FORMATION OF AN ARYLAMINE BY A SULFANILAMIDE-RESISTANT STRAIN OF *BACILLUS SUBTILIS*

BY SUDHA JOSHI AND A. SREENIVASAN, F.A.SC.
(*Department of Chemical Technology, University of Bombay*)

Received August 19, 1957

STETTEN AND FOX (1945) observed the accumulation of a diazotisable arylamine in the culture filtrate of *Escherichia coli* in presence of bacteriostatic concentrations of sulfonamides. It was characterised by Shive and coworkers (1947) as 5 (4) amino-4 (5) imidazole carboxamide which was suggested to be a precursor of the purine ring. The riboside is the predominant form in which the amine is known to accumulate (Greenberg, 1956). The compound is utilized by purine-requiring micro-organisms as a purine substitute (Shive, 1951). It was suggested (Shive *et al.*, 1947; Woolley and Pringle, 1950) that a folic acid derivative was concerned in the conversion of the arylamine to purine and Buchanan and Schulman (1953) have demonstrated the *in vitro* conversion of the arylamine-ribotide to inosinic acid with a liver enzyme preparation to be influenced by folinic acid.

Sevag and Green (1944) on the other hand observed that a sulfonamide-resistant strain of *Staphylococcus aureus* elaborated an arylamine which was identified as kynurenine. The formation of kynurenine was independent of attainment of resistance to sulfanilamide and even the susceptible strain could be induced to elaborate it after certain alterations in the medium, in particular, on addition of glucose and tryptophan. Love and Gots (1955) observed the accumulation in a purine-requiring mutant of *E. coli* of a new diazotisable arylamine which was probably an intermediate in carboxamide formation.

During studies (Joshi and Sreenivasan, present paper) on the possibility of acetylation of sulfonamides being associated with acquisition of resistance, it was observed that a strain of *Bacillus subtilis*, rendered resistant to 0·5 per cent. concentration of sulfanilamide by exposing the susceptible strain to gradually increasing concentrations of the drug, produced a diazotisable arylamine irrespective of the presence of the drug. The observations regarding the influence of various cultural factors on this amine accumulation are discussed herein.

EXPERIMENTAL

Organism.—Studies were carried out with *B. subtilis*, a local isolate used in the earlier studies (Joshi and Sreenivasan, 1958). The original susceptible strain was rendered resistant to 0·5 per cent. sulfanilamide by gradually exposing it to increasing concentrations of the drug. Unless otherwise stated the resistant organism indicates therefore 0·5 per cent. sulfanilamide-resistant strain of *B. subtilis*.

Culture medium.—The medium of Muir *et al.* (1942) with 1 per cent. casein hydrolysate stored in double strength under sterile conditions was used for growing the strains.

Testing methods.—For testing the effect of various substances on the arylamine formation, 5 ml. of double strength medium and solutions of the test materials were taken in bacteriological test-tubes, total volume made up to 10 ml. with water, adjusted to pH 7·2 and finally autoclaved at 15 lb. steam pressure for 15 minutes.

The susceptible and resistant strains were grown in the basal medium without sulfanilamide and with 0·5 per cent. sulfanilamide respectively for 18 hours. The cells were centrifuged and washed repeatedly with 0·9 per cent. saline under sterile conditions; in the case of the resistant strain, washings were continued till they were free from sulfanilamide as revealed by test with Bratton and Marshall reagent (1939). The cells were resuspended in 0·9 per cent. saline for use as inoculum.

The test solutions with the nutrient medium, tubed and sterilised, were inoculated with 2 drops of the inoculum and incubated at 37°. A complete set of similar tubes simultaneously filled and sterilised but not inoculated with the organism was also incubated alongside in every experiment. They served as controls for the basal medium with the various test materials. All observations were made at least in duplicate.

After incubation for about 48 hours (this time has varied to some extent in different experiments) at 37°, the cells were centrifuged, and the centrifugates diluted to suitable volumes. Aliquots were assayed for the arylamine which gave a violet colour with the Bratton and Marshall reagent (1939). The arylamine was calculated in terms of sulfanilamide from Klett readings with the help of a standard curve (Joshi and Sreenivasan, 1958).

In some experiments, additional sets of control and experimental tubes were run for measurements of cell growth. The contents of the tubes were mixed well and turbidity estimated after dilution where necessary with the Klett-Summerson photoelectric colorimeter with filter 660 mμ in position.

RESULTS

Formation of a diazotisable arylamine by the resistant strain.—The resistant strain was grown in 80 ml. medium in 250 ml. Erlenmeyer flasks at 37° for 48 hours in presence and absence of sulfanilamide. Flasks autoclaved after inoculation with the organism served as controls. About 2800 μg. of the arylamine was formed in absence of sulfanilamide while in presence of 400 μg. of the drug the arylamine concentration increased to 3300 μg. The presence of sulfanilamide in the medium is thus not necessary for arylamine production. The arylamine was formed maximally in 48 hours of incubation. The property of the arylamine formation was retained by the resistant strain more or less permanently and actual multiplication of cells appeared necessary. The susceptible strain did not produce any diazotisable arylamine under these conditions.

Effects of glucose and tryptophan.—Sevag and Green (1944) found that the arylamine formed in a resistant strain of *S. aureus* was an oxidation product of tryptophan and that it was increased by addition of glucose and tryptophan. The susceptible strain of *S. aureus* also produced the same arylamine in presence of glucose and tryptophan. Therefore the effects of glucose ($1 \cdot 1 \times 10^{-2}$ M) and tryptophan ($1 \cdot 0 \times 10^{-2}$ M) separately and together were noted on the arylamine formation with susceptible and resistant strains of *B. subtilis*; the concentrations of the substances were similar to those employed by Sevag and Green (1944). It was observed that both the substances did not induce any arylamine formation in the susceptible strain. With the resistant organism, the total arylamine formation (μg.) was 195, 197, 162 and 160 respectively when grown in the basal medium without any additions and with additions of glucose, tryptophan and glucose + tryptophan.

Thus, glucose has no influence on the arylamine formed with the resistant strain whether by itself or in the presence of tryptophan. The lower values with tryptophan indicate that either it helps in the further metabolisation of the amine or that it exerts an inhibitory influence on the arylamine formation.

Effects of pantothenic acid and methionine.—Sevag and Green (1944) observed that the diazotisable arylamine formed in *S. aureus* was increased by pantothenate probably through enhancement of tryptophan synthesis and pantothenic acid also reversed the effect of sulfonamides. A sparing action of methionine for pantothenic acid in antibody formation was noted by Ludovici *et al.* (1951). Methionine also prevents the toxicity of low concentrations of sulfonamides as reported by Kohn and Harris (1941) and

Bliss and Long (1941). The effects of calcium pantothenate ($0 \cdot 21 \times 10^{-2}$ M) and *dl* methionine ($1 \cdot 3 \times 10^{-2}$ M) were therefore studied on the arylamine formation in both the susceptible and resistant strains. In the former case, these additions did not induce any amine formation while, in the latter, the values for arylamine (μg.) formed with the pantothenate and methionine additions respectively were 66 and 198, the latter figure being the same as for the control basal medium. The inhibition by pantothenic acid and the absence of any effect due to methionine would therefore indicate that the amine synthesized by *B. subtilis* is different from kynurenine formed by *S. aureus* (Sevag and Green, 1944), or the carboxamide accumulated by *E. coli* under conditions of sulfonamide bacteriostasis (Bergmann *et al.*, 1952).

The effect of varying the concentration of pantothenic acid was studied. Since reduced formation of arylamine may be due to reduced growth, growth was simultaneously measured (Table I).

TABLE I

Effect of varying concentrations of calcium pantothenate on arylamine formation by resistant strain

Ca Pantothenate concentration (....M $\times 10^{-2}$) in basal medium	Total arylamine (μg.)	Turbidity (dilution 1 : 2) Klett readings
None	250	190
(0·01)	180	182
(0·02)	180	182
(0·10)	172	183
(0·15)	150	..
(0·21)	150	185

There is a gradual decrease in arylamine formation with increasing concentrations of pantothenic acid, the effect due to a low concentration of the vitamin being relatively more pronounced. The decrease in arylamine formation is not due to a decrease in growth of the organism.

This inhibition by pantothenic acid prompted a study of certain related substances. Those tried were β-alanine, a moiety of pantothenic acid, which,

as reported by Shive and Macow (1946), could serve as a precursor of pantothenic acid in some cases, and pantoyl taurine, an analogue antimetabolite of the vitamin (Snell, 1941; McIlwain, 1942).

TABLE II

Effects of β-alanine and pantoyl taurine on arylamine formation by the resistant strain

Addition to basal medium	Arylamine (μg.)
None	250
Ca pantothenate ($5 \cdot 0 \times 10^{-4}$ M)	164
β-alanine ($1 \cdot 1 \times 10^{-2}$ M)	248
Pantoyl taurine ($8 \cdot 0 \times 10^{-4}$ M)	248
Ca pantothenate + pantoyl taurine	248

β-alanine does not exert any influence on arylamine formation in the resistant strain of *B. subtilis*. This may be due to several possibilities. The organism may not synthesise pantothenic acid from β-alanine or the synthesis may be very slow. It may also have been blocked completely in the drug-resistant strain. Again the possibility is not excluded that endogenously formed pantothenic acid has no retarding influence on arylamine formation as is with added pantothenic acid.

Pantoyl taurine was without any influence by itself but reversed the inhibitory effect of pantothenic acid. Even in presence of the antogonist the susceptible strain did not produce any arylamine.

This reversal of inhibition by pantoyl taurine indicated that the disappearance of arylamine in presence of pantothenic acid is probably due to some reaction after formation of the arylamine in which the vitamin participates. The possibility whether this reaction is acetylation was therefore investigated. Estimations of arylamine were carried out both before and after hydrolysis with 4 N hydrochloric acid, thus giving free and acetylated arylamine. Turbidity was also measured (Table III).

Hydrolysis with 4 N HCl does not result in increased recovery of the arylamine; under these conditions, acetylated sulfanilamide is completely deacetylated. Hence if the arylamine is at all acetylated, it may be further

TABLE III

Free and acetylated arylamine in resistant strain in presence of pantothenic acid

Supplement to basal medium	Arylamine		Turbidity
	Free	Total	
None	204	200	155
Ca pantothenate ($0{\cdot}1\times10^{-2}$ M)	114	110	150

converted into a derivative of importance for some physiological processes so that the regeneration of the acetylated arylamine does not occur. Alternatively, less arylamine may be formed in presence of pantothenic acid. The susceptible strain was not able to utilize the arylamine to any extent, when added to the growth medium, during 48 hours of growth, even in presence of added pantothenic acid. The arylamine thus does not appear to be a normal metabolite. It is not formed as a result of blocking of its further utilization by sulfonamides as otherwise it would be utilized by the susceptible strain. Conclusive proof that the amine is not an intermediate in the biosynthetic pathway for purines or other essential metabolites would however need a study of its utilization or otherwise by purineless or other appropriate mutants of organisms (*cf.* Davis, 1951), since there is a possibility that the amine may not be utilized in preference to simpler precursors.

Effects of other B vitamins.—Several instances have been reported of modifications of sulfonamide action by thiamine (Sevag, 1945), riboflavin (Gots and Sevag, 1949), *p*-amino benzoic acid (Woods, 1940), nicotinic acid (Wood and Austrian, 1942), folic acid (PGA) (Lampen and Jones, 1947) and vitamin B_{12} (Jukes and Stokstad, 1951; Shive, 1950, 1951). The effects of these additions were therefore studied (Table IV).

Thus thiamine and pyridoxine inhibited significantly the elaboration of the arylamine whereas vitamin B_{12} slightly enhanced it. The lack of any effect by *p*ABA and PGA is interesting. It seems that the arylamine does not arise on account of a deficiency of *p*ABA nor is *p*ABA and hence presumably folic acid a limiting factor in its utilization. This observation further emphasizes that the arylamine in question is different from the carboxamide produced by *E. coli* since *p*ABA is known to decrease carboxamide accumula-

TABLE IV

Effects of certain B vitamins on arylamine formation by resistant strain of B. subtilis

Experiment	Supplement to basal medium	Arylamine (μg.)	Percentage change
1	None	140	..
	Thiamine HCl ($0{\cdot}30\times10^{-2}$ M)	91	−35
	Riboflavin ($0{\cdot}27\times10^{-4}$ M)	134	nil
	Pyridoxine HCl ($0{\cdot}29\times10^{-2}$ M)	84	−40
2	None	162	..
	Nicotinic acid ($0{\cdot}20\times10^{-2}$ M)	162	nil
	Biotin ($2{\cdot}04\times10^{-4}$ M)	164	nil
3	None	250	..
	*p*ABA ($0{\cdot}36\times10^{-2}$ M)	250	nil
4	None	52	..
	Pteroyl glutamic acid ($0{\cdot}48\times10^{-4}$ M)	50	nil
5	None	97	..
	Vitamin B_{12} (10 μg.)	115	+18
6	None	144	..
	Choline HCl ($0{\cdot}41\times10^{-2}$ M)	138	nil

tion in presence of bacteriostatic concentrations of sulfonamides (Stetten and Fox, 1945; Bergmann *et al.*, 1952).

The increase in the amine produced in presence of vitamin B_{12} further supports the view about its differential nature from the purine precursor accumulating in sulfonamide-inhibited *E. coli* which is counteracted by vitamin B_{12} (Ishai *et al.*, 1951).

Effects of certain amino acids.—In view of the important role of amino acids in the biogenesis of purines (Shive, 1951) and also in view of the present studies showing that arylamine from *B. subtilis* differs from the carboxamide precursor of purines, the influence of several amino acids was studied. Unlike the enhancing effect on the arylamine formation by *E. coli* (Ravel *et al.*, 1948), glycine had no effect in the present case. On the contrary, serine ($0{\cdot}45\times10^{-2}$ M) produced a marked (80 per cent.) enhancement. Serine is known to increase the sulfonamide to *p*ABA ratio needed to inhibit bacteria (Winkler and de Haan, 1948). The pronounced stimulation of the amine formation by serine would suggest a study of its possible precursorial role. Arginine, known to antagonize sulfanilamide action in *Streptococci* (Gale, 1945), was without any effect. Other amino acids did not also influence

amine accumulation. It was stated earlier that even though biosynthesis of methionine is one of the basic mechanisms affected by sulfonamides (Shive and Roberts, 1946; Winkler and de Haan, 1948; Alimchandani and Sreenivasan, 1955), availability of methionine was not a limiting factor on arylamine formation in *B. subtilis*.

Glycine and Serine are normally interconvertible; this conversion is facilitated by *p*ABA and folic acid (Shive and Macow, 1946; Holland and Meinke, 1949; Woods and Lascelles, 1950, 1954). The possibility that this interconversion may be blocked in the case of the resistant strain was tested by studying the effect of glycine in presence of these vitamins. However, in no case the normally no effect of added glycine was found to be altered.

Effects of nucleic acid derivatives.—Purines antagonize the inhibitory action of sulfa drugs in several micro-organisms (Shive and Roberts, 1946). The arylamine formed by *E. coli* is now recognized to be a purine precursor. Sulfonamides seem to interfere in the biosynthesis of purines and pyrimidines and hence of nucleic acids (Shive, 1951). Their effects on the arylamine formation were therefore studied (Table V).

TABLE V

Effects of nucleic acid derivatives on arylamine formation by resistant strain

Experiment	Addition to basal medium	Arylamine (μg.)	Percentage variation
1	None	132	..
	Xanthine ($0\cdot35\times10^{-2}$ M)	28	−80
2	None	123	..
	Uric acid ($0\cdot37\times10^{-2}$ M)	157	+27
	Adenine ($0\cdot37\times10^{-2}$ M)	123	nil
3	None	164	..
	Uracil ($0\cdot44\times10^{-2}$ M)	226	+38
	Thymine ($0\cdot32\times10^{-2}$ M)	216	+31
4	None	124	..
	Yeast nucleic acid (5 mg.)	190	+53

Both the pyrimidines tried and yeast nucleic acid increased the arylamine markedly. Uric acid also had an enhancing effect. However, xanthine depressed the arylamine elaboration by 79 per cent. Simultaneous turbidity measurements in all cases showed that there was no marked effect on growth except with xanthine where there was some 28 per cent. growth increase.

To elucidate whether xanthine depressed arylamine accumulation by facilitating its further utilization, experiments were tried with the culture filtrate on the susceptible strain. However, presence of xanthine did not help the strain to utilize the arylamine, all the added arylamine being recovered at the end of 48 hours of growth. Growth itself was not influenced under the conditions of these experiments.

The formation of the arylamine with the resistant strain of *B. subtilis* and its disappearance in presence of added xanthine might imply that the arylamine accumulated on account of a deficiency of xanthine. The effect of benzimidazole ($0 \cdot 17 \times 10^{-2}$ M), a structural antagonist of xanthine (Woolley, 1944) was therefore studied.

The purine antagonist did not induce the susceptible strain of *B. subtilis* to produce any arylamine. However, with the resistant strain, it decreased growth by 20 per cent. but arylamine formation was depressed by over 73 per cent. The formation of arylamine in the culture filtrate of the resistant strain cannot therefore be explained mainly by the hypothesis that sulfonamides condition purine deficiency.

Effects of various additions on arylamine formation.—The effects of various additions on arylamine formation by the resistant strain of *B. subtilis* are summarised in Table VI. No effect was observed with any of the substances

TABLE VI

Effect of various substances on arylamine formation by resistant strain

Substance	Concentration ($\times 10^{-2}$ M)	Percentage change in arylamine
Ca pantothenate	0·21	−60
Thiamine HCl	0·30	−35
Pyridoxine HCl	0·29	−40
Vitamin B_{12}	10 μg.	+18
Serine	0·45	+80
Xanthine	0·35	−70
Benzimidazole	0·17	−73
Uric acid	0·37	+27
Uracil	0·44	+38
Thymine	0·32	+31
RNA	5 mg.	+53

+ Indicates increase; − Indicates decrease.

tested on the susceptible strain. The following compounds had no effect on arylamine formation by the resistant strain as well. Glucose ($1 \cdot 1 \times 10^{-2}$ M), Tryptophan ($1 \cdot 0 \times 10^{-2}$ M), Methionine ($1 \cdot 3 \times 10^{-2}$ M), β-alanine ($1 \cdot 1 \times 10^{-2}$ M), Riboflavin ($0 \cdot 27 \times 10^{-4}$ M), Nicotinic acid ($0 \cdot 20 \times 10^{-2}$ M), Biotin ($2 \cdot 4 \times 10^{-4}$ M), *p*ABA ($0 \cdot 36 \times 10^{-2}$ M), Folic acid ($0 \cdot 48 \times 10^{-4}$ M), Choline ($0 \cdot 41 \times 10^{-2}$ M), Glycine ($0 \cdot 15 \times 10^{-2}$ M), Arginine ($0 \cdot 12 \times 10^{-2}$ M), Hydroxyproline ($0 \cdot 15 \times 10^{-2}$ M) and Adenine ($0 \cdot 37 \times 10^{-2}$ M).

DISCUSSION

The substances formed in the culture filtrates during growth of micro-organisms in presence of bacteriostatic concentrations of sulfonamides or by micro-organisms resistant to sulfonamides may arise in several ways. They may be essential metabolites specially elaborated as a result of the unfavourable environment. They may counterbalance the inhibitory action of the drug and modify the unfavourable environment. They may also be formed as secondary products and may thus represent possible alternate reactions which may or may not be of importance in the resistant strain. Finally, they could be intermediary products accumulating as a result of metabolic blocks.

In the present studies with *B. subtilis*, glucose and tryptophan neither induced any arylamine formation in the susceptible strain nor enhanced it in the resistant strain. Pantothenate had an inhibitory effect. These observations show therefore that the arylamine produced by the resistant *B. subtilis*, is different from kynurenine of the resistant *S. aureus* (Sevag and Green, 1944).

The arylamine was produced only by the resistant strain and was independent of the presence of sulfanilamide. Serine increased the arylamine formation but glycine had no effect. It was ascertained that this organism lacked the ability to bring about glycine-serine conversion (unpublished work). Methionine, choline, *p*ABA and folic acid exerted no effect. Vitamin B_{12} had a slight stimulatory effect. These observations are in contrast with those on the amine in *E. coli* (Bergmann *et al.*, 1952) and would suggest that the arylamine produced by resistant *B. subtilis* is different from the purine precursor 4-amino-5-imidazole carboxamide produced in *E. coli*.

Serine, uric acid, uracil, thymine and yeast nucleic acid increased the arylamine formation in resistant *B. subtilis*. These additions may be metabolized to an arylamine by the resistant strain and possibly the reactions do not exist in the susceptible strain.

Among the compounds which decrease arylamine formation it is probable that xanthine and benzimidazole may compete with a precursor of the arylamine and thereby decrease its formation. The decrease observed with thiamine or pantothenic acid could arise from oxidative changes in the arylamine.

The arylamine is not utilised by the susceptible strain and hence may not be a normal metabolite. However, it may happen that a biosynthesised metabolite is more effective than when it is supplied externally.

Purines are essential metabolites and their synthesis would govern growth rates. It was however observed that growth stimulation and decrease in arylamine formation were independent of each other. Thus, Bergmann *et al.* (1952) found that bacteriostatic action manifested itself after 4 hours, but the accumulation of the arylamine started almost immediately. Methionine and *p*ABA decreased the arylamine in *E. coli* without any simultaneous effect on growth. Acetic acid slightly enhanced arylamine in *E. coli* without affecting growth. Sevag and Green (1944) also found no correlation between kynurenine formation and growth of *S. aureus*. In the present work, xanthine increased growth to some extent but decreased arylamine to the extent of 80 per cent. Benzimidazole inhibited growth by 20 per cent. but decreased arylamine formation to the extent of 73 per cent. The lack of a correlation between arylamine accumulation and growth therefore indicates that drug action is not explicable solely in terms of the accumulation of inhibited products of metabolism.

It is now recognized that sulfonamide actions cannot be explained solely on the basis of *p*ABA antagonism (*cf.* Sevag, 1946). Sulfonamides have been shown to interfere in the biosynthesis of methionine, xanthine, serine, valine, thymine, glycine, etc. (Alimchandani and Sreenivasan, 1955) and in certain transformations like glycine into serine, *p*ABA into folic acid, etc. (Lampen and Jones, 1947). In supplying these metabolites preformed in the medium, the incapacity to carry out these basic biosynthetic reactions may no longer be effective and the growth inhibition may be overcome to some extent. The relative sensitivity of the synthetic reactions, towards sulfonamides is however different and depending on the exact quantity of the drug present one or the other product may assume relatively more importance.

There is also the circumstance that certain substances may create an overall favourable environment and may therefore antagonize the drug. Non-specific antagonists like peptone, yeast extract, albumin, urethane, nicotinic acid, etc., are in this category. Some of these antagonists can react with and directly neutralize the toxic effect of the drug. Others may combine with

and protect the enzyme proteins making them less easily available for sulfonamides (*cf.* Work and Work, 1948).

There are reports of changes in metabolic patterns associated with acquisition of drug-resistance. Thus, a strain originally capable of growing without methionine, required methionine as an essential growth factor during training in synthetic media containing methionine and increasing amounts of sulfanilamide; a resistant strain trained in the absence of methionine did not require it as a growth factor (Kohn and Harris, 1942). Lampen and Jones (1946) stated that strains which are able to synthesise folic acid are sensitive to sulfonamides under conditions where they are forced to synthesise the essential metabolite but insensitive when it is supplied in the medium. *S. aureus* trained until resistant to a sulfonamide in the presence of glucose is not resistant to the drug when glucose is replaced by pyruvate (Sevag and Green, 1944) indicating that the resistant organism develops an alternate metabolic process which bypasses pyruvate. The same workers also found that repeated subculture of resistant organism in a medium free of glucose resulted in a strain resistant to sulfonamides even in the absence of glucose. The results indicate that the nature of the medium employed has an important influence on the biochemical characteristics developed during formation of a resistant strain. Such a strain, as has been studied here, may be expected to have a different metabolic pattern from the parent organism and in this process develops an arylamine the formation of which in turn is affected differently by various metabolites.

The possibility that the resistant strain on being transferred to media containing, in addition to the basal medium, one or the other of the different additions studied and shown to have an enhancing or depressing effect on arylamine formation might have mutated to different forms with altered metabolism in each case and therefore with the formation of more than one arylamine was considered unlikely for the following reasons. In the first place, such wide alterations seem improbable in the course of a single transfer. Secondly, the growth rates of the resistant strain in presence of sulfanilamide before and after such a transfer, during 24 hours, to a medium containing added metabolites like glycine, pantothenate, etc., were more or less identical. Such would not be the case if during a transfer into such media containing added metabolites the organism had changed type.

The observations presented here are inadequate in elucidating the biosynthetic pathway for the arylamine formed, or its relationship to essential metabolites. Nevertheless, they serve to emphasise the many different types of metabolic interference in drug action.

SUMMARY

A diazotizable arylamine is formed in culture filtrates of a growing sulfanilamide-resistant strain of *B. subtilis*.

The arylamine accumulates under normal cultural conditions in peptone or in synthetic media. The presence of sulfanilamide is not necessary for its formation. Actual multiplication of cells however appears essential for the formation of the arylamine. There is no correlation between arylamine formation and growth of the organism.

Vitamin B_{12}, serine, uric acid, uracil, thymine or yeast nucleic acid increases the arylamine synthesis to an appreciable extent.

Pantothenic acid, thiamine, pyridoxine, xanthine or benzimidazole considerably decreases the arylamine formation.

Other additions like glucose, tryptophan, glucose + tryptophan, methionine, β-alanine, pantoyl taurine, riboflavin, nicotinic acid, biotin, *p*-aminobenzoic acid, folic acid, choline, glycine, glycine in presence of *p*-aminobenzoic acid or folic acid, arginine, hydroxyproline or adenine do not exert any appreciable influence on arylamine formation. Pantoyl taurine annulled the inhibitory influence of pantothenic acid.

The effects of glycine, serine, pantothenic acid and xanthine on the arylamine formation have been studied in some detail.

The parent susceptible strain of *B. subtilis* does not cause any detectable arylamine accumulation under normal cultural conditions or in presence of various metabolite additions. Also none of the substances, tested, could induce the susceptible strain to produce any arylamine.

The arylamine is not utilised by the susceptible strain under normal cultural conditions or in presence of pantothenic acid or xanthine.

The observations clearly distinguish the arylamine formed by the resistant *B. subtilis* from the two known arylamines, kynurenine and 4-amino-5-imidazole-carboxamide accumulating respectively in resistant strains of *S. aureus* and of *E. coli* under sulfonamide bacteriostasis.

ACKNOWLEDGEMENT

Our grateful thanks are due to the Raptakos Medical Research Board for a research grant.

REFERENCES

Alimchandani, H. R. and Sreenivasan, A. — *Nature*, 1955, **176**, 702; *J. Bact.*, 1957, **73**, 538.

Bergmann, E. D., Volcani, B. E. and Ishai, R. B. — *J. Biol. Chem.*, 1952, **194**, 521, 531.

Bliss, E. A. and Long, P. H. .. *Johns Hopk. Hosp. Bull.*, 1941, **69**, 14.

Bratton, A. C. and Marshall, E. K. Jr. — *J. Biol. Chem.*, 1939, **138**, 537.

Buchanan, J. M. and Schulman, M. P. — *Ibid.*, 1953, **202**, 241.

Davis, B. .. Cited in *Vitamins and Hormones*, (Academic Press), 1951, **9**, 84.

Gale, E. F. .. *Brit. J. Exp. Path.*, 1945, **26**, 225.

Gots, J. S. and Sevag, M. G. .. *J. Bact.*, 1949, **58**, 585.

Greenberg, G. R. .. *J. Biol. Chem.*, 1956, **219**, 411.

Holland, B. R. and Meinke, W. W. — *Ibid.*, 1949, **178**, 7.

Ishai, R. B., Volcani, B. E. and Bergmann, E. D. — *Arch. Biochem.*, 1951, **32**, 229.

Joshi, S. and Sreenivasan, A. — *Proc. Ind. Acad. Sci.*, 1958, **47 B**, 48.

Jukes, T. H. and Stokstad, E. L. R. — *Vitamins and Hormones*, (Academic Press), 1951, **9**, 15.

Kohn, H. I. and Harris, J. S. .. *J. Pharmacol. and Exptl. Therap.*, 1941, **73**, 343.

——— .. *J. Bact.*, 1942, **44**, 717.

Lampen, J. O. and Jones, M. J. .. *J. Biol. Chem.*, 1946, **164**, 485; **166**, 435.

——— .. *Ibid.*, 1947, **170**, 333.

Love, J. S. and Gots, J. S. .. *Ibid.*, 1955, **212**, 650.

Ludocivi, P. P., Axelrod, A. E. and Carter, B. B. — *Proc. Soc. Exp. Biol. Med.*, 1951, **76**, 665.

McIlwain, H. .. *Biochem. J.*, 1942, **36**, 417.

Muir, R. D., Shamleffer, V. J. and Jones, L. R. — *J. Bact.*, 1942, **44**, 95.

Ravel, J. M., Eakin, R. E. and Shive, W. — *J. Biol. Chem.*, 1948, **172**, 67.

Sevag, M. G. .. *J. Bact.*, 1945, **49**, 65.

——— .. *Advances in Enzymology*, (Interscience Publishers), 1946, **6**, 34.

—— and Green, M. N. .. *J. Bact.*, 1944, **48**, 615, 623, 631.

Shive, W. .. *Ann. N.Y. Acad. Sci.*, 1950, **52**, 1212.

——— .. *Vitamins and Hormones*, (Academic Press), 1951, **9**, 97.

Shive, W., Ackermann, W. W., Gordon, M., Getzendoner, M. E. and Eakin, R. E. *J. Amer. Chem. Soc.*, 1947, **69**, 725.

—— and Macow, J. .. *J. Biol. Chem.*, 1946, **162**, 451.

—— and Roberts, E. C. .. *Ibid.*, 1946, **162**, 463.

Snell, E. E. .. *Ibid.*, 1941, **141**, 121.

Stetten, M. and Fox, C. L. .. *Ibid.*, 1945, **161**, 333.

Winkler, K. C. and de Haan, P. G. *Arch. Biochem.*, 1948, **18**, 97.

Wood, W. B. and Austrian, R. .. *J. Exp. Med.*, 1942, **75**, 383.

Woods, D. D. .. *Brit. J. Exp. Path.*, 1940, **21**, 74.

—— and Lascelles, J. .. *Nature*, 1950, **166**, 649.

———— .. *J. Gen. Microbiol.*, 1954, **10**, 267.

Woolley, D. W. .. *J. Biol. Chem.*, 1944, **152**, 225.

—— and Pringle, R. B. .. *J. Amer. Chem. Soc.*, 1950, **72**, 634.

Work, T. S. and Work, E. .. *The Basis of Chemotherapy*, (Oliver and Boyd Ltd.), 1948.