REFERENCES

- Bagdy, D., Banga, I. & Horváth, M. (1959). *Kísérletes Orvostud.* (in the Press).
- Baló, J. & Banga, I. (1950). *Biochem. J.* **46**, 384.
- Baló, J. & Banga, I. (1954). *Acta physiol. hung.* **6**, 235.
- Banga, I. (1951). *Z. Vitam.-Horm.-u. Fermentforsch.* **4**, 49.
- Banga, I. (1952). *Acta physiol. hung.* **3**, 317.
- Banga, I. & Schuler, D. (1953). *Acta physiol. hung.* **4**, 13.
- Grant, N. A. & Robbins, K. C. (1957). *Arch. Biochem. Biophys.* **66**, 396.
- Hall, D. A. (1953). *Biochem. J.* **55**, xxxv.
- Hall, D. A. (1955). *Biochem. J.* **59**, 459.
- Hall, D. A. & Gardiner, J. E. (1955). *Biochem. J.* **59**, 465.
- Hall, D. A., Reed, R. & Tunbridge, R. E. (1952). *Nature, Lond.*, **170**, 264.
- Holmes, W. (1943). *Anat. Rec.* **86**, 163.
- Labella, F. S. (1957). *Nature, Lond.*, **180**, 1360.
- Lansing, A. I., Rosenthal, T. B., Alex, M. & Dempsey, E. V. (1952). *Anat. Rec.* **114**, 555.
- Lewis, U. J., Williams, D. E. & Brink, N. G. (1956). *J. biol. Chem.* **222**, 705.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Michaelis, L. (1930). *J. biol. Chem.* **87**, 33.
- Partridge, S. M. & Davis, H. F. (1955). *Biochem. J.* **61**, 21.
- Robert, L. & Samuel, P. (1957). *Experientia*, **13**, 167.
- Sachar, L. A., Winter, K. K., Sicher, N. & Frankel, S. (1955). *Proc. Soc. exp. Biol., N.Y.*, **90**, 323.
- Sols, A. (1947). *Nature, Lond.*, **160**, 89.
- Sørensen, S. P. L. (1909). *Biochem. Z.* **21**, 131.

The Degradation of Natural Polyamines and Diamines by Bacteria

BY S. RAZIN, I. GERY AND U. BACHRACH

Department of Clinical Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

(Received 18 June 1958)

The polyamines spermine and spermidine are known to be widely distributed in animal tissues (Rosenthal & Tabor, 1956), yeasts (Dudley & Rosenheim, 1925) and bacterial cells (Tabor, Rosenthal & Tabor, 1956). The biosynthesis of the natural polyamines is still obscure, although a pathway for their formation by some micro-organisms has recently been described (Tabor, Rosenthal & Tabor, 1957). The natural diamines putrescine, agmatine and cadaverine are known to be produced from amino acids by decarboxylation (Gale, 1946).

Little is known about the biological function of the natural polyamines. They have been shown to be growth factors for several micro-organisms (Herbst & Glinos, 1955; Martin, Pelczar & Hansen, 1952; Sneath, 1955; Traub, Mager & Grossowicz, 1955; Kihara & Snell, 1957). On the other hand, spermine and spermidine inhibit the growth of various bacteria (Rozansky, Bachrach & Grossowicz, 1954). The growth of *Mycobacterium tuberculosis* is inhibited by the degradation products of spermine or spermidine (Hirsch & Dubos, 1952).

Zeller (1938) studied the enzymic degradation of the natural polyamines by animal tissues; the enzymes were not purified and the oxidation products were not identified. Hirsch (1953*a, b*) found that sheep and bovine plasma contain an enzyme oxidizing spermine and spermidine. This enzyme was purified (Tabor, Tabor & Rosenthal, 1954), and its properties were studied (Werle & Roewer, 1954). However, the degradation pro-

ducts of the polyamines by this enzyme were not identified.

The oxidation of the natural polyamines by bacteria has not yet been thoroughly investigated. The only bacteria which have been found to metabolize these amines are *Pseudomonas aeruginosa* (Silverman & Evans, 1944) and *Mycobacterium smegmatis* (Roulet & Zeller, 1945). The diamines putrescine, agmatine and cadaverine are known to be oxidized by some bacteria (Gale, 1942). This study deals with the distribution of enzymes oxidizing the natural polyamines and diamines among bacteria. The mechanism of the oxidation is described.

MATERIALS AND METHODS

Organisms. The organisms used were obtained from the Diagnostic Laboratory of this department. Their identification was carried out according to Bergey (1948).

Media. The organisms were grown on brain-heart-infusion agar (Difco) or on an 'adaptation medium' composed of: yeast extract (Difco) 1 g.; K_2HPO_4 2 g.; KH_2PO_4 1 g.; $MgSO_4 \cdot 7H_2O$ 0.2 g.; amine for adaptation 3 g.; agar 15 g.; water 1 l.; final pH 7.2.

Cell suspensions and enzyme preparations. Bacteria grown on the above-mentioned media for 20 hr. at 37° were harvested, then washed twice with 0.85% NaCl soln. and kept at 4° until used.

Dried cell preparations were obtained by drying the cells over P_2O_5 at 0.3 mm. Hg (centrifugal freeze-drier, W. Edwards and Co.). Cell-free extracts were obtained by subjecting washed cell suspensions to the action of the Mickle tissue-disintegrator (Mickle, 1948) for 1 hr. with

Ballotini beads size no. 14. The intact cells and debris were removed by centrifuging at 11 000 *g* for 20 min.

Chemicals. Spermine tetrahydrochloride and spermidine phosphate were obtained from Hoffman-La-Roche and Co. Ltd. (Basle, Switzerland). Propane-1:3-diamine was the product of L. Light and Co. Ltd. (Colnbrook, Bucks). The other amines and amino acids were obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). Unless otherwise stated, the hydrochlorides of the amines were used; the pH of the solutions was adjusted to 7.0–7.5.

Buffer solutions. The phosphate buffers used were mixtures of 0.067 M- Na_2HPO_4 and 0.067 M- KH_2PO_4 . 0.05 M-2-Amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7.2, was prepared according to Gomori (1955).

Manometric methods. Oxygen uptake and CO_2 output were measured by conventional Warburg manometric techniques with air as gas phase and bath temperature 30° (Umbreit, Burris & Stauffer, 1949); the 'direct' method of Warburg was used for CO_2 determination and correction was made for bound CO_2 by tipping acid at the end of the experiment. All manometric data quoted have been corrected for the endogenous rates. In anaerobic experiments hydrogen was the gas phase.

Chromatographic methods. Paper chromatography was used for qualitative and quantitative determination (Giri, Radhakrishnan & Vaidyanathan, 1952) of the amines and their degradation products. Unless otherwise stated, butanol-acetic acid-water (50:25:25, by vol.) was the solvent (Baker, Harborne & Ollis, 1952) and papers were sprayed with 0.2% (w/v) ninhydrin in butanol. Other solvents used were: ethanol 70% (v/v) (Miller & Rockland, 1952); pyridine-acetic acid-water (50:35:15, by vol.; Decker & Riffart, 1950); 95% ethanol-amyl alcohol-water (70:50:30, by vol.).

For the identification of β -alanine and γ -aminobutyric acid an additional paper-chromatographic method was employed (Kalyankar & Snell, 1957). Ion-exchange chromatography was used for the separation of amines. The column contained Amerlite IRC 50 (Bergström & Hansson, 1951). The amines were eluted by $\text{N-H}_2\text{SO}_4$ and estimated by quantitative paper chromatography.

Paper electrophoresis. This was carried out by a modification of the method described by Bronk & Fisher (1956). Electrophoresis was carried out for 3 hr. with Whatman no. 3 paper strips (40 mm. width) and 0.05 M-phthalate buffer, pH 3.2. A current of 0.5 mA/cm. width of paper was employed. The paper strip was then air-dried and sprayed with 0.2% (w/v) ninhydrin in butanol.

Ammonia. This was estimated by distillation (Markham, 1942) or by nesslerization (Umbreit *et al.* 1949).

Aldehydes. These were determined by the method described by Friedemann & Haugen (1943).

Bioassay of β -alanine. This compound was identified and determined by the method of Billen & Lichstein (1949) with *Saccharomyces fragilis* as test organism.

RESULTS

Various bacteria were tested for their ability to oxidize the polyamines spermine and spermidine and the diamines putrescine, cadaverine and agmatine. *P. aeruginosa* oxidized all the amines tested, whereas *Serratia marcescens* oxidized spermidine, putrescine and agmatine; spermine was metabolized by a non-oxidative process. *Corynebacterium pseudodiphtheriticum* oxidized putrescine only (Table 1). Putrescine and spermidine were not oxidized by the following bacteria: *Escherichia coli* O₁₁₁, *E. coli* no. 23, *Klebsiella* sp., *Proteus vulgaris*, *Shigella flexneri*, *Shigella ambigua*, *Shigella shigae*, *Shigella sonnei*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella schottmuelleri*, *Salmonella typhimurium*, *Staphylococcus aureus* no. 23, *Micrococcus lysodeikticus*, *Streptococcus lactis*, *Streptococcus faecalis* and *Mycobacterium phlei*.

Oxidation of polyamines and diamines by *Pseudomonas aeruginosa*

Factors affecting the rate of oxidation. Cells of *P. aeruginosa* grown on brain-heart-infusion agar oxidized spermine and spermidine slowly during the first hour of the experiment. The addition of these amines to the culture medium enhanced the rate of oxidation. The effect was more pronounced when a semi-synthetic adaptation medium was used (Fig. 1). Cells adapted to spermine were simultaneously adapted to spermidine, and vice versa. The optimum pH for the oxidation of spermine and spermidine was 7.0–7.2.

Oxidation products and intermediates. Spermine-adapted cells consumed about 8 μ moles of O_2 and set free 2.5 μ moles of NH_3 and 6 μ moles of CO_2 for each μ mole of spermine oxidized. When spermidine served as substrate, 9.5 μ moles of O_2 were consumed,

Table 1. Oxidation of polyamines and diamines by various bacteria

Cells were grown on brain-heart-infusion agar. Each Warburg vessel contained 10 μ moles of substrate, 1 ml. of cell suspension (approx. 5 mg. dry wt.), 1 ml. of 0.067 M-phosphate buffer, pH 7.0. In the centre well was 0.2 ml. of 15% (w/v) KOH; total vol. 3.2 ml. Incubation was carried out in air at 30° for 5 hr.

Organism	Uptake of O_2 (μ l.) during the oxidation of					
	Spermine	Spermidine	Putrescine	Agmatine	Cadaverine	No substrate
<i>Pseudomonas aeruginosa</i>	1880	2140	570	533	870	79
<i>Serratia marcescens</i>	0*	923	612	580	0	51
<i>Corynebacterium pseudodiphtheriticum</i>	0	0	452	0	0	43

* Metabolized by a non-oxidative process.

3 μ moles of NH_3 and 6 μ moles of CO_2 were set free for each μ mole of substrate. It had been shown by paper chromatography that all the spermine or spermidine disappeared during these experiments. It is remarkable that the total consumption of O_2 and production of NH_3 were higher with spermidine than with spermine (Fig. 1).

On oxidation of spermine or spermidine by adapted cells, an intermediate giving a positive ninhydrin reaction was detected by paper chromatography. Fig. 2 shows that the concentration of this intermediate was maximal at the end of the first hour of the experiment; on further incubation its concentration decreased and finally the intermediate disappeared. This compound was shown to be β -alanine by paper chromatography, paper electrophoresis and by microbiological assay.

Quantitative estimation of β -alanine by bioassay and paper chromatography showed a maximal formation of about 2 μ moles of β -alanine/ μ mole of spermine and about 1 μ mole of β -alanine/ μ mole of spermidine consumed. These results made us assume that β -alanine was formed from the $[\text{CH}_2]_3$ fragments of the polyamine molecule. This was supported by the fact that propane-1:3-diamine was also oxidized to β -alanine by *P. aeruginosa*. 1 μ mole of β -alanine was formed/ μ mole of propane-1:3-diamine consumed.

When spermine and spermidine were oxidized by unadapted cells several ninhydrin-positive spots appeared on the paper chromatogram. As seen

from Fig. 3, after incubation of cells with spermidine for 45 min., spots corresponding to propane-1:3-diamine, β -alanine and γ -aminobutyric acid appeared. After incubation for 240 min. spermidine completely disappeared, whereas the intermediates were still present. The upper spot of propane-1:3-diamine apparently does not represent an

Fig. 2. Formation of β -alanine from spermine and spermidine by *Pseudomonas aeruginosa*. Cells were grown on adaptation medium containing spermine. Experimental conditions were as given in Table 1. Substrate (2.5 μ moles) was spermine or spermidine. Samples were taken at the times indicated and centrifuged, and the supernatants analysed by paper chromatography. ■, Spermine; ▲, spermidine; ●, β -alanine formed from spermine; ○, β -alanine formed from spermidine.

Fig. 1. Oxidation of spermine and spermidine by *Pseudomonas aeruginosa*. Experimental conditions were as given in Table 1; ●, 2.5 μ moles of spermine; ○, 2.5 μ moles of spermidine. ---, Cells grown on adaptation medium without spermine; —, cells grown on adaptation medium containing spermine.

Fig. 3. Oxidation of spermidine by unadapted cells of *Pseudomonas aeruginosa*. Cells were grown on brain-heart-infusion medium. Experimental procedure was as given in Fig. 2. Spermidine (2.5 μ moles) served as substrate. The figure represents a photograph of a chromatogram.

impurity as it always appeared when synthetic or biological products were tested. This upper spot coincides in its position with that of β -alanine, but it might easily be distinguished by its different colour with ninhydrin. The intermediates were identified by paper chromatography, with different solvents, and by paper electrophoresis. When spermine was used as the substrate an additional spot of spermidine appeared at the beginning of the reaction.

The occurrence of β -alanine, γ -aminobutyric acid and propane-1:3-diamine as intermediates was also proved by simultaneous-adaptation technique (Stanier, 1947). Cells grown on brain-heart-infusion medium hardly oxidized propane-1:3-diamine or β -alanine, and γ -aminobutyric acid was oxidized initially at a slow rate. Cells adapted to spermine oxidized propane-1:3-diamine or β -alanine without a lag period and oxidation of γ -aminobutyric acid was enhanced (Fig. 4).

Aldehydes are formed during the oxidation of amines by mammalian amine oxidase (Zeller, 1951). In our experiments aldehydes could be detected during the oxidation of spermine or spermidine, only by the use of 'aged' or freeze-dried cells.

Fig. 4. Oxidation of the degradation products of spermine by *Pseudomonas aeruginosa*. Experimental conditions were as given in Table 1; ■, 5 μ moles of γ -aminobutyric acid; ●, 5 μ moles of propane-1:3-diamine; ▲, 5 μ moles of β -alanine. —, Cells grown on adaptation medium containing spermine; ---, cells grown on brain-heart-infusion medium.

Oxidation of polyamines and diamines by *Serratia marcescens*

Factors affecting the rate of oxidation. Cells grown on brain-heart-infusion medium oxidized putrescine and agmatine after a lag period of about 2 hr. The oxidation of spermidine occurred without such a long lag. The lag period could be eliminated by growing the cells on adaptation medium containing the corresponding amine. Cells adapted to spermidine were not adapted simultaneously to putrescine, and vice versa (Fig. 5).

Oxidation products and intermediates. During the oxidation of putrescine, agmatine or spermidine, O_2 was taken up and CO_2 and NH_3 were evolved. No ninhydrin-positive compounds could be detected during the oxidation of putrescine or agmatine. When spermidine served as substrate several ninhydrin-positive compounds were formed (Fig. 6). The identity of the lower spots (R_f 0.20, 0.55) with propane-1:3-diamine was proved by paper chromatography and paper electrophoresis. This oxidation product was also separated by ion-exchange chromatography and its chloroplatinate was prepared. This derivative blackened at 230° and sintered at 240° like the chloroplatinate of authentic propane-1:3-diamine (Wrede, 1926). As expected, this degradation product was oxidized to β -alanine by cells of *P. aeruginosa*.

The upper spots (R_f 0.60, 0.69) seen in the chromatogram (Fig. 6) apparently represent one compound (as with propane-1:3-diamine) since the

Fig. 5. Oxidation of spermidine and putrescine by *Serratia marcescens*. Experimental conditions were as given in Table 1; ●, 5 μ moles of spermidine; ○, 5 μ moles of putrescine. —, Cells grown on brain-heart-infusion medium; ---, cells grown on adaptation medium containing putrescine.

spots always appeared and disappeared simultaneously. On prolonged incubation the compound was metabolized. This intermediate could not be obtained in sufficient quantity to be identified. During the oxidation of spermidine in the presence of 3 mM-semicarbazide a new ninhydrin-positive compound was formed instead of the unidentified intermediate. This new compound was identified chromatographically and electrophoretically as γ -aminobutyric acid. γ -Aminobutyric acid was obtained also by the oxidation of the unidentified intermediate in the presence of semicarbazide in the following manner: spermidine was incubated with cells of *S. marcescens* and the reaction was interrupted when spermidine completely disappeared; at that time the intermediate reached its maximum concentration (Fig. 6). Cells were then removed by centrifuging and discarded. Semicarbazide in a final concentration of 6 mM and fresh cells were then added. During further incubation (6 hr.) the intermediate compound disappeared and γ -aminobutyric acid was formed instead, as shown by paper chromatography. Oxygen was taken up during this reaction. γ -Aminobutyric acid also accumulated during the oxidation of putrescine and agmatine in the presence of semicarbazide. γ -Aminobutyric acid was oxidized very slowly by *S. marcescens*. The oxidation was enhanced by the addition of glucose, glutamic acid, α -oxoglutaric acid, succinic acid or pyruvic acid.

Spermine was slowly metabolized by cells of *S. marcescens*. This process was non-oxidative as no O_2 uptake was observed (cf. Table 1). Several

ninhydrin-positive compounds were formed. One of them seemed to be propane-1:3-diamine and the other was similar to the unidentified intermediate (R_f 0.60, 0.69) obtained during the oxidation of spermidine. The same compounds were also obtained in very small amounts when cells of *S. marcescens* were incubated under anaerobic conditions with both spermine and spermidine.

Oxidation of putrescine by Corynebacterium pseudodiphtheriticum

C. pseudodiphtheriticum oxidized putrescine only (Table 1). This diamine was oxidized without a lag period by cells grown on brain-heart-infusion medium. Carbon dioxide and NH_3 were evolved and no ninhydrin-positive compounds appeared during the reaction.

Effect of inhibitors on the oxidation of the polyamines and diamines

Carbonyl reagents are known to inhibit diamine oxidase (Zeller, 1951). The effect of these reagents on the oxidation of the amines by *P. aeruginosa* and *S. marcescens* was therefore tested. Table 2 shows that hydroxylamine and semicarbazide inhibited the oxidation of the amines by both organisms, the latter being less active. *iso*Nicotinic acid hydrazide and dihydrostreptomycin were also tested for inhibitory activity; the first was found to be moderately active, whereas the second hardly affected the oxidation of the amines (Table 2). Amines which were not oxidized by the bacteria exerted an inhibitory effect on the degradation of the oxidizable amines (Table 3). In order to establish the nature of this inhibition, the influence of cadaverine on the oxidation of various amounts of putrescine by *S. marcescens* was tested. Results, given according to Lineweaver & Burk (1934), show the competitive nature of this inhibition (Fig. 7). The oxidation of putrescine by a cell-free extract of *C. pseudodiphtheriticum* was also inhibited by spermine.

DISCUSSION

A proposed pathway for the degradation of spermine and spermidine by *P. aeruginosa* is given in the scheme shown in Fig. 8.

The first step in the degradation of spermine is oxidative, as it was not metabolized under anaerobic conditions. The oxidation is carried out by an amine oxidase apparently responsible also for the oxidation of spermidine and propane-1:3-diamine. According to Zeller (1951) the first step in amine oxidation is the formation of an imine which spontaneously hydrolyses to the corresponding aldehyde. Such an imine is likely to be formed also during the oxidation of spermine by

Fig. 6. Oxidation of spermidine by *Serratia marcescens*. Cells were grown on brain-heart-infusion medium. Experimental procedure was as given in Fig. 2. Spermidine (10μ moles) served as substrate. The figure represents a photograph of a chromatogram.

Table 2. *Effect of inhibitors on the oxidation of amines by Pseudomonas aeruginosa and Serratia marcescens*

Experimental conditions were as given in Table 1, but the cells were preincubated with the inhibitor for 30 min. *P.*, *P. aeruginosa*; *S.*, *S. marcescens*.

Inhibitor	Concn. (mm)	Organism	Substrate (μmoles)	Inhibition of uptake of O ₂ (%)
Hydroxylamine	0.5	<i>P.</i>	Spermine 2.5	100
	0.5	<i>S.</i>	Spermidine 10.0	73
	0.8	<i>S.</i>	Agmatine 10.0	96
Semicarbazide	60	<i>P.</i>	Spermine 2.5	82
	1	<i>P.</i>	Spermine 2.5	37
	4	<i>S.</i>	Spermidine 10.0	87
	4	<i>S.</i>	Putrescine 10.0	87
isoNicotinic acid hydrazide	10	<i>P.</i>	Spermine 2.5	68
	2	<i>P.</i>	Spermine 2.5	42
	2	<i>S.</i>	Spermidine 10.0	19
	2	<i>S.</i>	Agmatine 10.0	84
Dihydrostreptomycin	2	<i>P.</i>	Spermine 2.5	20
	0.2	<i>P.</i>	Spermine 2.5	15
	0.4	<i>S.</i>	Agmatine 10.0	0

Table 3. *Inhibition of amine oxidation by non-oxidizable amines*

Experimental conditions were as given in Table 2. *S.*, *S. marcescens*; *C.*, *C. pseudodiphtheriticum*.

Inhibitor (4 mm)	Organism	Substrate (3.3 mm)	Inhibition of uptake of O ₂ (%)
Spermine	<i>S.</i>	Spermidine	64
Spermine	<i>S.</i>	Agmatine	73
Spermine	<i>S.</i>	Putrescine	71
Spermine	<i>C.</i>	Putrescine	68
Spermine	<i>C.*</i>	Putrescine	68
Spermidine	<i>C.</i>	Putrescine	53
Cadaverine	<i>S.</i>	Agmatine	88
Cadaverine	<i>C.</i>	Putrescine	32
Arcaine	<i>S.</i>	Agmatine	47
Arcaine	<i>C.</i>	Putrescine	78

* Cell-free extract.

P. aeruginosa (Fig. 8, step 1). The imine is then converted into spermidine and into an aldehyde (step 2). This aldehyde and those formed during the oxidation of spermidine (step 5) or propane-1:3-diamine (step 8) are rapidly further oxidized by the fresh cells. Aldehydes could be demonstrated with 'aged' or freeze-dried cells. Similar results were obtained by Silverman & Evans (1944). The aldehyde formed in step 4 (4a) is oxidized to β-alanine, which is slowly metabolized. The oxidation of spermidine proceeds through propane-1:3-diamine (step 5), which is oxidized to β-alanine (steps 6, 8, 4a). Another oxidation product of spermidine is γ-aminobutyric acid, which is further metabolized.

It follows from the suggested scheme that β-alanine is formed from the [CH₂]₃ fragment of the

Fig. 7. *Effect of cadaverine on the oxidation of putrescine by Serratia marcescens*. Cells were grown on adaptation medium containing putrescine. Experimental conditions were as given in Table 1, but 1.0, 2.5, 5.0 or 10.0 μmoles of putrescine were used as substrate. Cadaverine (10.0 μmoles) served as inhibitor. [S], Substrate concentrations (in μmoles/3 ml.); V, reaction velocity (μl. of O₂ taken up in 30 min.). ●, Putrescine without inhibitor; ○, putrescine and cadaverine.

polyamine molecule, whereas the [CH₂]₄ fragment contributes to the formation of γ-aminobutyric acid. The possible importance of this new pathway for the formation of β-alanine has already been discussed (Razin, Bachrach & Gery, 1958). Further

investigations into the degradation of β -alanine and γ -aminobutyric acid by *P. aeruginosa* are in progress.

With spermidine as substrate, the rate of oxidation and the total uptake of oxygen and evolution of ammonia and carbon dioxide were higher than with spermine. No explanation could be offered for these unexpected observations. Spermine and spermidine obtained from different commercial sources behaved similarly. The same results were obtained also when 0.05M-tris buffer, pH 7.2, was used instead of phosphate buffer in the reaction mixture.

S. marcescens oxidized putrescine, agmatine and spermidine, i.e. compounds which contain a $[\text{CH}_2]_4$ fragment in their molecule. Cadaverine, $\text{H}_2\text{N}\cdot[\text{CH}_2]_5\cdot\text{NH}_2$, was not oxidized. As shown by adaptation experiments, the enzymes responsible for the oxidation of the diamines putrescine and agmatine apparently differ from those oxidizing spermidine.

During the oxidation of spermidine by *S. marcescens* propane-1:3-diamine was formed. Unlike *P. aeruginosa*, *S. marcescens* was unable to oxidize propane-1:3-diamine, and this diamine accumulated as an end product. γ -Aminobutyric acid seems also to be an intermediate in the oxidation of spermidine by *S. marcescens*, as it is with *P. aeruginosa*, although its presence could be demonstrated after addition of semicarbazide only. The ninhydrin-positive intermediate (R_f 0.60, 0.69)

probably originates from the $[\text{CH}_2]_4$ fragment of spermidine molecule, as it was converted into γ -aminobutyric acid in the presence of semicarbazide. The oxidation of γ -aminobutyric acid by cells of *S. marcescens* was enhanced in the presence of other oxidizable compounds. Similar results have been reported by Higashi, Horio & Okunuki (1957). The oxidation of putrescine by *S. marcescens* apparently proceeds via γ -aminobutyric acid. A similar mechanism has already been described for the oxidation of putrescine by mammalian tissues (Zeller, 1942).

Inhibition of the oxidation of poly- and di-amines by carbonyl reagents is in agreement with the known effect of these reagents on diamine oxidase activity (Zeller, 1951). According to Davison (1956) isonicotinic acid hydrazide inhibited diamine oxidase activity. In our experiments this compound exerted a weak effect only. Dihydrostreptomycin, which strongly inhibits diamine oxidase activity of *Mycobacterium smegmatis* (Owen, Karlson & Zeller, 1951), had no significant effect on the oxidation of the polyamines by *P. aeruginosa* and *S. marcescens*.

Another kind of inhibition is shown by non-oxidizable amines. For example, cadaverine, $\text{H}_2\text{N}\cdot[\text{CH}_2]_5\cdot\text{NH}_2$, inhibited the oxidation of putrescine, $\text{H}_2\text{N}\cdot[\text{CH}_2]_4\cdot\text{NH}_2$, and spermine inhibited the oxidation of spermidine and putrescine by *S. marcescens*. Similar observations have been reported for *M. smegmatis* (Roulet & Zeller, 1945).

Fig. 8. A proposed pathway for the degradation of spermine and spermidine by *Pseudomonas aeruginosa*.

Spermidine inhibited the oxidation of putrescine by cell-free extract from *C. pseudodiphtheriticum*, whence it is suggested that this polyamine directly interfered with the activity of the amine oxidase.

SUMMARY

1. The oxidation of natural polyamines and diamines by bacteria was studied. Out of twenty species tested only *Pseudomonas aeruginosa*, *Serratia marcescens* and *Corynebacterium pseudodiphtheriticum* were active. *P. aeruginosa* oxidized spermine, spermidine, putrescine, agmatine and cadaverine, *S. marcescens* oxidized spermidine, putrescine and agmatine, whereas *C. pseudodiphtheriticum* oxidized putrescine only.

2. Spermine was degraded by *P. aeruginosa* to spermidine, which was further oxidized to propane-1:3-diamine, β -alanine and γ -aminobutyric acid.

3. Propane-1:3-diamine accumulated during the oxidation of spermidine by *S. marcescens*.

4. Carbonyl reagents inhibited the degradation of the poly- and di-amines. *iso*Nicotinic acid hydrazide and dihydrostreptomycin were less active. Amines which were not oxidized by the bacteria exerted an inhibitory effect on the degradation of the oxidizable amines.

The authors would like to thank Professor J. Gurevitch for his interest and encouragement, and Dr R. Rozansky for helpful advice.

This investigation was supported by a grant from the Hadassah Medical Organization Research Fund.

REFERENCES

- Baker, W., Harborne, J. B. & Ollis, W. D. (1952). *J. chem. Soc.* p. 3215.
- Bergey, D. H. (1948). *Manual of Determinative Bacteriology*, 6th ed. Baltimore: Williams and Wilkins Co.
- Bergström, S. & Hansson, G. (1951). *Acta physiol. scand.* **22**, 87.
- Billen, D. & Lichstein, H. C. (1949). *J. Bact.* **57**, 267.
- Bronk, J. R. & Fisher, R. B. (1956). *Biochem. J.* **64**, 106.
- Davison, A. N. (1956). *Biochem. J.* **64**, 546.
- Decker, P. & Riffart, W. (1950). *Chemikerztg.* **74**, 261. Cited in *Chem. Abstr.* (1951) **45**, 6528.
- Dudley, H. W. & Rosenheim, O. (1925). *Biochem. J.* **19**, 1034.
- Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
- Gale, E. F. (1942). *Biochem. J.* **36**, 64.
- Gale, E. F. (1946). *Advanc. Enzymol.* **6**, 1.
- Giri, K. V., Radhakrishnan, A. N. & Vaidyanathan, C. S. (1952). *Nature, Lond.*, **170**, 1025.
- Gomori, G. (1955). In *Methods in Enzymology*, vol. 1, p. 144. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Herbst, E. J. & Glinos, E. B. (1955). *J. biol. Chem.* **214**, 175.
- Higashi, T., Horio, T. & Okunuki, K. (1957). *J. Biochem., Tokyo*, **44**, 735.
- Hirsch, J. G. (1953a). *J. exp. Med.* **97**, 327.
- Hirsch, J. G. (1953b). *J. exp. Med.* **97**, 345.
- Hirsch, J. G. & Dubos, R. J. (1952). *J. exp. Med.* **95**, 191.
- Kalyankar, G. D. & Snell, E. E. (1957). *Nature, Lond.*, **180**, 1069.
- Kihara, H. & Snell, E. E. (1957). *Proc. nat. Acad. Sci., Wash.*, **43**, 867.
- Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Martin, W. H. jun., Pelczar, M. J. jun. & Hansen, P. A. (1952). *Science*, **116**, 483.
- Mickle, H. (1948). *J. R. micr. Soc.* **68**, 10.
- Miller, J. M. & Rockland, L. B. (1952). *Arch. Biochem. Biophys.* **40**, 416.
- Owen, C. A. jun., Karlson, A. G. & Zeller, E. A. (1951). *J. Bact.* **62**, 53.
- Razin, S., Bachrach, U. & Gery, I. (1958). *Nature, Lond.*, **181**, 700.
- Rosenthal, S. M. & Tabor, C. W. (1956). *J. Pharmacol.* **116**, 131.
- Roulet, F. & Zeller, E. A. (1945). *Helv. chim. acta*, **28**, 1326.
- Rozansky, R., Bachrach, U. & Grossowicz, N. (1954). *J. gen. Microbiol.* **10**, 11.
- Silverman, M. & Evans, E. R. jun. (1944). *J. biol. Chem.* **154**, 521.
- Sneath, P. H. A. (1955). *Nature, Lond.*, **175**, 818.
- Stanier, R. Y. (1947). *J. Bact.* **54**, 339.
- Tabor, C. W., Tabor, H. & Rosenthal, S. M. (1954). *J. biol. Chem.* **208**, 645.
- Tabor, H., Rosenthal, S. M. & Tabor, C. W. (1956). *Fed. Proc.* **15**, 367.
- Tabor, H., Rosenthal, S. M. & Tabor, C. W. (1957). *J. Amer. chem. Soc.* **79**, 2978.
- Traub, A., Mager, J. & Grossowicz, N. (1955). *J. Bact.* **70**, 60.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*. Minneapolis: Burgess Publishing Co.
- Werle, E. & Roewer, F. (1954). *Biochem. Z.* **325**, 550.
- Wrede, F. (1926). *Hoppe-Seyl. Z.* **153**, 291.
- Zeller, E. A. (1938). *Helv. chim. acta*, **21**, 1645.
- Zeller, E. A. (1942). *Advanc. Enzymol.* **2**, 93.
- Zeller, E. A. (1951). In *The Enzymes, Chemistry and Mechanism of Action*, vol. 2, part 1, p. 536. Ed. by Sumner, J. B. & Myrback, K. New York: Academic Press Inc.