102. THE PRODUCTION OF AMINES BY BACTERIA

2. THE PRODUCTION OF TYRAMINE BY *STREPTOCOCCUS FAECALIS*

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IN the first paper of this series, the author [Gale, 1940] showed that washed suspensions of *Bact. coli* will decarboxylate *l*(+)-arginine, *l*(+)-lysine, *l*(+)-ornithine, *l*(−)-histidine and *l*(+)-glutamic acid to form agmatine, cadaverine, putrescine, histamine and γ-aminobutyric acid respectively. Of 14 strains of coliform organisms investigated, 12 decarboxylated arginine, 12 histidine, 13 lysine, 12 ornithine and 9 glutamic acid. The decarboxylases involved proved to be very thermolabile and were formed more easily in organisms grown at 27 than in those grown at 37°. The decarboxylase activity of the organisms was shown to depend upon the *p*H of the medium during growth: organisms grown at *p*H 7 have little decarboxylase activity but this is greatly increased (20–100-fold) by growing the organisms at *p*H 5. In most cases the activities were also increased by the presence of glucose in the growth medium but this increase in activity is due to the fall in *p*H produced during growth by the fermentation acids. The decarboxylases are active over a very restricted *p*H range, the optimum value in each case being: for arginine, 4·0; lysine, 4·5; histidine, 4·0; ornithine, 5·0 and glutamic acid, 4·0. None of the strains investigated decarboxylated any of the common amino-acids, other than the five mentioned, under the experimental conditions used.

Emerson [1902] showed the presence of tyramine in autolysing pancreas, while Rosenheim [1909] and Barger & Walpole [1909] showed it to be one of the main pressor substances in placental extracts and putrefying meat respectively. Sasaki [1914] obtained tyramine from a medium of salts, tyrosine and glycerol heavily inoculated with *Bact. coli* and incubated, while Hanke & Koessler [1924] obtained this amine by the growth of pure cultures of organisms isolated from the intestine in synthetic media containing tyrosine, salts, glycerol and lactose. As a result of their investigations they suggest that organisms producing histamine are incapable of forming tyramine and *vice versa*.

The present communication deals with the production of tyramine from tyrosine by washed suspensions of *Strep. faecalis* and describes the properties of the tyrosine decarboxylase present in certain strains of that organism.

Methods and organisms used

The methods used in this investigation for the preparation of washed bacterial suspensions and the investigation of their decarboxylase activities were essentially the same as those described previously [Gale, 1940]. Of the strains of *Strep.*

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faecalis used, no. 1 is the stock strain of the laboratory; nos. 2–5 were freshly isolated from faeces and given to us by Dr Carruthers of the Cambridge University Pathology Dept. and nos. 6, 7 were obtained from the National Institute of Type Cultures.

Preliminary work

Warburg manometers were set up as follows: in the main compartment, 1 ml. washed suspension of stock *Strep. faecalis* prepared from a 17 hr. culture in 2% glucose broth (tryptic digest of casein) grown at 27°, 1·5 ml. 0·05 *M* phthalate buffer and in the side cup 0·5 ml. *M*/30 amino-acid solution. The manometers were filled with N_2, equilibrated in a thermostat at 30° and the contents of the side cup tipped into the main compartment after equilibration. The following amino-acids were tested at the *p*H values shown—the values chosen being the optimum *p*H in those cases where the decarboxylase has already been studied in *Bact. coli* and *p*H 5·0 in other cases as a probable experimental *p*H.

	*p*H		*p*H
Glycine	5·0	*l*(−)-Aspartic acid	5·0
dl-Alanine	5·0	*l*(−)-Proline	5·0
dl-Valine	5·0	*dl*-Serine	5·0
l(−)-Leucine	5·0	*l*(−)-Cysteine	5·0
l(−)-Tyrosine	5·0	*l*(+)-Arginine	4·0
l(−)-Tryptophan	5·0	*l*(+)-Ornithine	5·0
l(+)-Glutamic acid	4·0	*l*(−)-Histidine	4·0
l(+)-Phenylalanine	5·0	*l*(+)-Lysine	4·5

The only substrate for which any gas evolution occurred ($Q_{CO_2} < 1$ regarded as not significant) was *l*(−)-tyrosine. Repetition of the experiment with 0·3 ml. 20% NaOH in the absorption cup gave no net gas output, showing that the liberated gas is CO_2. At the completion of the experiment, the contents of the cups were tested for NH_3 formation by distillation in Conway vessels followed by nesslerisation, with completely negative results. Controls with organism alone gave negligible CO_2 output or NH_3 formation at *p*H 5·0 under the experimental conditions.

Tyrosine decarboxylase of Strep. faecalis

p*H optimum*. A series of manometers was set up with the usual contents but with phthalate buffers of *p*H 3·5–6·5. The amount of organism used in each cup was 2–3 mg. dry wt. determined with a photoelectric turbidimeter previously calibrated against the organism in question. Fig. 1 shows the variation of the rate of decarboxylation of tyrosine expressed as Q_{CO_2} (= μl. CO_2 liberated/hr./mg. dry wt. of organism) with the reaction *p*H. The optimum value is *p*H 5·0 when $Q_{CO_2} = 244$ for this organism at 30°. The tyrosine decarboxylase thus falls into line with the other amino-acid decarboxylases investigated in being optimally active between *p*H 4·0 and 5·0.

Temperature conditions. The decarboxylation was next investigated at various temperatures; manometers containing the usual quantities at the optimum *p*H of 5·0 being set up in thermostats at 18, 25, 31, 37 and 44° respectively. After the addition of the substrate readings were taken every 10 min. for 2 hr. From the figures obtained the velocity of reaction (expressed as Q_{CO_2}) at various times after the addition of the substrate was calculated; Fig. 2 shows the variation of this velocity with time at various temperatures. The temperature coefficient calculated for maximum velocities attained over the range 20–30° is 2·1. Fig. 2 shows that the velocity fluctuates rapidly for the first 10–20 min. of the reaction before a fairly steady rate is achieved; for con-

venience an experimental temperature of 30° has been adopted as in previous work and Q_{CO_2} values quoted below are calculated from the steady rate attained 15–20 min. after addition of the substrate.

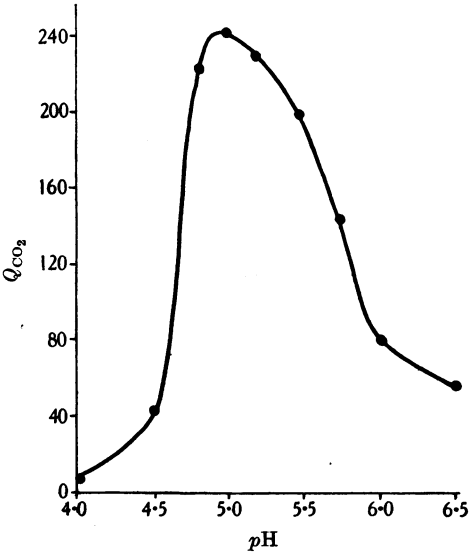

Fig. 1. Variation of tyrosine decarboxylase activity (Q_{CO_2}) with pH, *Strep. faecalis*.

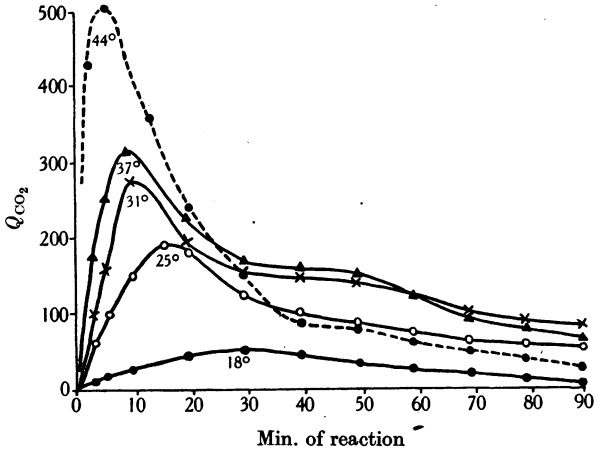

Fig. 2. Tyrosine decarboxylase activity of washed suspensions of stock *Strep. faecalis*: variation of velocity of reaction (expressed as Q_{CO_2}) with time of reaction at different temperatures.

Growth conditions

Growth temperature. With the decarboxylases of *Bact. coli*, it was found that organisms grown at 27° in glucose broth were markedly more active than organisms grown at 37°. Investigation of the effect with the tyrosine decarboxylase of stock *Strep. faecalis* shows that the activity of the washed suspension does not vary significantly whether the culture is grown at 27 or 37°. To conform

with the previous work, however, all cultures were grown, unless otherwise stated, at 27°.

Age of culture. Table 1 shows the variations of *p*H medium, dry weight of organism in culture, and tyrosine decarboxylase activity of the washed suspension, with the "age of the culture" for stock *Strep. faecalis* growing in 2% glucose broth at 27°. The experimental details are the same as those previously described [Gale, 1940]. In other cases quoted, the organism was harvested between the 17th and 18th hr. of growth when its activity is maximal.

Table 1. *Variation of tyrosine decarboxylase activity with age of culture*

Hr. of growth	*p*H of medium	Dry wt. of organism in culture mg./ml.	Tyrosine decarboxylase Q_{CO_2}
8	7·5	0·005	103
10	7·2	0·01	143
12	7·0	0·055	286
14	6·5	0·275	325
16	6·1	0·350	330
18	5·7	0·420	286
20	5·6	0·470	243
30	5·4	0·490	180

pH of growth medium. It has been shown [Gale, 1940] that the decarboxylases of *Bact. coli* are formed most readily when the organism is grown in a medium of low *p*H. Thus when the organism is grown in a medium containing glucose, washed suspensions of high activity are obtained but the activity is due to the low *p*H produced in the medium during growth by fermentation acids. In order to investigate whether similar conditions hold with the tyrosine decarboxylase of *Strep. faecalis*, the stock organism was grown in flasks of plain broth, the *p*H having been previously adjusted in various cases to 8·0, 7·0, 6·0 and 5·0. These cultures were incubated at 27° and the organism harvested at the time, in each case, when the turbidity ceased to increase. *Strep. faecalis* does not grow readily in non-carbohydrate media but sufficient organism was obtained in each case for washed suspensions to be prepared and their tyrosine decarboxylase activities to be investigated at *p*H 5·0 and 30°. The results obtained are shown in Fig. 3 where it can be seen that this enzyme is formed by the organism in response to an acid growth environment, resembling in this respect the other decarboxylases that have been studied. The activity of the organism grown in glucose broth can thus be explained by the fall in *p*H during growth due to fermentation acids.

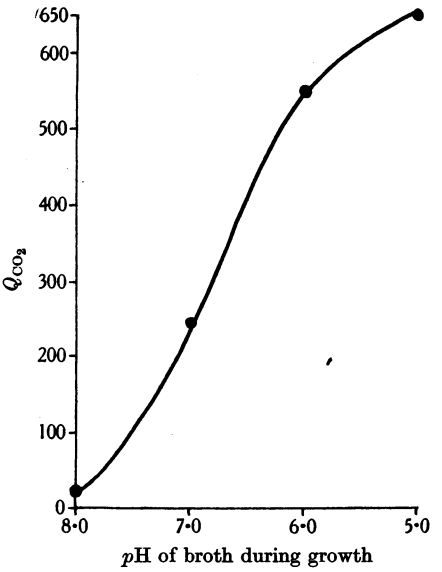

Fig. 3. Variation of tyrosine decarboxylase activity (Q_{CO_2}) of washed suspensions of stock *Strep. faecalis* with the *p*H of the plain broth in which the organism was grown.

Isolation and identification of tyramine as the dibenzoyl derivative

The amount of tyrosine used in the manometric experiments so far described was 0·5 ml. *M*/30 suspension. Decarboxylation of this to completion would liberate 373 μl. CO_2 and the total volume of gas evolved under the experimental conditions varied between 360 and 380 μl. Exact theoretical output cannot be expected as the amino-acid is used in suspension, but the results are sufficiently correct to show that the decarboxylation is quantitative. Isolation of the tyramine as the dibenzoyl compound is carried out as follows: 1 l. of 2 % glucose broth is inoculated with stock *Strep. faecalis* and incubated at 27° for 18 hr. The organism is then spun out of culture, washed on the centrifuge and made up in distilled water to a washed suspension of strength 10 mg./ml. 30 ml. of this suspension are then added to 30 ml. 0·05*M* phthalate buffer *p*H 5·0 and 30 ml. *M*/60 tyrosine suspension in a Krebs's vessel. This is filled with N_2 and then incubated at 30° until a control manometric experiment indicates that the reaction has gone to completion (1·5–2 hr.). The organism is then removed by centrifuging and passage through a Seitz filter. The filtrate is acidified to about *p*H 1 and evaporated *in vacuo* to small bulk. The *p*H is then brought to 7·5–8 by the addition of solid $NaHCO_3$ and the mixture cooled in ice to 10–12°. Benzoyl chloride is then added with vigorous shaking, a few drops at a time until about 3 molecular equivalents have been added, the *p*H being maintained in the region of 8 by addition of $NaHCO_3$. The dibenzoyltyramine slowly precipitates out and the mixture is left overnight, when the precipitate is filtered off, washed with water and dried *in vacuo*. Twice recrystallized from abs. alcohol: M.P. 171–2° (corr.). Found: C, 76·4; H, 5·33; N, 4·03 % (Weiler). Calc.: C, 76·50; H, 5·54; N, 4·06 %. Yield 132 mg.

Decarboxylation of tyrosine by various strains of Strep. faecalis

The action of washed suspensions of other strains of *Strep. faecalis* has been tested under the usual experimental conditions on the 16 amino-acids listed above. The organisms were grown in 2 % glucose broth for 18 hr. at 27°; washed suspensions were prepared and their actions tested anaerobically at *p*H 5, etc. as indicated. In no case was any amino-acid, other than tyrosine, decarboxylated with the formation of an amine and in every case the action with tyrosine was optimal at *p*H 5·0. Arginine was attacked in some cases with the liberation of

Table 2. *Formation of tyramine and ornithine by* Strep. faecalis *grown in 2 % glucose broth*

Strain	Final *p*H in medium	Q_{CO_2} Tyrosine	Q_{CO_2} Arginine
1. Stock	4·95	243*	0
2. Grant A	4·84	221*	0
3. Grant B	5·38	68*	24
4. Sargent	5·70	14	61
5. Double	6·02	—	5
6. Hucker (N.T.C. 2703)	5·29	43	83
7. Stubbs (N.T.C. 370)	5·03	44*	17

* Tyramine isolated as dibenzoyl derivative and identified.

It can be seen that, generally speaking, the strains which attack tyrosine rapidly are those that produce acid rapidly in the growth medium.

2 mol. NH_3 and 1 mol. CO_2 per mol. arginine. This reaction has been studied by Hills [1939] for various *Streptococci* and the product is stated to be ornithine. Table 2 shows the Q_{CO_2} obtained with tyrosine at the optimum *p*H 5·0, the Q_{CO_2} obtained with arginine at *p*H 6·0 for the reaction arginine → ornithine + $(NH_4)_2CO_3$ and the *p*H attained in the medium on harvesting in representative cases.

Specificity of tyrosine decarboxylase

The strict specificity of this enzyme is emphasized by the list of amino-acids not attacked by any of the organisms decarboxylating tyrosine. Thus, as none of phenylalanine, tryptophan, alanine or serine is attacked, it would appear that the enzyme is specific for the complete *p*-hydroxyphenylalanine molecule. Although all the above tests were carried out anaerobically in the first place, the decarboxylation at *p*H 5·0 is not affected in any way by the presence of oxygen.

Variation of amino-acid constitution of growth medium

Table 3 gives the tyrosine decarboxylase activity (Q_{CO_2}) of *Strep. faecalis Grant A* grown in the following media:

Medium A: Stephenson's inorganic medium; 2% glucose; 0·1% marmite.
Medium B: as A + 0·3% tyrosine.
Medium C: acid hydrolysate of gelatin; 2% glucose; 0·1% marmite.
Medium D: tryptic digest of casein; 2% glucose.
Initial *p*H in all cases = 7·3.

Table 3. *Variation of tyrosine decarboxylase activity with constitution of growth medium* (Strep. faecalis Grant A)

Medium	Final *p*H	Q_{CO_2} (Tyrosine)
A	4·85	8
B	5·55	20
C	5·23	69
D	4·85	218

SUMMARY

1. Washed suspensions of stock *Strep. faecalis* decarboxylate *l*(−)-tyrosine to form tyramine.
2. The decarboxylase involved is optimally active at *p*H 5·0.
3. Cultures grown at 27° have the same activity as those grown at 37°.
4. The activity of the washed suspension varies with the age of the culture, maximum activity occurring between the 16th and 18th hr. when growth takes place in 2% glucose broth (tryptic digest of casein) at 27°.
5. The activity of the washed suspension depends upon the *p*H of the growth medium. Cultures grown in non-carbohydrate media at *p*H 7 have little activity, this being increased 30–40-fold by growth at *p*H 5. In carbohydrate media the activity obtained is explained by the fall in *p*H during growth due to fermentation acids.
6. The decarboxylation is quantitative and the tyramine has been isolated and identified as the dibenzoyl derivative.
7. Of 7 strains of *Strep. faecalis* investigated, 6 decarboxylated tyrosine. These active strains appear to be the more rapid acid-producing strains; 5 strains also formed ornithine by liberation of $(NH_4)_2CO_3$ from arginine.

8. In no case was any other amino-acid decarboxylated to form an amine under the experimental conditions described. The tyrosine decarboxylase is thus strictly specific.

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