103. THE PRODUCTION OF AMINES BY BACTERIA

3. THE PRODUCTION OF PUTRESCINE FROM *l*(+)-ARGININE BY *BACTERIUM COLI* IN SYMBIOSIS WITH *STREPTOCOCCUS FAECALIS*

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IN the first paper of this series, the author [Gale, 1940, 1] showed that washed suspensions of *Bact. coli* acting at pH 4.0 carry out a simple decarboxylation of *l*(+)-arginine to produce agmatine. Previous work on amine production by bacteria growing in culture in the presence of arginine had given putrescine as the product [Ackermann, 1910; Hirai, 1936; Akasi, 1938; etc.]. It was suggested that this result was obtained, where mixed cultures were used as by Ackermann [1910], owing to the presence of organisms containing an "arginase" which produced ornithine from the arginine before decarboxylation occurred. Ackermann [1908] obtained ornithine from arginine in the presence of decomposing pancreas and the reaction has recently been obtained in the presence of washed suspensions of various streptococci by Hills [1939]. The reaction by *Strep. faecalis* has been confirmed by Gale [1940, 2] who obtained ornithine from arginine in the presence of 5 out of 7 strains tested. The enzyme responsible does not appear to be a true arginase as urea is not an intermediate product [Hills, 1939] and the actual course of the breakdown is obscure. An enzyme possessing the same properties has also been found in certain strains of *Lactobacillus acidophilus* by the author.

In the present communication the action of mixtures of *Bact. coli* and *Strep. faecalis* in washed suspension is discussed, the suspensions being obtained by artificial mixture of cultures grown separately and also from cultures grown in symbiosis.

Methods

The organisms were grown in a tryptic digest of casein to which glucose was added to a concentration of 2% and at a temperature of 27°. The preparation and investigation of activity of washed suspensions were essentially the same as described in the previous papers.

Organisms used

Bact. coli Esch. This organism has been shown [Gale, 1940, 1] to decarboxylate *l*(+)-arginine to agmatine at an optimum pH 4.0, *l*(+)-ornithine to putrescine at pH 5.0, *l*(+)-lysine to cadaverine at pH 4.5 and *l*(-)-histidine to histamine at pH 4.0.

Strep. faecalis Sargent. This organism produces ornithine from *l*(+)-arginine at an optimum pH 5.5 and also decarboxylates tyrosine to tyramine at a slow rate ($Q_{CO_2} = 14$ at pH 5.0) [Gale, 1940, 2].

Strep. faecalis Grant A is a rapid tyramine producer ($Q_{CO_2} = 221$) but does not attack arginine under the usual experimental conditions.

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*Effect of artificial mixture of washed suspensions of Bact. coli Esch.
and Strep. faecalis Sargent*

Fig. 1 shows the variation with pH of (1) arginine decarboxylase activity of *Bact. coli*, (2) ornithine decarboxylase activity of *Bact. coli* and (3) "arginase" activity of *Strep. faecalis*. It can be seen that at pH 4.0 the arginine decarboxylase is optimally active whereas the other two enzymes are only slightly active, while at pH 5.5, the optimal value for the "arginase" enzyme, the arginine decarboxylase is almost inactive. It is to be expected that, if the properties of

Fig. 1. Variation of activity (Q_{CO_2}) with pH of (1) arginine decarboxylase of *Bact. coli* Esch., (2) ornithine decarboxylase of *Bact. coli* Esch., (3) "arginase" of *Strep. faecalis* Sargent.

these enzymes remain unchanged by mixture of the organisms containing them, then the mixed culture should produce agmatine at pH 4.0 and putrescine at pH 5.5 from arginine, the latter reaction occurring with ornithine as intermediate product. The "arginase" of *Strep. faecalis* is inactive towards agmatine.

In the first place, washed suspensions of *Bact. coli* Esch. and of *Strep. faecalis* Sargent were prepared from cultures grown separately in 2% glucose broth and their activities determined against the various amino-acids attacked. The suspensions were then mixed and these activities again determined as shown in Table 1.

From Table 1 it can be seen that in those cases where the substrate is attacked by only one of the organisms, the mixture of the organisms has no effect upon the rate of attack by the enzymes concerned. In the case of arginine at pH 5.5, the rate of attack by the mixed cultures is, however, greater than the sum of the rates of their separate attacks. This is due, presumably, to the fact that three reactions are occurring in this case:

1. Arginine → agmatine by the arginine decarboxylase of *Bact. coli*.
2. Arginine → ornithine by the "arginase" of *Strep. faecalis*.

3. Ornithine $\rightarrow$ putrescine by the ornithine decarboxylase of *Bact. coli* reacting with the ornithine formed in reaction 2. Thus the third reaction is made possible by the mixture of the organisms.

Table 1. *Decarboxylase activities of organisms before and after mixing*

Substrate	pH	<i>Bact. coli</i> mg./ml.	<i>Strep. faec.</i> mg./ml.	μ l. CO ₂ evolved per 10 min.
Ornithine	5.0	10.25	—	153
		—	13.5	0
		10.25	13.5	152
Lysine	4.5	3.4	—	140
		—	13.5	0
		3.4	13.5	128
Histidine	4.0	10.35	—	18
		—	13.5	0
		10.35	13.5	20
Tyrosine	5.0	10.25	—	0
		—	13.5	15
		10.25	13.5	15
Arginine	4.0	3.4	—	104
		—	13.5	3
		3.4	13.5	108
Arginine	5.5	10.3	—	48
		—	13.5	51
		10.3	13.5	141

The quantity of arginine used in the above experiment was 0.25 ml. of *M/30* *l*(+)-arginine; thus if reaction (1) proceeds to completion, a total of 186 μ l. CO₂ will be liberated, whereas if both reactions (2) and (3) go to completion, then 372 μ l. CO₂ will be given off. From the rates of reaction given above, it appears that all three reactions occur simultaneously and the actual amount of gas evolved is 250–260 μ l. At pH 4.0, when neither reaction 2 or 3 occurs at an appreciable rate, the total volume of CO₂ evolved in the presence of the mixed culture = 175 μ l. The extent to which reaction 2 has taken place can be determined at the end of the experiment by estimation of the NH₃ liberated. The decarboxylases of *Bact. coli* are very thermolabile in washed suspensions and it is not to be expected that, under the experimental conditions used, all the ornithine formed by reaction 2 will be decarboxylated to putrescine. Calculations based upon the rates and quantities of CO₂ and NH₃ formation during the action of the pure and mixed cultures at pH 5.5 show that the products depend upon the ratio of the dry weights of *Bact. coli*/*Strep. faecalis* present; when this ratio = 1 the products appear to be agmatine, ornithine and putrescine in approximately equal amounts.

*Action of a washed suspension of Bact. coli Esch. and
Strep. faecalis Sargent grown symbiotically*

1 l. 2% glucose broth was inoculated with approximately equal quantities of pure cultures of *Bact. coli* Esch. and *Strep. faecalis* Sargent and incubated at 27° for 18 hr.; the organisms were centrifuged off and made into washed suspension. At the same time a control suspension of *Bact. coli* Esch. alone was prepared. The activities (vol. CO₂ liberated/10 min.) of the symbiotic and pure cultures were determined against arginine, ornithine, lysine, histidine and tyrosine, the activity for arginine being determined at pH 4.0 and 5.5 while the other activities were determined at the known optimum pH values. In the case of the pure culture of *Bact. coli*, the dry weight of organism used was determined as usual

and the various Q_{CO_2} values calculated. Since the previous work has shown that mixing of the organisms does not affect the rate of attack of the enzymes, the dry weight of *Bact. coli* present in the symbiotic mixture can now be calculated. Table 2 shows an example of such an experiment:

Table 2. *Decarboxylating activities of symbiotic culture*

Substrate	pH	Q_{CO_2} <i>Bact. coli</i>	ml. symbiot. culture used	μ l. CO_2 / 10 min.	Calc. dry wt. <i>coli</i>	Calc. dry wt. <i>coli</i> /ml. mixture
Lysine	4.5	230	0.25	109	2.83	11.32
Histidine	4.0	22.5	1.0	37	9.9	9.9
Arginine	4.0	179	0.25	82	2.74	10.96
Arginine	5.5	—	1.0	69	—	—
Ornithine	5.0	151	0.1	69	2.76	27.6
Tyrosine	5.0	0	1.0	71	—	—

From the activities with three of the substrates for which the mixing of the organisms has no effect—arginine at pH 4.0, lysine and histidine—the calculated amount of *Bact. coli* present/ml. of mixed culture = 10.7 ± 0.8 mg. However, the amount of *coli* present calculated from the ornithine activity is 27.6 mg., roughly 3 times as much. This figure is too high to agree with the turbidity measurements. It would thus appear that the symbiosis of *Strep. faecalis* Sargent with *Bact. coli* Esch. results in the ornithine decarboxylase activity of the latter organism being increased 250–300%. This effect is presumably due to the production in the growth medium of ornithine by the action of the *Strep.* on arginine. In order to check this assumption, the experiment was repeated using stock *Strep. faecalis* (which contains no “arginase”) in the place of *Sargent*; in this case, the calculations based upon the ornithine activity agreed with the others to within 7%.

To obtain an idea of the relative numbers of the two organisms in the symbiotic culture, the washed suspension was serially diluted and plated out on (1) broth agar, (2) glucose-broth agar. After 24 hr. incubation, only *coli* colonies were visible on (1) while both types of colony could be counted on (2). In 3 cases, the ratios of *Bact. coli*/*Strep. faecalis* in suitable dilution were 127/109, 122/88, 52/40 respectively.

With regard to the reaction with arginine: at pH 4.0 the manometric results showed a simple decarboxylation unaccompanied by any significant NH_3 formation; at pH 5.5 the CO_2 and NH_3 produced indicated that the arginine had been mainly broken down through ornithine to putrescine and this proved to be the case upon isolation as will be shown. This more clean-cut result obtained with the symbiotic mixture as compared with the artificial mixture is presumably due to the effect of symbiosis upon the enzymes concerned.

Isolation of the products of the action of the symbiotic culture on arginine

The organisms obtained from 1 l. of glucose broth as above were made up into 20 ml. washed suspension. Of this 10 ml. were mixed with 10 ml. 0.05 *M* phthalate buffer pH 4.0 and 10 ml. *M*/60 *l*(+)-arginine, and incubated anaerobically in a Krebs vessel. The remainder of the washed suspension was set up similarly but using buffer pH 5.5. The vessels were incubated at 30° for 2 hr. and the products then isolated as the picrates as previously described [Gale, 1940, 1]. The yields were not quantitative as in each case the product was contaminated with traces of either putrescine or agmatine but these traces could be removed by two or three recrystallizations.

The product obtained from the experiment at pH 4.0 gave orange needles, $m.p.$ 238° ; found (Weiler) N , 23.65 %: calc. for agmatine picrate, N , 23.81 %. Yield 56 mg.

The crystals obtained from the experiment at pH 5.5 appeared in the first place as yellow needles tinted red at the edges. Three recrystallizations from water gave bright yellow needles, $m.p.$ 252° (decomp.); found (Weiler) N , 20.5 %; calc. for putrescine picrate, N , 20.44 %. Yield 73 mg.

Thus the action of a mixed culture of *Bact. coli* Esch. and *Strep. faecalis* Sargent, grown in symbiosis, can be represented diagrammatically as:

SUMMARY

1. The mixture of washed suspensions of *Bact. coli* Esch. and *Strep. faecalis* Sargent has no effect upon the decarboxylation of $l(+)$ -lysine, $l(-)$ -histidine, $l(+)$ -ornithine by the former or of $l(-)$ -tyrosine by the latter. With $l(+)$ -arginine, three reactions can occur: (1) decarboxylation of arginine to agmatine, (2) breakdown of arginine to ornithine, (3) decarboxylation of ornithine to putrescine. At pH 4.0, only the first reaction occurs to any extent; at pH 5.5, all three reactions occur, the ratio of the products depending upon the ratio of *Bact. coli*/*Strep. faecalis* present.

2. When these two organisms are grown in symbiosis, the activities of *Bact. coli* towards lysine, histidine and arginine at pH 4.0 appear to be the same as those of the pure culture. Towards ornithine, the activity increases 250–300 %; probably as a result of the production of ornithine during growth.

3. The organisms grown in symbiosis attack arginine at pH 4.0 to produce agmatine and at pH 5.5 to give putrescine.

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