The Production of γ -Aminobutyric Acid by *Bacterium coli* Wilson, Type I

BY H. K. KING AND L. I. FLETCHER

The Bacteriology Department, University of Edinburgh

SUMMARY: Examination by paper partition chromatography of casein-hydrolysate/glucose culture filtrates of forty-seven strains of coliform organisms indicates that the decarboxylation of glutamic acid is characteristic of Wilson's *Bacterium coli* Type I.

Linggood & Woiwod (1948) have drawn the attention of bacteriologists to the potentialities of paper partition chromatography as a means of investigating the nitrogen metabolism of bacteria. The amino-acids present in a single drop of a culture filtrate may be recognized by this simple and elegant technique, and a large number of samples investigated simultaneously. During a study of amino-acid utilization by a variety of bacteria it was observed that certain members of the *Bacterium coli* group readily utilized glutamic acid with production of a substance whose chromatographic behaviour led to its identification as γ -aminobutyric acid. Gale (1940) has shown that some strains of *Bact. coli* possess a glutamic decarboxylase enzyme and demonstrated its action in washed suspensions. Through the courtesy of Dr J. P. Duguid and Miss M. J. D. Macpherson, of this Department, we obtained a number of strains of coliform organisms of known taxonomic characteristics. We found that the production, on a casein-hydrolysate glucose medium, of what is most probably γ -aminobutyric acid, is characteristic of Wilson's (1935) '*Bact. coli* Type I'.

EXPERIMENTAL

Bacteria. These are listed in Table 1. Most of them were recently isolated by Miss M. J. D. Macpherson from the sources indicated, or obtained from the National Collection of Type Cultures without information as to their origin. The classification of Wilson (1935) has been followed where it is applicable.

Media. Casein was hydrolysed with 6N hydrochloric acid for 45 min. at 120°, and the bulk of the acid removed by repeated vacuum distillation. Phosphates were removed by precipitation at pH 9. The medium was finally diluted to contain the equivalent of 2% casein, the pH adjusted to pH 7.4, and 0.5% of glucose added. Sterilization was by a single steaming for 1½ hr., sterility being tested by incubation before use. Enrichment with growth factors (cf. Proom & Woiwod, 1949) was not necessary, since we were investigating only Gram-negative organisms with relatively simple nutritional needs.

The sodium chloride present in this medium produced a yellow spot (R_F 0.20) on the collidine chromatograms. This salt can be removed if desired by careful alternate treatment with cation- and anion-exchange resins; the medium should then be re-examined chromatographically to ensure that no amino-acids have been lost.

Procedure. A tube of the casein-hydrolysate glucose medium was inoculated

with the appropriate strain from an agar slope culture and incubated for 72 hr. at 37°. The bacteria were removed by centrifugation and the supernatant fluid examined chromatographically; the pH of the fluid was noted.

Chromatographic methods. A general account of the technique has been given by Consden, Gordon & Martin (1944), Dent (1948), and Woiwod (1949), so no detailed description is needed. Single-dimensional chromatograms were run on

Table 1. *Strains of bacteria used*

Classification	Reactions					Origin						
	Methyl red	Voges Proskauer	Elkman	Indole	Citrate	Faeces	Wounds	Urine	Water	Milk	Laboratory Stock	N.C.T.C.
<i>Bact. coli</i> Type I	+	-	+	+	-	E48-9, 55, 93, 97-8, 101-4, 106	E50, 51, 52	E57, 58, 92	E53, 54	.	E56	123, 414, 419, 5928, 7275
<i>Bact. coli</i> Type II	+	-	-	-	-	E91, 95, 96	.	.	.	.	.	.
Intermediate I	+	-	-	-	+	.	.	.	.	.	.	3735
<i>Bact. aerogenes</i> Type I	-	+	-	-	+	.	E59	.	.	.	E1, 10, 14, 20, 61	418
<i>Bact. aerogenes</i> Type II	-	+	-	+	+	.	.	.	.	.	.	5936
<i>Bact. anaerogenes</i>	+	-	-	+	-	.	.	.	E119	E121	.	.
<i>Bact. cloacae</i>	-	+	-	-	+	.	.	.	.	.	.	5920
Irregular	-	-	-	-	+	.	.	.	.	.	.	7271
	+	-	+	+	+	E60	.	.	.	.	.	.
<i>Bact. pneumoniae</i>	+	-	-	-	-	.	.	.	.	.	E30	5054
	+	+	-	-	+	.	.	.	.	.	.	5055
	-	+	-	-	+	.	.	.	.	.	.	5056
	+	-	-	-	+	.	.	.	.	.	.	5056
<i>Bact. ozaenae</i>	+	-	-	-	+	.	.	.	.	.	.	5050

strips of Whatman No. 1 filter paper, 8 × 16 in., suspended from troughs 9 in. long made from Pyrex tubing (Atkinson, 1948). These glass troughs were strong, easily made and avoided the expense of stainless steel or the difficulties arising from the use of less resistant metals. The samples (0.01 ml.) were measured from an 0.1 ml. blood pipette on to the paper strip, the end of which was folded round a glass strip which was then lowered into the trough. Five samples were run on each strip, with a control sample of the uninoculated medium at each end. The troughs were held in carriers cut from tinplate, care being taken that the papers did not come into contact with the metal. The carriers were suspended in glass pathological specimen tanks, 18 × 16 × 15 in., covered with a sheet of glass or asbestos. This simple improvised apparatus proved wholly satisfactory.

Each culture-supernatant was examined in two solvent systems: (1) phenol-water with 0.1 % ammonia (redistilled phenol being used); (2) collidine-water, the commercial collidine, containing a mixture of isomers, being redistilled once before use.

The solvent phase was placed in the trough, with a beaker containing both phases in the bottom of the tank. Phenol chromatograms were run until the

solvent-front had nearly reached the end of the paper strip (about 24 hr.). The collidine chromatograms were run for 4 days, i.e. the solvent-front was allowed to run right off the strip. This was desirable since none of the amino-acids have R_F values greater than 0.60 and most are crowded into the region above R_F 0.85.

The papers, after removal from the troughs, were dried by suspending over a radiator or electric hot-plate, no special drying chamber being available. The solvent fumes would, however, cause annoyance if many papers were treated thus. The dry papers were sprayed with 0.1 % ninhydrin in *n*-butanol, allowed to dry at room temperature, and then placed in the 37° incubator for 3 hr. to develop the spots. Like Dent (1948) we found that heating in an oven as recommended by the original authors (Consden *et al.* 1944) was liable to cause rapid fading. Our chromatograms were stable for about a month, and were photographed when permanent records were required.

No constant-temperature room was available, but an inner room without windows was found to keep within 1° over a 24 hr. period and seldom varied by more than 2° even during the 4-day runs with the collidine system. The actual temperature is less important than its constancy during a run, though collidine does not give satisfactory results above about 18°, and temperature must always be taken into account in interpreting collidine chromatograms.

RESULTS

Decarboxylation of glutamic acid. With certain strains the glutamic acid spot was absent from the chromatograms of the culture filtrate and a new spot was seen. Experiments with shorter incubation periods showed that these changes occurred concurrently. The R_F value of the new spot was 0.80 in phenol—i.e. it appeared as a strengthening of the valine spot; in collidine the R_F value was 0.20 and its position immediately below that of glutamic acid itself. The production from glutamic acid of material giving the new spot was confirmed when one of the strains concerned, *Bact. coli* E 56, was grown on a chemically defined medium with glucose and glutamic acid as the sole organic compounds present: neither alone gave rise to the new material. Hydrolysis of culture fluid (8 hr. at 100° with 2N-hydrochloric acid) did not alter the chromatographic behaviour of the new spot; it was not therefore a peptide. The R_F values were those given for γ -aminobutyric acid by Dent (1948). We considered this sufficient to identify the material as γ -aminobutyric acid since this compound is known to be produced from glutamic acid by some strains of *Bact. coli* (Gale, 1940). Thus it was apparently this decarboxylation which was being recorded on our chromatograms by the disappearance of the glutamic acid and the concomitant appearance of the γ -aminobutyric acid spot. Recently, Woiwod & Proom (1950) read a paper demonstrating chromatographically that *Shigella paradysenterae* produces γ -aminobutyric acid by specific decarboxylation of glutamic acid, and suggested that the corresponding spot in some of their *Bact. coli* filtrates was due to the same cause.

Distribution of γ -aminobutyric acid production. Of the twenty-five strains of

Bact. coli Type I (Wilson, 1935) all produced γ -aminobutyric acid except N.C.T.C. 414. The Eijkman test did not give consistent results with this organism, so that its exact classification was open to some doubt. Results with all the other coliforms were negative save for the anomalous strain E 60 which differed from *Bact. coli* in utilizing citrate. All negative results were confirmed by a duplicate experiment in which incubation was continued for 8 days. In our series the production of the γ -aminobutyric acid spot was correlated with a positive Eijkman reaction.

Some experiments on growth in simple chemically defined media were also carried out. Gale (1940), using washed suspensions, found that glutamic decarboxylase was formed only at acid pH. This was usually achieved by adding glucose to the medium, though carbohydrate itself was not necessary for the formation of the enzyme. We found that decarboxylation accompanied growth at pH 5.6 in a heavily buffered medium with glucose and glutamic acid as sole carbon compounds, but not with glutamic acid alone. Growth at pH 7.6 in a heavily buffered medium was not accompanied by decarboxylation save for a very slow reaction in the presence of glucose.

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