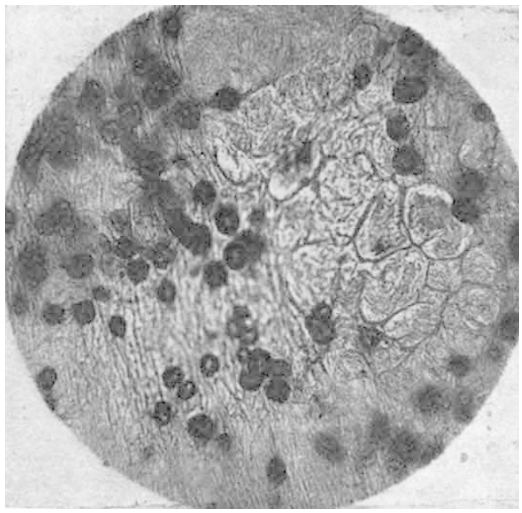

Fig. 1. Photomicrograph of part of a section of a proglottid of *Taenia saginata* showing the calcareous corpuscles stained with nuclear fast red. × 160

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Inhibition Steps in Sulphonamide Bacteriostasis

THE activity of several compounds, structurally unrelated to *p*-aminobenzoic acid, in reversing growth inhibition of *Escherichia coli* by sulphonamides, was explained by Shive and Roberts[1] as due to their being products of enzymes associated with *p*-aminobenzoic acid. Using increasing concentrations of sulphanilamide, these authors showed that it inhibits sequentially the synthesis of methionine and xanthine by the organism. Winkler and de Haan[2] extended this study to higher concentrations of the inhibitor, and reported further involvement of serine and thymine (the latter, interchangeably with pteroyl glutamic acid) in that order in the *p*-aminobenzoic acid action; further addition of valine was stimulatory to growth.

During studies on the reversal of growth inhibition of *Esch. coli* (McLeod) by sulphadiazine, we observed that, in a concentration of the drug up to 30 mgm. per cent, a combination of the foregoing five metabolites was necessary and effective; however, at its maximum solubility (50 mgm. per cent) in the medium employed[3] they could no longer overcome growth inhibition. When, in addition to them, mixtures of purines and pyrimidines, amino-acids, or the *B*-group of vitamins were tried (see Table 1), it was found that the amino-acid mixture, and to a less extent the vitamins, could reverse considerably the inhibition of growth by the drug. Using individual amino-acids,

Table 1. REVERSAL EFFECTS ON GROWTH INHIBITION OF *Esch. coli* BY SULPHADIAZINE

	Additions to 10 c.c. of basal medium[a]	Growth[b] without sulphadiazine	Growth[b] with sulphadiazine (5 mgm./10 c.c.)
1	None	47	0
2	*p*-Aminobenzoic acid (0·5 mgm.)	47	46
3	Methionine (0·5 mgm.) + xanthine (0·25 mgm.) + serine (0·2 mgm.) + thymine (0·25 mgm.) + valine (0·5 mgm.)	49	0
4	As in (3) + amino-acid mixture[c]	72	53
5	As in (3) + vitamin mixture[d]	46	25
6	As in (3) + purine–pyrimidine mixture[e]	48	0
7	As in (3) + glycine (0·25 mgm.)	48	27
8	As in (3) + threonine (0·4 mgm.)	47	19
9	As in (7) + threonine (0·4 mgm.)	48	28
10	As in (3) + vitamin B_{12} (10 mμgm.)	49	23
11	As in (7) + vitamin B_{12} (10 mμgm.)	47	40
12	As in (11), but with 0·1 mgm. each of adenine, xanthine and guanine in place of 0·25 mgm. of xanthine	50	48

[a] Green and Sevag (ref. 3).
[b] Represents 48 hr. growth in terms of turbidity measured on a Klett-Summerson photocolorimeter at 660 mμ.
[c] 0·2 mgm. each of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, *iso*leucine, leucine, lysine phenyl alanine, proline, threonine, tryptophan and tyrosine.
In all experiments, racemic forms of the optically active amino-acids were used.
[d] Thiamine hydrochloride, riboflavin, nicotinic acid, pantothenic acid, and pyridoxine hydrochloride (10 μgm. each), pteroyl glutamic acid (3 μgm.), 'Leucovorin', Lederle (3 μgm.) and vitamin B_{12} (10 mμgm.).
[e] 0·1 mgm. each of adenine, guanine and uracil.

it was ascertained that the active member was either glycine or threonine; the former was more effective than the latter on a molar basis, and there was no additive effect among the two. Vitamin B_{12} alone, among the B vitamins, showed a reversal effect. A combination of glycine and vitamin B_{12} was nearly as active as *p*-aminobenzoic acid, and substitution of xanthine by a mixture of purines (xanthine, adenine and guanine) gave reversal of growth inhibition comparable to that with *p*-aminobenzoic acid (Table 1).

It would seem from the above that synthesis of glycine is yet another reaction mediated by *p*-aminobenzoic acid and blocked by sulphonamides. The organism *Esch. coli* is apparently capable of converting threonine to glycine (cf. ref. 4). Such a possibility is also indicated from the observation of Ravel *et al.*[5] that glycine and threonine both increase synthesis of 4-amino-5-imidazole carboxamide by *Esch. coli* under conditions of sulphonamide bacteriostasis.

It is difficult to explain the relationship observed here between the action of *p*-aminobenzoic acid and that of vitamin B_{12} solely on the basis of an effect of the former on the synthesis of the latter[6]. Shive[6,7] had also reported on the potentiating action of vitamin B_{12} on methionine, xanthine and serine, at each stage in their reversal of sulphanilamide action, and on the replaceability of thymine by pteroyl glutamic acid or by vitamin B_{12}.

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