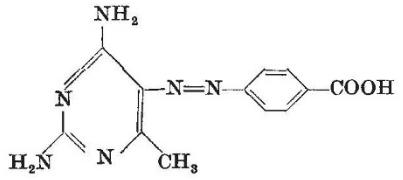
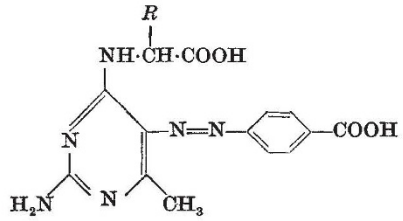
N-(5-Arylazo-4-Pyrimidyl)-Amino-Acids as Growth Inhibitors of *Streptococcus faecalis*

It was shown by Tanaka *et al.*[1,2] that 5-arylazo pyrimidines having different groups (for example, hydroxyl, methyl or amino) at the 2, 4 and 6 positions are strong folic acid antagonists and that some of these possess marked anti-neoplastic properties. They also found that the nature of substituents in the benzene ring of the phenylazo radical was the determining factor for this activity.

We considered it to be of interest to investigate the biological properties of pyrimidine systems containing a 5-arylazo as well as an amino-acid moiety. It is expected that in the proposed compounds the amino-acid side-chain would increase penetration and help maintain a higher blood-level and thus render the molecule as a whole more effective. In continuation of our synthesis of *N*-pyrimidyl amino-acids[3,4], we have, therefore, synthesized several *N*-(2-amino-5-*p*-carboxybenzeneazo-6-methyl-4-pyrimidyl)-amino-acids (compounds II–VII) and have examined their action on *S. faecalis*. The parent compound (I) was also synthesized for comparison. (So far as we know, these compounds (I–VII) are being reported for the first time.)



(I)



(II–VII) (Table 1)

We chose the *p*-carboxybenzeneazo moiety because pyrimidines having this group in the 5-position show marked anti-tumour activity although their inhibitory action on certain micro-organisms are somewhat less pronounced than that of some other compounds of this type[1,2].

All the compounds synthesized were found to inhibit the growth of *S. faecalis*. However, the amino-acid moiety was found to decrease the inhibitory effect to some extent. Thus compounds (II–VII) are less inhibitory than the parent compound (I). It has also been found that among the compounds II–VII the inhibitory activity decreases with the increase of the bulk of the amino-acid moiety (Table 1).

Inhibition studies have also been carried out using other concentrations of pteroylglutamic acid (PGA) and leucovorin and it has been observed that all these compounds can be regarded as competitive antagonists of both PGA and leucovorin in regard to the growth of *S. faecalis*. These compounds also inhibit the growth of *S. faecalis* when PGA in medium (†) is replaced by a combination of adenine and thymine, but to a much lesser extent. The inhibition can be reversed by certain metabolites.

Table 1. Inhibition of *S. faecalis* by *N*-(5-Arylazo-4-pyrimidyl)-Amino-Acids

Compound	Concentration (μg/ml.) required for 50 per cent inhibition of growth* PGA†	Leucovorin‡
I	0·105	2·17
II : R = – H	1·07	50·00
III§ : R = – CH_3	2·25	178·00
IV§ : R = – $CH_2.CH_3$	2·70	380·00
V§ : R = – $CH(CH_3)_2$	7·19	¶
VI§ : R = – $CH(CH_3)CH_2CH_3$	7·80	¶
VII§ : R = – $CH_2.C_6H_5$	22·80	¶

* Growth measured turbidimetrically after 18 h at 37° C (micro-organism used was *Streptococcus faecalis* A.T.C.C. 8043).

† Folic acid assay medium (ref. 5) excluding the purine and pyrimidine metabolites was used. Medium contained pteroylglutamic acid (PGA) (0·001 μg/ml.).

‡ Medium same as in †. No PGA used. Concentration of leucovorin (0·001 μg/ml.) refers to 50 per cent of the weight of 'leucovorin' used, since synthetic leucovorin is half as active as naturally occurring citrovorum factor due to the presence of an inactive isomer.

§ All these compounds are of DL-variety.

¶ Half-inhibition concentration could not be reached because the compound was not sufficiently soluble in the basal medium to permit the attainment of the required high concentration.

The metabolites used in the reversal experiments were adenine, guanine, xanthine, uracil, thymine, orotic acid, thymidine, cytidine, PGA, leucovorin, serine and methionine. The inhibition caused by the compounds could, however, be reversed only by thymine, thymidine, PGA and leucovorin. Although PGA and leucovorin are both equally effective for the growth of *S. faecalis*, these *N*-pyrimidyl amino-acid derivatives are much more inhibitory in presence of PGA than in presence of leucovorin (Table 1). These facts suggest that the compounds exert their inhibitory activity mainly by interfering with the enzymatic conversion of PGA to citrovorum factor in the metabolic pathway of *S. faecalis* cell.

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Glucose-6-Phosphate and 6-Phosphogluconate Dehydrogenase Activities in Erythrocytes of Monkeys infected with *Plasmodium knowlesi*

Certain drugs, when administered to individuals deficient in erythrocyte glucose-6-phosphate (G-6-P) dehydrogenase, can cause hæmolytic crises. Episodes of acute hæmolytic anæmia caused by a variety of infections have been reported in enzyme-deficient individuals[1]. To investigate if the presence of a similar deficiency in monkeys could explain the severe hæmolysis which is a frequent feature of acute *Plasmodium knowlesi* infections in monkeys, quantitative estimations of erythrocyte G-6-P dehydrogenase were carried out. Fifty-eight rhesus monkeys, *Macaca mulatta*, of both sexes, were examined, and no evidence of dehydrogenase deficiency was found. This finding is in agreement with the recent report of Lie-Injo Luan Eng[2].

Blood was obtained from the saphenous veins of monkeys under general anæsthesia and was stored at 4° C in acid–citrate–dextrose solution. The test was normally performed within 3 h. Glucose-6-