effectiveness of the arylazo compounds is significantly reduced. The data indicate that these compounds are similar to the folic acid antagonists reported by Hitchings *et al.*[33]

The effect of the arylazo compounds on the growth of *L. arabinosus* is shown in Table 6. It is apparent that the compounds inhibit the growth of *L. arabinosus* to a lesser extent than *S. faecalis*.

The ability of adenine, guanine, adenosine, guanosine, uracil, orotic acid, thymine, thymidine, cytidine, PGA, and ^{5}N-formyl THF to reverse the effect of the arylazo compounds on the growth of *L. arabinosus* was also investigated. Thymine, thymidine, and ^{5}N-formyl THF partially reverse the inhibition of compounds XVIII–XX (Table 7), whereas PGA and other metabolites have no effect. Folic acid enzyme systems are known to be involved in the biosynthesis of thymidine in *L. arabinosus*.[34] From these facts and since the arylazopyrimidines have been found to exert their effect on *S. faecalis* primarily by acting as folic acid antagonists, it would be expected that these compounds also affect folic acid metabolism in *L. arabinosus*.

Four N-pyrimidyl amino acids (I, II, VI, VII) were tested by Dr. Chester Stock at the Sloan-Kettering Institute, New York, on mouse sarcoma 180 but only one of them, compound VI, was found to possess some activity against sarcoma 180. Compound VI was further tested for its effect on the growth of a number of anaerobic

TABLE 7. REVERSAL OF THE INHIBITORY EFFECTS OF THE COMPOUNDS XVIII–XX BY ^{5}N-FORMYL THF, THYMINE, AND THYMIDINE IN *L. arabinosus*

Metabolites* added	Concentration of metabolites (μg/ml)	Per cent growth† Inhibitor XVIII Conc. (20 μg/ml)	Inhibitor XIX Conc. (100 μg/ml)	Inhibitor XX Conc. (200 μg/ml)
Nil		28	66	44
N^5-formyl THF	200	42	75	57
	400	42	77	57
	600	45	77	57
Thymine	200	60	76	60
	600	60	78	62
Thymidine	200	58	80	62
	400	60	82	62
	600	65	82	63

* The addition of these metabolites to the culture medium does not result in stimulation of growth in the absence of inhibitor.

† Growth of *L. arabinosus* in the absence of inhibitor has been taken as 100.

bacteria, and compounds I and XIX were tested against B82A and B82T (solid form) leukemia respectively in the same laboratory. The compounds were ineffective at the doses tested.

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