52. THE PRODUCTION OF AMINES BY BACTERIA

1. THE DECARBOXYLATION OF AMINO-ACIDS BY STRAINS OF *BACTERIUM COLI*

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(*Received 29 January 1940*)

ACKERMANN [1910; 1911], in work on bacterial putrefaction, showed that amines can be formed from certain amino-acids by mixed cultures of organisms. His method was to inoculate a synthetic medium, containing salts, peptone, glucose and an amino-acid, with decomposing pancreas and then after an incubation of some weeks to isolate the amine from this medium. Thus he demonstrated the formation of putrescine and δ-aminovaleric acid from arginine, of histamine, cadaverine, γ-aminobutyric acid and β-alanine from their corresponding amino-acids. This work is typical of the early investigations on bacterial amine formation [Ellinger, 1900; Abderhalden *et al.* 1913] showing the formation of such substances without giving information as to the processes, organisms or enzymes involved.

Later work has been concerned with the amines formed by pure strains of identified organisms, in particular those isolated from the intestine. Thus Berthelot & Bertrand [1911; 1912] isolated a "*Bacillus aminophilus intestinalis*" which proved capable of the formation of histamine, tryptamine and tyramine. Strains of *Bact. coli* and *Proteus vulgaris* form tyramine from tyrosine [Sasaki, 1914] and *iso*amylamine from leucine [Arai, 1921]. The formation of putrescine from arginine by *Bact. coli* has been reported by Akasi [1938] and Hirai [1936], while the formation of histamine from histidine has been the subject of many papers [Mellanby & Twort, 1912; Kendall & Gebauer, 1930; Hirai, 1933; Matsuda, 1933]. The technique in most of this work is the inoculation of a synthetic medium containing salts, the amino-acid in question and either glycerol or carbohydrate, with a pure strain of the organism. This is followed by a lengthy incubation and chemical isolation of the amine produced. This method is usually not quantitative and the long incubation may entail considerable variations in the enzymic make-up of the organisms as the constitution of the medium alters.

Koessler & Hanke [1919] studied the formation of histamine by a "colon bacillus" isolated from a case of cystitis and used an extraction method to remove histamine from the culture medium, followed by colorimetric estimation. They showed that whenever the amine is produced, the medium first becomes distinctly acid and that "histamine is never formed except in the presence of an easily available source of C such as glycerol or glucose". This conclusion was later [Hanke & Koessler, 1922; 1924] found to apply to many strains of "colon bacilli" isolated from faeces. Eggerth *et al.* [1939] improved the method of extraction of histamine from culture media and Eggerth [1939] investigated the histamine production by many strains of several intestinal species. The organisms were grown in various determined media containing inorganic salts, glucose and,

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in some cases, asparagine or peptone to assist growth. In favourable conditions, histamine production began within 24 hr. and continued for 4 or 5 days. Experiments in which the *p*H of the medium was adjusted during growth showed that histamine is produced most rapidly, in most cases, between *p*H 5·0 and 5·5 and not at all at reactions more alkaline than *p*H 6·5. The results reveal the large number of intestinal organisms capable of producing histamine, but as they deal with organisms growing in culture, do not indicate the exact enzymic conditions necessary for the reaction or the extent to which other amino-acids are attacked to form amines under similar conditions.

Work of the nature quoted has shown that (*a*) carbohydrate is necessary for amine formation during growth and that (*b*) the medium becomes acid during the incubation. Such experiments however cannot show whether the action of carbohydrate is essential as such, or is due to the acid formed from it by fermentation, or whether the action of such acid is to cause the organism to form amines as a defensive "buffer reaction" as suggested by Hanke & Koessler [1924] or if its effect is due to the properties of the enzymes involved. Virtanen & Laine [1937] and Virtanen *et al.* [1938] have shown the formation of β-alanine and γ-aminobutyric acid from aspartic and glutamic acids respectively by the legume bacteria, and have also shown the quantitative formation of cadaverine from lysine by *Bact. coli*. They have avoided the difficulty of interpreting experiments in which growth is occurring by using thick suspensions of the organism and incubating these in a non-nutrient medium containing phosphate and lysine only. The long period of incubation used (3 weeks), however, does not allow of exact conditions being determined. The cadaverine produced was estimated by isolation as the picrate.

The present series of investigations has been planned to answer, by combination of washed suspension, manometric and chemical techniques, the questions: (1) what amines are produced by certain intestinal bacteria? (2) what enzymes are involved and what are their properties? (3) what environmental conditions are required for the production of such enzymes? and (4) to what extent can such findings be generalized? This communication deals with the findings with a representative selection of *Bact. coli* strains and it is intended to follow this, if circumstances permit, with the results of similar investigations with other groups of intestinal bacteria.

Preliminary work. In the above literature, *Bact. coli* is often cited as being an amine former and it is noticeable that in every case where it has proved to be such, the strains have been grown in a medium containing either glucose or glycerol. In the work of this department on bacterial deamination [Stephenson & Gale, 1937; Gale & Stephenson, 1938] it has been consistently noted that the formation of the deaminases of *Bact. coli*, which have optimum *p*H values between 7·5 and 8·0, is almost completely inhibited when glucose is present in the growth medium. It was thought that, under such conditions, the organism might proceed to attack the amino-acids of the growth medium in another manner—namely, by decarboxylation without deamination. Accordingly, the stock strain of *Bact. coli* was grown in glucose broth and the activities of the washed suspension towards various amino-acids investigated as set out below. At first the experiments were carried out in slightly acid buffers at *p*H 6·0–6·5, with completely negative results. When the experiments were repeated at *p*H 5·5, a small evolution of gas was obtained in the presence of arginine; this gas proved to be CO_2 and no NH_3 was liberated under the conditions in which it was evolved. When the reaction with arginine was studied over the *p*H range 5·0–2·0, it immediately became obvious that previous work was probably negative as the

experiments were carried out at too alkaline a reaction. Hence the work was repeated with the results presented below.

Methods. In order to obtain as wide a range of activities with each organism as possible, the cultures were grown in a tryptic digest of casein as a basal medium providing a fairly complete mixture of amino-acids. The temperature and time of incubation are dealt with below. At the end of the incubation the organisms were centrifuged out of the growth medium, washed in distilled water and then made up into suspension in distilled water. The dry weight of organism per ml. suspension was determined in each case by a photoelectric turbidimeter [Clifton *et al.* 1935] previously calibrated against various strains of *Bact. coli*. The evolution of CO_2 from amino-acids under the action of the washed suspensions was studied in Warburg manometers. As the reactions all occur at *p*H values more acid than 5·5, the CO_2 is quickly liberated into the gas phase and the pressure increase can be followed directly. The following quantities were used: in the main compartment, 1 ml. 0·05 *M* phthalate buffer, 0·5 ml. water (1 ml. in controls), 1 ml. washed suspension of organism and, in the side bulb, 0·5 ml. *M*/30 solution of the amino-acid under investigation. Thus complete decarboxylation of the amino-acid liberated 373 μl. CO_2. The manometers were filled before use with N_2 and the amino-acid solution tipped into the main compartment of the manometer after equilibration in the thermostat. The various buffers used were made from 0·2 *M* potassium hydrogen phthalate by the addition of standard acid or alkali and the *p*H checked potentiometrically every few days. The isolation of the amines from large scale experiments is described later.

Strains of Bact. coli *used*

Bact. coli (stock). The stock strain in use in this laboratory which has been kept on tryptic broth agar for several years.

Bact. coli Esch. National Institute of Type Cultures no. 86; "original *Escherichia coli*".

Bact. coli Sheffield. Isolated from horse faeces and given to us by the University of Sheffield Bact. Dept.

Bact. coli Dunn. Freshly isolated by us from a case of cystitis.

Bact. coli 201. Strain isolated from water in 1922.

Bact. coli 210 and 211. Haemolytic urinary strains, isolated 1936.

Bact. coli 216. Faecal *coli* isolated from Cam water.

Bact. coli 217. Wilson's Type I.

Bact. coli Faecal A, B, C. Faecal *coli* (Type I) freshly isolated.

Intermediate A, B. Freshly isolated coliform intermediate, Type I.

The organisms 201, 210, 211, 216 and 217 were given to us by Dr Spooner, and the freshly isolated coliforms A, B, C, Int. A and Int. B, by Dr Carruthers of the Cambridge University Pathology Dept., to whom the author is grateful for their assistance.

Decarboxylation of amino-acids by Bact. coli (*stock*)

The preliminary experiments indicated that washed suspensions of *Bact. coli* (stock), grown in 2% glucose broth, attacked arginine when incubated with it anaerobically in the presence of phthalate buffer at *p*H 5·5, causing an evolution of CO_2 ($Q_{CO_2}=7=\mu$l. CO_2 evolved/mg. dry weight of bacteria/hr.). Manometers were therefore set up containing similar amounts of organism, etc., but in the presence of buffers ranging from *p*H 2·0 to 6·0 with intervals of 0·5 *p*H unit. The resulting activities (expressed as Q_{CO_2}) are shown in Fig. 1. The optimum *p*H for CO_2 evolution is about 4·0 ($Q_{CO_2}=83$). The experimental temperature

was 30°. Repetition of the experiment at *p*H 4·0 with 0·3 ml. 20% NaOH in the centre cup of the manometer gave no gas output, showing that the evolved gas is CO_2. In each case, at the completion of the experiment, the contents of the cups were tested for NH_3 formation by distillation in Conway vessels followed by nesslerization, with completely negative results. Controls with organism alone gave no CO_2 output or NH_3 formation at *p*H 4·0 under the experimental conditions. Thus the washed suspension decarboxylates arginine without coincident deamination under these conditions.

Having established the conditions with arginine, the experiments were repeated with other amino-acids. The action of washed suspensions at *p*H 4·0 and 5·0 was tested upon the following amino-acids:

Glycine.	*l*-(+)Aspartic acid.
dl-Alanine.	*l*-(−)Proline.
dl-Valine.	*dl*-Serine.
l-(−)Leucine.	*l*-(−)Cysteine.
l-(−)Phenylalanine.	*l*-(+)Arginine.
l-(−)Tyrosine.	*l*-(+)Ornithine.
l-(−)Tryptophan.	*l*-(−)Histidine.
l-(+)Glutamic acid.	*l*-(+)Lysine.

The amino-acid solutions were prepared from the products of Hoffmann La Roche, Ltd.

The Q_{CO_2} of the organism alone was negligible but in no case has a Q_{CO_2} of <1 been taken as significant. Evolution of CO_2 was obtained with glutamic acid, histidine, lysine and ornithine in addition to arginine: in no case was there any coincident deamination. In the positive cases, the optimum *p*H was determined in a manner similar to that used for arginine and the results are also shown in Fig. 1.

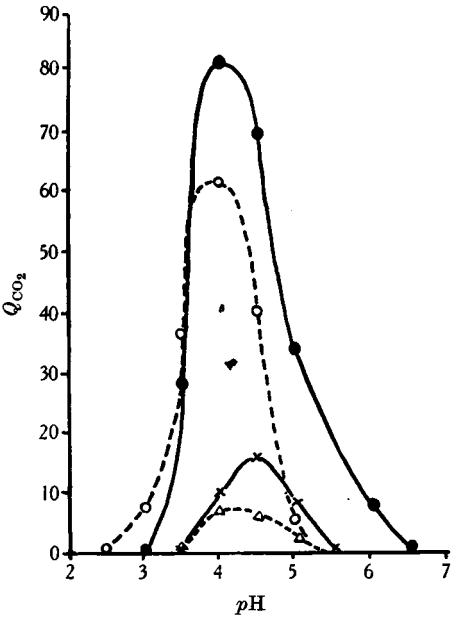

Fig. 1. Decarboxylation of amino-acids by washed suspensions of *Bact. coli* (stock): variation of activity (Q_{CO_2}) with *p*H. •—• Arginine. o - - - o Glutamic acid. ×—× Lysine. △- -△ Histidine.

Glutamic acid is decarboxylated optimally at *p*H 4·0 when $Q_{CO_2}=62$; the optimum is sharp, for the activity is almost completely inhibited at *p*H 3·0 on the acid side and at *p*H 5·0 on the alkaline. The activities towards the other three amino-acids are much lower than towards arginine and glutamic acid and do not permit of such an accurate determination of the *p*H optimum. Lysine and histidine have Q_{CO_2} of the order 8–15 between *p*H 4·0 and 4·5, while with ornithine the rate is scarcely significant, showing a Q_{CO_2} of 2 at about *p*H 4·5. In order to obtain a linear decarboxylation in these experiments the following quantities of organism were used in each manometer: for arginine and glutamic acid, 5–7 mg.; for lysine and histidine, 17–20 mg.; and for ornithine about 30 mg.

dry weight of organism. The Q_{CO_2} figures are calculated from the linear rate of CO_2 evolution for the first 20–30 min. after the amino-acid has been tipped in. All activities in the following experiments were determined at the optimum *p*H for the decarboxylase in question.

The properties of the reactions and the enzymes involved are discussed in detail later: the demonstration of decarboxylation without deamination would indicate that amines are being formed under the above experimental conditions and this is confirmed by the isolation of the products, but before dealing with this it is necessary to establish the optimal conditions for the production of such substances by the organism.

Temperature conditions. Early experiments carried out at 37° showed that although the decarboxylation reactions occurred at this temperature with an initially high Q_{CO_2}, the rate rapidly fell off and it seemed probable that this was due to the thermolability of the enzymes involved. To investigate this, manometers were set up in four thermostats set at 19, 26, 31 and 38·5° respectively. The contents of the cups were as usual and the effect investigated for the decarboxylation of arginine and glutamic acid (dry weight of organism = 4·9 mg.), lysine and histidine (dry weight of organism = 19·2 mg.), each at its optimum *p*H value. The washed suspension was prepared from a culture grown for 18 hr. in 2% glucose broth at 27°. After the addition of the amino-acid in each case, readings were taken at 10 min. intervals for 3 hr. Fig. 2 shows the course of the

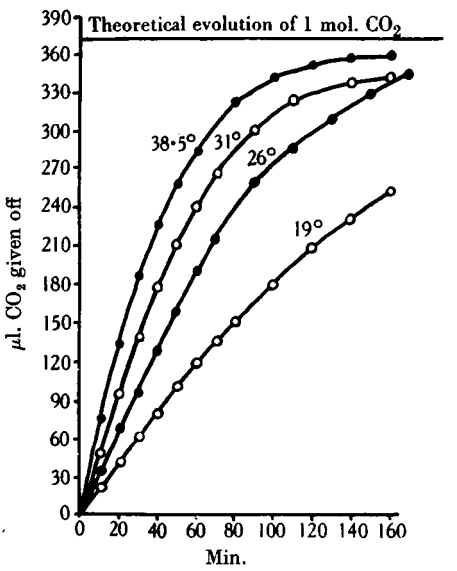

Fig. 2. Decarboxylation of arginine by washed suspensions of *Bact. coli* (stock) (4·9 mg.) at *p*H 4·0 and various temperatures.

decarboxylation of arginine at the various temperatures: the reaction is not linear but becomes more nearly linear the lower the temperature. At all temperatures the decarboxylation ceases at about 360 μl.: 373 μl. correspond to the removal of one molecule of CO_2 from the arginine molecule.

The results obtained with the other decarboxylation reactions are similar except in the case of glutamic acid. Fig. 3 shows the variation with temperature

of the initial Q_{CO_2}, measured over the first 10 min. of the reaction. The decarboxylations of arginine, lysine and histidine give an initial Q_{CO_2} increasing steadily with temperature, the temperature coefficients over the range 20–30° being: arginine 2; lysine 6·5; histidine 6. The reaction with glutamic acid is, however, much more sensitive to temperature, temperatures above 26° being inhibitory so that the rate does not increase above 26° and falls rapidly above 31°. Apart

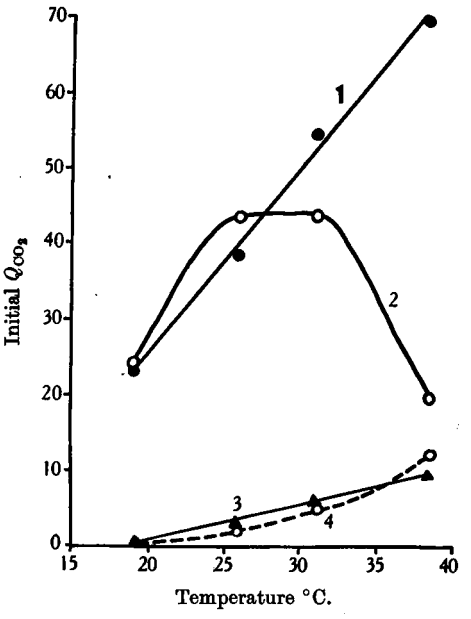

Fig. 3.

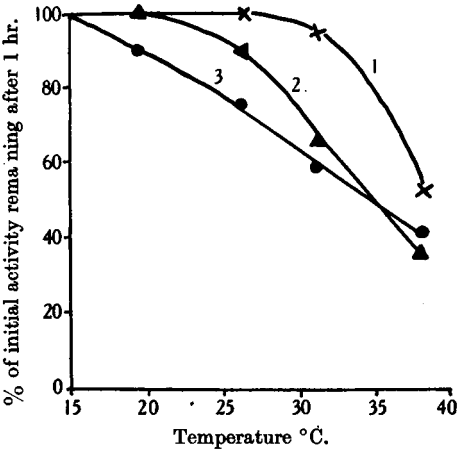

Fig. 4.

Fig. 3. Effect of temperature on initial velocity (Q_{CO_2}) of decarboxylation of amino-acids by washed suspensions of *Bact. coli* (stock). 1, Arginine. 2, Glutamic acid. 3, Histidine. 4, Lysine.

Fig. 4. Effect of temperature on amino-acid decarboxylases of *Bact. coli* (stock). 1, Lysine. 2, Histidine. 3, Arginine.

from this case, the effect of temperature is seen not in the initial Q_{CO_2} but in the diminution of the rate with time. Fig. 4 shows the percentage activities remaining after 1 hr. reaction at the various temperatures. Thus to maintain the full activity of the washed suspension towards most of the amino-acids in question, it is necessary to carry out the experiments below 20°. However, owing to the low activities at such temperatures, it has been found more convenient to use an experimental temperature of 30°, planning the quantities so that the reaction is completed in about 1 hr. and measuring rates over the early linear portion of the curve.

Growth conditions

Effect of growth temperature. Since temperature has such a marked effect upon the activities of the washed suspension, it is to be expected that the temperature at which the culture is grown will affect the activities of the organism. Table 1 shows the activities at 30° and optimum *p*H towards various amino-acids of washed suspensions of *Bact. coli* (stock) and *Bact. coli Esch.*

Table 1. *Effect of growth temperature on decarboxylase activity*

Organism	Substrate	Culture grown in 2% glucose broth: (*a*) at 27° Q_{CO_2}	(*b*) at 37° Q_{CO_2}
Bact. coli (stock)	Arginine	72	37
	Glutamic acid	65	66
	Lysine	21	10
	Histidine	9	3
Bact. coli Esch.	Arginine	240	128
	Lysine	210	68
	Histidine	18	12
	Ornithine	90	8

All activities determined at the optimum *p*H and 30°.

prepared from cultures grown in 2% glucose broth for 18 hr. at (*a*) 27° and (*b*) 37°. The higher growth temperature results in significantly smaller activities and all cultures were grown in future at 27°.

Age of culture. In studies on certain deaminases, the activity of the organism has been found to vary enormously with the time for which the culture was incubated before harvesting [Gale & Stephenson, 1938]. To investigate whether such variations occurred with the decarboxylases, 2 l. of 2% glucose broth were inoculated with a 20 hr. culture of *Bact. coli* (stock), incubated at 27° and samples

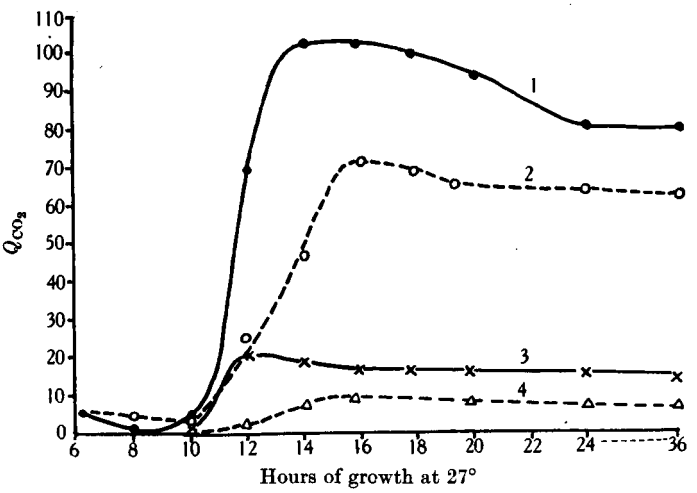

Fig. 5. Variation of decarboxylase activity (Q_{CO_2}) with "age of culture": *Bact. coli* (stock) grown in 2% glucose broth at 25°; growth ceases 15–17th hr. 1, Arginine. 2, Glutamic acid. 3, Lysine. 4, Histidine.

withdrawn at 2 hr. intervals from the 6th hr., when turbidity was first visible, up to the 20th hr. and then at 24 and 36 hr. The samples were withdrawn with sterile precautions, the turbidity and *p*H measured, the organisms centrifuged out of the culture, washed and made up into washed suspension. The activities of the suspensions toward arginine, glutamic acid, lysine and histidine were then determined in each case under optimal conditions and the results are shown in Fig. 5. Early cultures have little activity, the activity increasing rapidly between 11th–14th hr. of growth, becoming maximal from 14th to 16th hr.

when active cell division has finished. After this the activity declines slowly as the culture ages. There is no abrupt alteration in *p*H during the growth period but the *p*H falls slowly until it eventually stabilizes at about 5 after 48 hr. The variation in activity with age of culture is similar to that found with aspartase [Gale, 1938] which was shown to be due to some undefined change in the medium brought about by the metabolic activities of the organism. As in that case, the maximum activity coincides with the cessation of active cell division, and in all cases mentioned later where there is doubt about the correct growth period, the culture was harvested at the time when the turbidity ceased to increase—so that all cultures are harvested in approximately the same biological phase.

Nature and presence of carbohydrate in growth medium. It has been noted that whenever bacteria have been shown to produce amines in culture media, some form of carbohydrate or glycerol has been present and in all the experiments, so far quoted, the organisms have been grown in 2% glucose broth. Having determined the optimal conditions for the investigation of decarboxylation by the washed suspension, it is now necessary to find what part, if any, the carbohydrate in the growth medium plays. To do this, cultures of the organism were grown in the following ways: on the surface of broth agar in Roux bottles; in liquid broth under strictly anaerobic conditions; and in broth in flasks with the addition, separately, of glycerol, glucose, fructose, mannose and galactose to a final concentration of 2% in each case. In certain cases powdered chalk was included in the glucose medium. In all cases the inoculated medium was incubated at 27° until the turbidity ceased to increase (16–20 hr.), the final *p*H in the medium measured potentiometrically and washed suspensions prepared from the harvested organism. The activities towards arginine, glutamic acid, lysine and histidine were then determined under optimal conditions. Fig. 6 sets out diagrammatically the mean results obtained in each case.

When the organism is grown on the surface of broth agar, it has negligible decarboxylase activity. Strictly anaerobic conditions result in a small activity while the presence of glycerol in the growth medium produces the first marked decarboxylase activity. The presence of glucose in the growth medium results in the highest activity in each case, the effect being diminished by the presence of chalk. The presence of fructose, mannose or galactose has approximately the same effect as that of glucose, but not quite so marked in some cases, galactose having only a small enhancing effect in the case of arginine decarboxylase.

Fig. 6 shows that glucose produces an increase of activity that cannot be accounted for by the anaerobiosis produced by fermentation gases and that the effect is by no means specific for glucose as this can be replaced by other sugars and, to a smaller extent, by glycerol. When the final *p*H in the medium is considered in each case, it appears that there is a correlation between the *p*H of the medium and the activity of the organism, which accounts for the smaller effect of glycerol and the effect of the presence of chalk in the glucose medium.

Accordingly, a series of flasks of plain broth was set up and their *p*H values adjusted roughly to 8·5, 7·5, 7, 6, 5 and 4 respectively. The media were inoculated with a 20 hr. broth culture of the stock strain and incubated at 27°. The cultures were harvested in each case when the turbidity ceased to increase and the final *p*H determined. The periods of incubation varied from 16 hr. at *p*H 7 to 22 hr. at *p*H 5, the total crop of organism was less the further the growth *p*H diverged from 7·0 to 7·5, and at *p*H 4·0, although a slight turbidity was produced in 30 hr., insufficient organism was obtained to harvest. The activities of the washed suspensions were determined as usual and the results are shown in Fig. 7. The production of the four decarboxylases increases with decrease of growth *p*H,

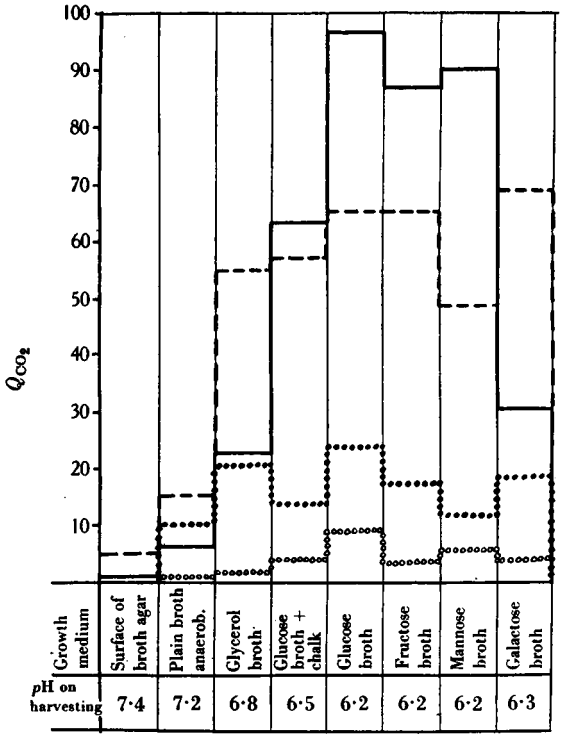

Fig. 6. Variation of decarboxylase activities of washed suspensions of *Bact. coli* (stock) with constitution of growth medium. All cultures grown at 27°. Activities determined at 30° and at optimum *p*H. —— Arginine. - - - - Glutamic acid. • • • Lysine. o o o Histidine.

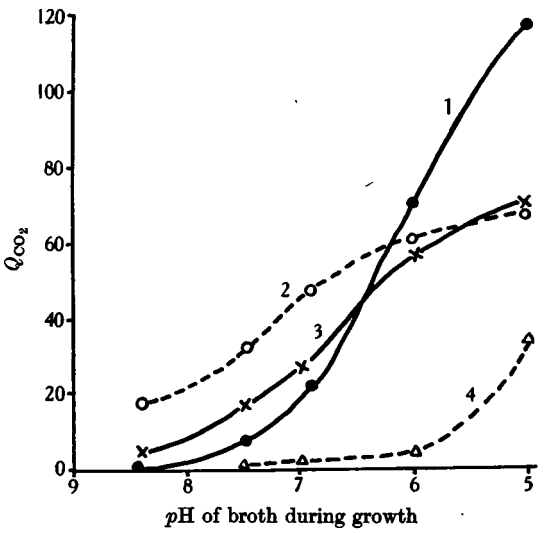

Fig. 7. Variation of decarboxylase activity (Q_{CO_2}) of washed suspensions of *Bact. coli* (stock) with the *p*H of the plain broth in which the organism was grown. 1, Arginine. 2, Glutamic acid. 3, Lysine. 4, Histidine.

that of the enzymes for arginine, lysine and histidine being negligible at *p*H 8 but reaching values higher than any obtained in the carbohydrate media when grown at *p*H 5. If the activities shown in Fig. 6 are superimposed on those in Fig. 7 it can be seen that the activities in the various media (Fig. 6) are fully accounted for by the *p*H reached in those media during growth. Thus the presence of carbohydrate in the medium has no specific effect but acts through the production of acid from it by fermentation, the organism actually producing more decarboxylase in a non-carbohydrate medium at an acid reaction. In many of the general experiments quoted later the organism was grown in 2% glucose broth as by this means a larger crop is obtained than by growing in an acid medium.

The findings so far are related to the decarboxylases of arginine, glutamic acid, lysine and histidine. *Bact. coli* (stock) also probably decarboxylates ornithine but at a rate too slow to allow of detailed investigation; this amino-acid is however attacked rapidly by *Bact. coli Esch.* Hence the properties of this organism will next be considered.

Decarboxylation of amino-acids by Bact. coli Esch.

Investigation of the decarboxylase activities of washed suspensions of this organism grown in 2% glucose broth at 27° for 18 hr. showed that, of the list

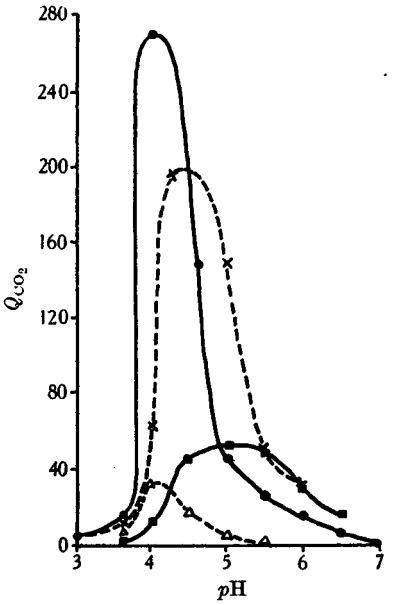

Fig. 8.

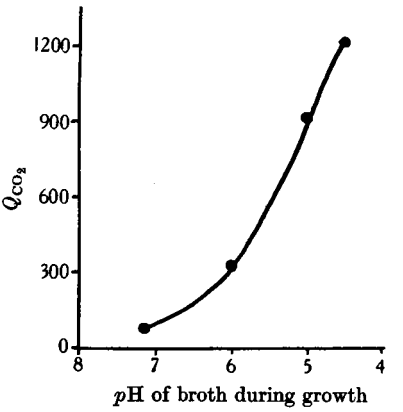

Fig. 9.

Fig. 8. Decarboxylation of amino-acids by washed suspensions of *Bact. coli Esch.*: variation of activity (Q_{CO_2}) with *p*H. •—• Arginine. × - - - - × Lysine. △ - - - - △ Histidine. ■—■ Ornithine.

Fig. 9. Variation of ornithine decarboxylase activity (Q_{CO_2}) of washed suspensions of *Bact. coli Esch.* with the *p*H of the broth in which the organism was grown.

of amino-acids set out above, the only ones attacked significantly are arginine, lysine, ornithine and histidine. Fig. 8 shows the *p*H-activity curve obtained for these amino-acids with the washed suspension. The activities are in general much higher than those shown by *Bact. coli* (stock), and this is possibly due to

the fact that *Esch.* is a more rapid acid former, the final *p*H in glucose medium after 18 hr. growth being 5·2 compared with 6·2 for the stock strain. Glutamic acid is not attacked. Fig. 8 shows an exceptionally sharp optimum at *p*H 4·0 for the arginine decarboxylase; at 4·5–4·7 for lysine; at 4·0 for histidine, and a less sharply defined optimum at about *p*H 5·0 for the ornithine enzyme. The Q_{CO_2} values obtained at the optimum *p*H and 30° are: arginine 272; lysine 198; ornithine 48 and histidine 33.

Fig. 9 shows the increase in Q_{CO_2} for the ornithine decarboxylase of the washed suspension with the decrease in *p*H of the plain broth growth medium (cf. Fig. 7 for stock). The value when grown in broth at *p*H 7·2 is 47 and this increases very rapidly to the extremely high figure of 1200 when the growth *p*H is 4·5. This enzyme thus gives a remarkable demonstration of the effect of the growth *p*H. It is noticeable in this case that the glucose growth medium which reaches a final *p*H of 5·2 gives rise to an organism which has an ornithine decarboxylase activity of only 48—in this case, the glucose medium is apparently inhibitory to the enzyme formation. This point will be discussed later.

General properties of the amino-acid decarboxylases

Quantitative nature of the decarboxylation. Fig. 10 shows the course of the decarboxylation of the five amino-acids so far studied by washed suspensions of either *Bact. coli* (stock) or *Esch.* under optimal conditions. The theoretical

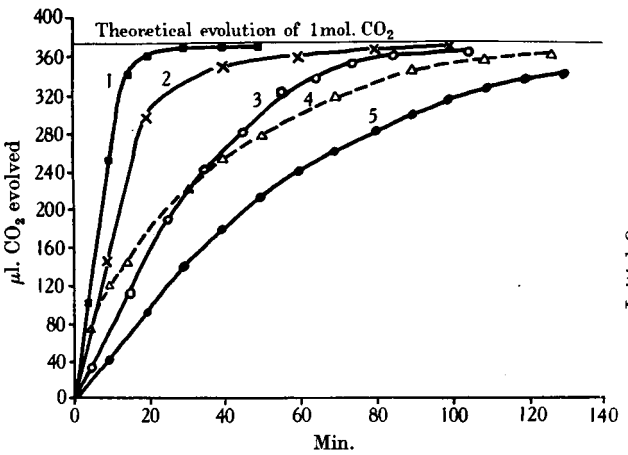

Fig. 10.

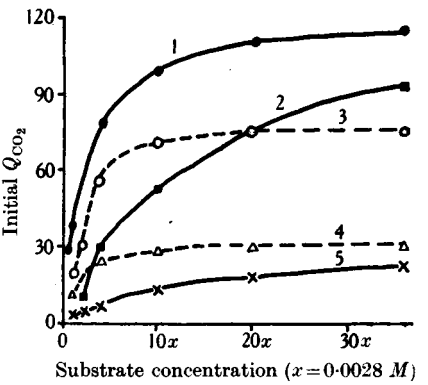

Fig. 11.

Fig. 10. Quantitative decarboxylation of amino-acids: action of washed suspensions on 0·5 ml. *M*/30 amino-acid. 1, Ornithine: 1·9 mg. *Bact. coli Esch.* (culture from broth at *p*H 5). 2, Lysine: 11·4 mg. *Bact. coli Esch.* (2% glucose broth culture). 3, Glutamic acid: 8·0 mg. *Bact. coli* (stock) (2% glucose broth culture). 4, Histidine: 30·0 mg. *Bact. coli Esch.* (2% glucose broth culture). 5, Arginine: 4·9 mg. *Bact. coli Esch.* (2% glucose broth culture).

Fig. 11. Variation of initial rate of decarboxylation (Q_{CO_2}) with concentration of substrate. 1, Arginine (stock). 2, Ornithine (*Esch.*) 3, Glutamic acid (stock). 4, Histidine (*Esch.*). 5, Lysine (stock).

evolution of CO_2 corresponding to the removal of 1 mol. of CO_2 from the molecule of amino-acid is 373 μl. and it can be seen that in every case the decarboxylation ceases within a few μl. of this figure. Thus the reaction is quantitative.

Affinities of the decarboxylases. Fig. 11 represents the variation of initial Q_{CO_2} with the substrate concentration for the five enzymes in *Bact. coli* (stock) and *Esch.* The approximate concentration for half maximum reaction velocity in each case is given in Table 2.

Table 2. *Apparent Michaelis constant for amino-acid decarboxylases*

Substrate	Organism	Michaelis constant
Arginine	*Bact. coli* (stock)	0·00056 *M*
Glutamic acid	*Bact. coli* (stock)	0·00056 *M*
Lysine	*Bact. coli* (stock)	0·0028 *M*
Histidine	*Bact. coli Esch.*	0·00075 *M*
Ornithine	*Bact. coli Esch.*	0·003 *M*

Action of glucose on activities of washed suspension. The action of glucose in the growth medium has been stated to be due to the acids produced from it by fermentation. Table 3 shows the effect of the presence of glucose during the decarboxylation by washed suspensions prepared from cultures in 2% glucose broth. The presence of glucose during the decarboxylation by washed suspensions therefore has no significant effect.

Table 3. *Decarboxylation of amino-acids in presence of 1% glucose*

The values for Q_{CO_2} are corrected for the glucose blank.

Organism	Substrate	Q_{CO_2} Alone	Q_{CO_2} + Glucose
Bact. coli (stock)	Arginine	67	56
	Glutamic acid	50	45
	Lysine	6	6
	Histidine	6	6
Bact. coli Esch.	Arginine	253	247
	Lysine	244	261
	Histidine	16	17
	Ornithine	54	63
	Glucose at *p*H 4·0	—	0

Presence of oxygen. Two sets of manometers were set up to test the decarboxylations carried out by the stock organism under optimal conditions, one set filled with N_2 and the other with air. No significant difference in the rate of CO_2 evolution occurred and no deamination could be shown in the aerobic experiments—probably since the experimental *p*H diverges widely from the *p*H optimum for the deaminases [Stephenson & Gale, 1937]. To avoid any strain differences in this matter, however, all other experiments were carried out anaerobically.

Coenzyme effect. In previous investigations on deaminases [Gale & Stephenson, 1938; Gale, 1938] evidence has been obtained from the loss in activity of washed suspensions on standing under various conditions that has led to the postulation of coenzymes required by these enzymes. Similar experiments have been carried out with the decarboxylases: as a source of possible coenzymes a suspension of baker's yeast in phosphate buffer (*p*H 6) was prepared and boiled for 10 min., 0·5 ml. of this preparation was then added to the contents of the Warburg cups involved. The activities of a washed suspension of *Bact. coli* (stock) towards arginine, glutamic acid, lysine and histidine were determined (*a*) immediately after preparation of the washed suspension and under the usual optimal conditions; (*b*) as (*a*) but with the addition of the boiled yeast preparation; (*c*) after

the washed suspension had been incubated (in water suspension) for 4 hr. at 27°, and (*d*) as (*c*) with the addition of yeast. The resulting values of Q_{CO_2} in each case are set out in Table 4.

Table 4. *Effect of* (*i*) *addition of boiled yeast,* (*ii*) *incubation at 27°, on the decarboxylase activities of washed suspensions of* Bact. coli (*stock*)

For key to column headings see text.

	Q_{CO_2}			
Substrate	(*a*)	(*b*)	(*c*)	(*d*)
Arginine	69	82	52	86
Glutamic acid	57	66	33	80
Lysine	18	23	14	18
Histidine	4	4·5	3	4·5
Boiled yeast control	0	0	0	0

Comparison of (*c*) with (*a*) shows that the incubation of the washed suspension in water leads to a loss of activity although this is not very marked. As a result of this, the activities of all other suspensions were determined, unless otherwise stated, as soon after preparation as possible. Comparison of (*d*) with (*c*) and (*a*) shows that this lost activity can be fully restored by the presence of boiled yeast, the activity returning to a figure higher than that shown by the original washed suspension alone—but which is shown by the initial suspension in the presence of the yeast (*b*). Thus there is some factor present in boiled yeast which increases the decarboxylase activities of the organism and which brings about restoration of the activity lost by the washed suspension on standing. The factor cannot be replaced by a pure preparation of cocarboxylase (aneurin pyrophosphate).

Isolation of the amines found

The general technique evolved for the isolation of the amines formed by the bacterial decarboxylation of amino-acids is as follows: from the knowledge obtained by the manometric experiments, quantities are worked out for repeating the manometric experiment on a scale sufficiently large to decarboxylate an amount of amino-acid to completion and to provide 100–200 mg. of the amine picrate on isolation. The actual quantities vary with the activity of the organism and examples are set out below. The solutions are put in a "Kreb's pot", gassed with N_2 and then incubated at 30°; at the same time a control is carried out manometrically and the incubation is continued until the manometer indicates that the decarboxylation has reached completion. At the end of the incubation, the organism is removed by filtration through a Seitz pad and the filtrate then evaporated to dryness *in vacuo*. The dry residue is extracted several times with absolute alcohol made slightly alkaline by the addition of a drop of 10 % NaOH. To obtain quantitative recovery it is necessary to carry out the extraction in a reflux apparatus some seven or eight times, but in the majority of isolations described below four ½-hr. extractions were found to yield about 80 % of theory. The alcoholic extracts are filtered, made just acid to bromothymol blue with acetic acid and saturated alcoholic $HgCl_2$ then added until precipitation ceases. After standing overnight, the clear supernatant is retested against alcoholic $HgCl_2$. The precipitate is then centrifuged down, resuspended in about 10 ml. water and decomposed with H_2S. The filtrate is boiled to remove H_2S, adjusted to *p*H 6 and the theoretical amount of 10 % picric acid in methyl alcohol then added. The solution is brought to the boil and then allowed to cool slowly, when crystals of the amine picrate are precipitated. These are recrystallized from

water until their melting points agree with the values given in the literature as below. The analyses are by Weiler, the amine being isolated as the picrate in each case.

Isolation of agmatine from arginine. Quantities: 40 ml. washed suspension *Bact. coli* (stock) prepared from 1 l. 2% glucose broth culture grown for 18 hr. Total dry weight of organism 340–350 mg. 40 ml. 0·05 *M* phthalate buffer *p*H 4·0. 40 ml. *M*/60 *l*-(+)arginine (neutral). Yield: 256 mg. orange needles; on heating these crystals soften and appear to melt at 228–229°, decomposing sharply at 235–236°. Found: C, 34.83%; H, 3·47%; N, 24·03%. Calc.: C, 34·69%; H, 3·41%; N, 23·81%.

Isolation of cadaverine from lysine. Quantities: 20 ml. washed suspension *Bact. coli* (stock) as above. 20 ml. 0·05 *M* phthalate buffer *p*H 4·5. 20 ml. *M*/60 *l*-(+)lysine dihydrochloride (neutral). Yield: 158 mg. deep yellow needles: M.P. 221°. Found: C, 36·35%; H, 3·63%; N, 19·65%. Calc.: C, 36·42%; H, 3·57%; N, 20·0%.

Isolation of histamine from histidine. Quantities: 20 ml. washed suspension *Bact. coli Esch.* prepared from 1 l. 2% glucose broth culture grown for 18 hr. Total dry weight of organism 580 mg. 20 ml. 0·05 *M* phthalate buffer *p*H 4·0. 20 ml. *M*/60 *l*-(−)histidine monohydrochloride (neutral). Yield: 120 mg. orange-yellow needles: M.P. 235° (uncorr.), 240° (corr.). Found: C, 36·28%; H, 2·81%; N, 21·8%. Calc.: C, 35·97%; H, 2·64%; N, 22·2%.

Isolation of putrescine from ornithine. Quantities: 40 ml. washed suspension *Bact. coli Esch.* prepared from a culture grown in plain broth at *p*H 5·0 for 20 hr. Total dry weight of organism 50 mg. 40 ml. 0·05 *M* phthalate buffer *p*H 5·0. 40 ml. *M*/60 *l*-(+)ornithine dihydrochloride (neutral). Yield: 163 mg. light yellow needles: on heating these do not melt but decompose with blackening at 248–250°. Found: C, 35·20%; H, 3·65%; N, 20·35%. Calc.: C, 35·03%; H, 3·65%; N, 20·44%.

Isolation of γ-aminobutyric acid from glutamic acid. Quantities: 40 ml. washed suspension *Bact. coli* (stock) prepared from 2% glucose broth culture as above. 40 ml. 0·05 *M* phthalate buffer *p*H 4·0. 40 ml. *M*/60 *l*-(+)Na glutamate. The usual technique was followed as far as alcoholic extraction of dry residue: then $AgNO_3$ was added until precipitation ceased. Alcoholic extraction was continued until the filtered extract gave no precipitate with $AgNO_3$. Centrifuged down and dried *in vacuo*; yield 136 mg. Found: 51·1% Ag. Calc. for Ag aminobutyrate: 51·4%.

For the isolation of the γ-aminobutyric acid as such, the above experiment was repeated on a 10 times larger scale using the organism harvested from 10 l. of 2% glucose broth. The alcoholic extract was prepared as before and the acid precipitated as its Hg salt by the addition of saturated alcoholic Na acetate and $HgCl_2$ [Ackermann, 1910]. After standing overnight the precipitate was centrifuged down, redissolved in alcohol, filtered and reprecipitated. The centrifuged precipitate was washed in alcohol, suspended in water, decomposed with H_2S and the filtrate reduced *in vacuo* to small bulk. This was then placed in a vacuum desiccator and allowed to evaporate to dryness; very hygroscopic crystals appearing in the last stages. To obtain the free acid Ag_2O, 50% in excess of theory, was added to the aqueous solution: after shaking in the cold for some time, the AgCl was filtered off, the filtrate concentrated to small bulk and left standing in a desiccator containing alcohol. Crystals separated in a few days: M.P. 192–194° (uncorr.). The M.P. of γ-aminobutyric acid is variously given in the literature as 183–202° while that of α-aminobutyric acid is 283°. Further distinctions between the two aminobutyric acids are discussed later.

Decarboxylation of amino-acids by various strains of Bact. coli

The properties of the decarboxylases of arginine, ornithine, lysine, histidine and glutamic acid have been studied for those enzymes appearing in *Bact. coli* strains stock and *Esch.* These findings are now applied to other strains of the coliform group. The procedure was as follows: each organism was first grown in 2% glucose broth and the activities of its washed suspension were then investigated at *p*H 4·0 (and in some cases at *p*H 5·0) under standard conditions for the decarboxylation of the sixteen amino-acids listed early in this paper. In those cases where a positive evolution of CO_2 was obtained, unaccompanied by deamination, not explicable by the organism blank and having a $Q_{CO_2} > 1$, the reaction was then studied over the *p*H range 3·0–6·0 and the optimum *p*H so determined. A list of the optimum activities with the various strains is given in Table 5.

Table 5. *Decarboxylase activities of* Bact. coli *strains grown for 18 hr. in 2% glucose broth at 27°*

Activities expressed as Q_{CO_2} determined with washed suspensions at the optimum *p*H and 30°.

		Q_{CO_2}				
No.	Organism	Arginine	Histidine	Lysine	Ornithine	Glutamic acid
1	*Bact. coli* stock	90*	*10**	*19**	2	67*
2	*Bact. coli Esch.*	272*	33*	198*	*48**	– †
3	*Bact. coli Sheff.*	*146**	14*	59*	*4**	63
4	*Bact. coli Dunn*	5	2	206*	1	– †
5	*Bact. coli* 201	– †	*2*	121*	– †	152*
6	*Bact. coli* 211	*158**	*12*	53	*4*	107
7	*Bact. coli* 210	98*	– †	*24*	?	86
8	*Bact. coli* 216	95*	4	44*	6	130
9	*Bact. coli* 217	96*	*5*	*28*	*5**	105
10	*Bact. coli faecal* A	98*	*6*	*34**	*1**	95
11	*Bact. coli faecal* B	– †	– †	– †	5	– †
12	*Bact. coli faecal* C	116*	9	72*	*6*	82
13	*Coliform intermed.* A	7	*3*	4	*6*	?
14	*Coliform intermed.* B	*45**	*1*	*1*	*6*	– †
15	*Bact. formicum*	15	6	*2*	*2*	– †
16	*Bact. friedländeri*	– †	3	34	8	– †

* Indicates amine isolated and identified: the isolations of putrescine were carried out with the organism grown in broth at *p*H 5 (see Table 6). Italicized figures indicate that value is more than doubled by growth in broth at *p*H 5.

† Indicates activity $Q_{CO_2} < 1$.

In no case did the washed suspension attack any of the listed amino-acids, other than the five in Table 5, at a significant rate under the experimental conditions. For the organisms 1–6 the *p*H curve was carried out for the decarboxylation activities with each amino-acid showing a positive result, but as the optimum *p*H obtained was always the same, namely: arginine *p*H 4·0; histidine *p*H 4·0; lysine *p*H 4·5; ornithine *p*H 5·0; glutamic acid *p*H 4·0; the activities were determined in the other cases only at these optimum *p*H values. In many cases the amines were isolated as the picrates as indicated above and the picrates then identified by M.P. determination and, in representative cases, by analysis. In the case of arginine decarboxylation the isolation was carried out in all cases showing an activity >10 owing to the confusion in the literature with regard to the product of this reaction (see later) and the product in every case was agmatine and never putrescine.

Of the 14 coliform organisms investigated, 7 attacked all five amino-acids and 11 attacked the four excluding glutamic acid: 12 decarboxylated arginine; 12 histidine; 13 lysine; 12 ornithine and 9 glutamic acid. Histidine and ornithine are attacked much more slowly than the other three amino-acids but ornithine decarboxylase is better produced under different growth conditions, as shown below.

Effect of growth p*H on the formation of the decarboxylases.* The preliminary studies with *Bact. coli* (stock) and *Esch.* showed that the activities of the organism obtained from culture in glucose broth were due to the low *p*H produced during growth by the acid formed from the glucose. Organisms grown in plain broth previously adjusted to *p*H 5·0 had higher activities than those grown in glucose broth, this effect being particularly marked in the case of ornithine decarboxylase (*Bact. coli Esch.*). To test the general application of this finding cultures of each of the other strains mentioned were grown (*a*) in plain broth at *p*H 7·2 and (*b*) in plain broth at *p*H 5·0; the activities of the washed suspension were determined as usual, and these were compared with the values obtained with the glucose broth organism. The results are set out in Table 6. As the results are best shown with ornithine as substrate, the organisms are put down in order of the activity towards ornithine of their cultures grown at *p*H 5. The organisms are also numbered to compare with Table 5 and the Q_{CO_2} values are italicized in those cases where the activity of the *p*H 5 culture is more than twice that of the glucose culture. All activities are determined under optimal conditions.

Table 6. *Effect of growth* p*H on decarboxylase activity* (Q_{CO_2})

In each case the Q_{CO_2} is given for washed suspensions prepared from cultures grown at *p*H 7·2 (7), at *p*H 5·0 (5), and in 2% glucose broth (GB).

No.	Organism	Final *p*H (GB)	Ornithine			Arginine			Lysine			Histidine			Glutamic acid		
			7	5	GB	7	5	GB	7	5	GB	7	5	GB	7	5	GB
2	*Bact. coli Esch.*	5·25	47	*960*	48	2	338	272	53	194	198	3	26	33	—	—	1
9	*Bact. coli* 217	5·3	59	*840*	6	2	133	96	23	*113*	28	5	*17*	5	23	96	105
10	*Bact. coli faecal* A	5·1	40	*612*	1	7	83	98	6	*91*	34	—	*12*	6	23	87	95
3	*Bact. coli Sheff.*	5·15	13	*200*	3	95	*380*	146	18	76	60	3	16	14	32	54	63
13	*Coliform inter.* A	5·4	4	*47*	6	15	*149*	7	2	5	4	1	*11*	3	8	2	—
12	*Bact. coli faecal* C	5·6	2	*30*	6	4	115	116	18	110	72	1	10	9	10	99	82
14	*Coliform inter.* B	5·2	1	*29*	6	14	*135*	53	—	4	1	—	14	1	—	—	—
6	*Bact. coli* 211	5·3	—	*20*	4	28	*302*	154	29	58	53	4	*30*	12	36	72	107
15	*Bact. formicum*	5·05	—	*10*	2	5	22	15	4	*15*	2	2	6	6	—	—	—
16	*Bact. friedländeri*	5·1	—	4	8	—	*4*	—	5	51	34	—	2	3	—	—	—
8	*Bact. coli* 216	5·2	1	3	6	4	114	95	7	82	44	—	6	4	23	71	130
1	*Bact. coli* (stock)	6·25	—	*2*	1	8	120	90	12	*70*	20	1	*30*	10	20	66	67
7	*Bact. coli* 210	5·55	1	*2*	1	2	50	98	9	*102*	24	—	1	3	19	40	86
4	*Bact. coli Dunn*	5·1	—	1	2	—	5	1	22	373	206	—	1	3	—	—	—
11	*Bact. coli faecal* B	5·65	3	1	5	—	—	—	—	—	—	—	—	—	—	—	—
5	*Bact. coli* 201	5·3	—	—	—	—	—	—	56	142	121	2	*10*	2	48	81	182

The results given in Table 6 show that:

(*a*) Organisms grown at *p*H 7·2 have little decarboxylase activity.

(*b*) This activity is greatly increased by growing the organisms at *p*H 5·0 or by the presence of glucose in the growth medium.

(*c*) The increase in activity produced by the presence of glucose is fully explained by the fall in *p*H produced by the fermentation acid.

(*d*) In the case of ornithine decarboxylase the presence of glucose in the growth medium does not increase the formation of enzyme but appears to inhibit this in some cases. The effect of growth *p*H is particularly marked with this enzyme.

Specificity of the decarboxylases. Table 7, derived from Table 5, shows the distribution of the decarboxylases among various *Bact. coli* strains and associated organisms and, since no one enzyme is constantly associated with any other in this distribution, this would indicate their strict specificity.

Table 7. *Distribution of the decarboxylases*

Organism	Substrate				
	Ornithine	Arginine	Lysine	Histidine	Glutamic acid
Bact. coli 217	+	+	+	+	+
Bact. coli Esch.	+	+	+	+	−
Bact. coli 210	−	+	+	−	+
Bact. coli 201	−	−	+	+	+
Bact. friedländeri	+	−	+	+	−
Bact. coli faecal B	+	−	−	−	−

Characteristics of the amino-acid decarboxylases

Arginine decarboxylase. Optimum *p*H 4·0; temp. coeff. 2; Michaelis constant (stock) 0·00056 *M*; product—agmatine.

As far as the author knows there is no previous record of the production of agmatine from arginine by bacteria. Linneweh [1931] isolated agmatine from amongst the products of bacterial decomposition of arcaine but the records of bacterial attack of arginine give putrescine as the amine produced [Ackermann, 1910; Hirai, 1936; Akasi, 1938; etc.]. Mueller [1925] states that agmatine cannot be obtained from arginine by putrefaction. The production of putrescine from arginine in the presence of mixed cultures [Ackermann, 1910] is probably due to the presence of organisms containing arginase and, where the period of incubation is lengthy [Hirai, 1936], coliform organisms may produce that enzyme by adaptation. In the work of Akasi [1938] 21 out of 54 strains of *Bact. coli* produced putrescine when grown in a synthetic medium containing arginine in which the arginine formed the sole source of N. Using washed suspensions as described in this work, the product is agmatine which can be easily differentiated from putrescine not only by the decomposition point of its picrate but also by the colour of the crystals, since agmatine picrate forms deep orange needles, and putrescine picrate forms yellow needles. Moreover, as can be seen from Table 5, in every case the rate of decarboxylation of arginine by organisms grown in glucose broth is considerably greater than that of ornithine, so that the latter cannot be an intermediate substance in the amine formation. Growth experiments differ from washed suspension experiments in that the *p*H may vary greatly in the former and it may be that ornithine is formed from arginine during the early stages of some growth experiments while the *p*H is near neutrality, but that the enzymes responsible for such a change are inactive at *p*H 4·0, the optimum *p*H for the decarboxylation of arginine by the washed suspension.

Agmatine is reported to possess marked physiological actions of an insulin-like nature and its properties have been studied by Frank *et al.* [1926]; Frank [1927]; Hazard [1929]; etc.

Ornithine decarboxylase. Optimum *p*H 5·0; Michaelis constant (*Esch.*) 0·003 *M*; product—putrescine.

Ornithine decarboxylase formation increases rapidly with decreasing *p*H of the growth environment (Fig. 9; Table 6). It differs from the other decarboxylases studied in that the presence of glucose in the growth medium does not,

by the production of acid, lead to an increased enzyme formation (Table 6). The presence of glucose does not affect the decarboxylation of ornithine by the washed suspension—this has been shown in Table 3 and has been confirmed by experiments with *Bact. coli* 217 grown at *p*H 5 in plain broth. Thus, since glucose does not affect the enzyme once it is formed, it must have some effect on the formation of the enzyme. Table 8 shows the effects of various growth conditions on the activity of the enzyme in *Bact. coli* 217.

Table 8. *Effects of* (a) *growth* p*H*, (b) *glucose, on the ornithine decarboxylase activity of* Bact. coli *217*

Growth medium	Initial *p*H	Final *p*H	Q_{CO_2} (ornithine) (1)	(2)
Plain broth	7	7	59	75
Plain broth	5	5	840	645
2% glucose broth	7	5·3	9	6
2% glucose broth	5	4·8	142	128

The results show that not only does the presence of glucose during growth at *p*H 7, when vigorous fermentation occurs, inhibit the formation of the enzyme some 80–90%, but also when growth occurs at *p*H 5 and only slight fermentation occurs, its presence still causes an 80% reduction in the amount of enzyme formed. It would thus appear that in this case the presence of glucose inhibits the formation of the decarboxylase during growth, the effect being similar, superficially, to that noted for the deaminases [Stephenson & Gale, 1937].

Since the ornithine decarboxylase activity is so much greater in organisms grown in non-glucose media at *p*H 5 than when grown at *p*H 7, an attempt was made to increase the activity of a washed suspension, harvested from a culture grown in plain broth at *p*H 7, by incubating it at *p*H 5 either alone or in the presence of ornithine. Table 9 shows that the result was negative.

Table 9. *Attempt to increase activity of organism grown at* p*H 7*

Ornithine decarboxylase activity (Q_{CO_2}) of *Bact. coli Esch.*

	Q_{CO_2}
(*a*) Freshly prepared suspension	19
(*b*) Suspension after incubation at *p*H 7 for 3 hr. at 27°	12
(*c*) Suspension after incubation at *p*H 5 for 3 hr. at 27°	16
(*d*) Suspension as (*c*) in presence of *M*/10 ornithine	23
(*e*) Freshly prepared suspension from culture grown at *p*H 5	960

Lysine decarboxylase. Optimum *p*H 4·5; Michaelis constant (stock)—0·003 *M*; temp. coeff. 6·5; product—cadaverine.

Virtanen & Laine [1937] state that certain strains of *Bact. coli* decarboxylate lysine to give cadaverine, the optimum *p*H for the reaction being 7. Since their experiments continued over several weeks' incubation and their estimations depend on a final isolation of the cadaverine picrate, their method is not capable of exact determination of optimum conditions.

Histidine decarboxylase. Optimum *p*H 4·0; Michaelis constant (*Esch.*) 0·00075 *M*; temp. coeff. 6; product—histamine. The nature of the product has been confirmed by quantitative biological tests by Dr Feldberg of the Cambridge University Physiology Dept., to whom the author is grateful for his co-operation.

Glutamic acid decarboxylase. Optimum *p*H 4·0; Michaelis constant (stock) 0·00056 *M*; "optimum temp." 25°; product—γ-aminobutyric acid. The decarboxylation of glutamic acid ceases when 1 mol. of CO_2 has been removed from

the acid molecule. The properties of the product in the two cases studied in detail indicate that it is the γ-aminobutyric acid remaining. This is confirmed by (1) only 87% of the amino-N is given off in the Van Slyke estimation for α-amino-groups and (2) the dissociation constants of the decarboxylation product (Fig. 12) agree with those found for γ-aminobutyric acid by Neuberger [1937].

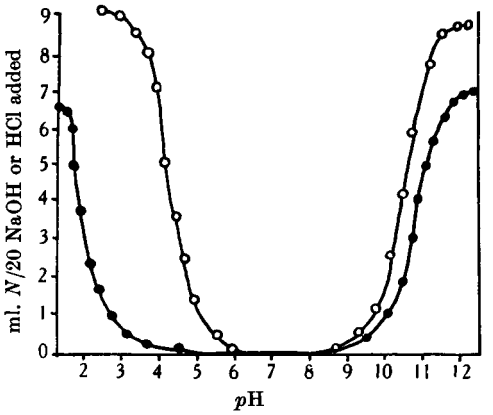

Fig. 12. Titration curve of α-aminobutyric acid •—• and product of decarboxylation of glutamic acid by *Bact. coli* (stock) o—o.

Table 10 indicates the specificity of the glutamic acid decarboxylase of *Bact. coli* (stock) (see also Table 7).

Table 10. *Specificity of glutamic acid decarboxylase*

Experiments with washed suspensions of *Bact. coli* (stock) at *p*H 4.

Substrate	Q_{CO_2}
l-Glutamic acid	58–67
l-Glutamine	25–30*
N-Acetylglutamic acid	0
l-Aspartic acid	0
l-α-Aminobutyric acid	0

* Preceded by deamidation.

Adaptive nature of the decarboxylases. Table 11 gives the activities (Q_{CO_2}) of washed suspensions of *Bact. coli Esch.* and 216 grown in the following synthetic media:

Medium A	Medium B
Stephenson's inorganic medium	Stephenson's inorganic medium
2% Na lactate	2% Na lactate
*p*H adjusted to 5·5	2% glucose
Medium C = medium A + 1% arginine	*p*H (initial) 6·8
Medium D = medium B + 1% arginine	
Medium E = medium B + 1% lysine	
Medium F = medium B + 1% glutamic acid	

Under present conditions, insufficient histidine or ornithine were available for use in growth media.

The decarboxylases are not developed in amino-acid-free media with the exception of the glutamic acid decarboxylase of 216 which would thus appear to be constitutive (in the sense used by Karstrom [1938]), although its production is increased by the presence of its substrate in the growth medium. The lysine

Table 11. *Decarboxylases of* Bact. coli *grown in various synthetic media*

Medium	Q_{CO_2} Arginine	Ornithine	Lysine	Histidine	Glutamic
Bact. coli Esch.					
A	0	1	0	0	—
B	1	2	3	0	—
C	0	—	—	—	—
D	27	—	—	—	—
E	—	—	270	—	—
Plain broth at *p*H 5	338	960	194	17	0
Bact. coli 216					
B	4	2	0	1	50
D	7	5	7	0	35
F	2	3	0	0	96
Plain broth at *p*H 5	114	3	82	6	71

decarboxylase of *Esch.* is strictly adaptive. The presence of arginine in the growth medium does lead to an increased production of the arginine decarboxylase but not to any marked extent; possibly the formation of this enzyme is dependent also on the production of some coenzyme that cannot be synthesized with ease in a simple medium. Owing to shortage of amino-acids, the results in this last section are based upon a few experiments and cannot be regarded as conclusive.

Reference to Fig. 6 shows that the organism, when grown on the surface of agar containing broth, possesses no significant decarboxylase activity. Attempts to produce arginine decarboxylase in such a washed suspension by incubating it at 27° for 4 hr. under the following conditions have proved negative:

(1) Anaerobically in phthalate buffer at *p*H 4·0.
(2) As (1) with the addition of *M*/100 arginine.
(3) As (1) with the addition of 1 % glucose.
(4) As (2) with the addition of 1 % glucose.
(5) Anaerobically in the presence of glucose at *p*H 6·0.
(6) As (5) with the addition of *M*/100 arginine.

Thus the enzyme is not produced in the non-dividing organism by incubation at the optimum *p*H with or without its substrate or in the presence of glucose, fermenting or non-fermenting.

Discussion

From the above work it would appear that *Bact. coli* produces enzymes capable of forming amines by the decarboxylation of certain amino-acids if the organism grows in the presence of the amino-acids at an acid reaction; the lower the *p*H the more enzyme being produced, within physiological limits. The enzymes once formed will act over a restricted *p*H range, being most active between *p*H 4 and 5. Hanke & Koessler [1924] obtained evidence that led to similar conclusions and wrote "the production of amines from amino-acids seems to be a protective mechanism and is resorted to when the accumulation of H ions within the organism's protoplasm is incompatible with its normal life processes. The amines can thus be thought of as reaction buffers". It must, however, also be remembered that when the reaction becomes acid, amino-acids can no longer be attacked by deamination, for the deaminases are inactivated and, at *p*H values lower than 5, carbohydrate itself is but slowly attacked; the amino-groups of the amino-acids become ionized but the —COOH groups become discharged and the production of decarboxylases may be the method

by which the organism extends its range of existence, utilizing amino-acid decarboxylation when other substrates and methods of attack are no longer available.

The extent to which conditions suitable for the production and activity of decarboxylases in bacteria in the intestine exist will depend upon the nature of the diet, presence of unabsorbed amino-acids, carbohydrate and acid-producing bacteria in the intestine. It is hoped to deal with some of these points in a later communication.

Summary

1. Washed suspensions of *Bact. coli* (stock) prepared from a culture grown in 2% glucose broth (tryptic digest of casein) decarboxylate arginine, lysine, histidine and glutamic acid to form agmatine, cadaverine, histamine and γ-aminobutyric acid respectively.

2. Washed suspensions of *Bact. coli Esch.*, similarly prepared, decarboxylate arginine, lysine, histidine and ornithine to form agmatine, cadaverine, histamine and putrescine respectively.

3. The decarboxylases have sharp *p*H-activity maxima, the optimum value in each case being: for arginine 4·0; lysine 4·5; histidine 4·0; glutamic acid 4·0 and ornithine 5·0.

4. The enzymes exhibit great sensitivity to rise of temperature and a convenient experimental temperature of 30° is used.

5. Washed suspensions prepared from organisms grown at 27° are more active than those prepared from organisms grown at 37°.

6. Young cultures have little activity, the activity rising to a maximum between the 14th and 16th hr. of incubation, after which it falls steadily to the 48th hr.

7. Experiments on the variation of growth conditions show that the presence of fermentable carbohydrate in the medium gives rise to an organism possessing far greater activity than one grown in a non-carbohydrate medium at *p*H 7. The action of the carbohydrate is due to the action of the acid produced from it by fermentation.

8. The production of the decarboxylases in the organism varies with the reaction of the growth medium: the lower the *p*H, the higher the activity of the resulting culture in washed suspension. This effect is particularly marked with ornithine decarboxylase, the culture grown at *p*H 5 having 20–100 times the activity of one grown at *p*H 7.

9. The decarboxylation is quantitative.

10. The affinities of the decarboxylases of *Bact. coli* (stock) and *Esch.* are determined.

11. Neither the presence of O_2 nor of glucose affects the course of the decarboxylations carried out by washed suspensions of *Bact. coli* (stock) and *Esch.* under optimal experimental conditions.

12. Evidence is given that the decarboxylases require a coenzyme, which is not aneurin pyrophosphate.

13. In every case the amine has been isolated and identified.

14. Investigation of the decarboxylation activities of 14 strains of coliform organisms shows that 12 strains decarboxylate arginine to form agmatine; 12 histidine to histamine; 13 lysine to cadaverine; 12 ornithine to putrescine, and 9 decarboxylate glutamic acid.

15. In all cases the optimum *p*H of the decarboxylases proved constant and equal to the values given in (3).

16. In all cases (*a*) organisms grown at *p*H 7 have little decarboxylase activity; (*b*) this activity is greatly increased by growing the organisms at *p*H 5 or by the presence of glucose in the growth medium; (*c*) the increase in activity produced by the presence of glucose is fully explained by the fall in *p*H during growth due to fermentation acids.

17. In contradistinction to the other four decarboxylases, the production of ornithine decarboxylase is inhibited by the presence of glucose in the growth medium but, as usual, greatly increased by a low growth *p*H in a non-carbohydrate medium.

18. The distribution of the decarboxylases indicates that each enzyme is specific for the decarboxylation of one amino-acid.

19. The characteristics of the individual enzymes are discussed.

20. The enzymes are not produced if the organisms are grown in an amino-acid-free medium.

21. In no case studied was any of the natural amino-acids, other than the five mentioned, decarboxylated at a significant rate ($Q_{CO_2} > 1$), by washed suspensions under the experimental conditions described.

The author wishes to thank Mr R. Davies for his assistance in the confirmatory investigations at the end of this paper, also Dr M. Stephenson and Prof. Sir Frederick Hopkins for their interest and encouragement throughout the course of this work. He is indebted to the Royal Commissioners for the Exhibition of 1851 for the Senior Studentship during the tenure of which this work has been carried out.

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