Nutritional Requirements of *Bacterium coli* at 44°

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SUMMARY: A strain of *Bacterium coli* capable of growth in broth and in an ammonium + glucose + salts medium at 37° and in broth at 44°, died in the defined medium at 44°. The addition of glutamic acid to this medium allowed the growth temperature to be raised slightly; the further addition of nicotinamide allowed a further increase in growth temperature.

One of the criteria on which Public Health bacteriologists rely to identify strains of *Bacterium coli* as being of faecal origin is the ability to ferment lactose with the evolution of gas in a bile salt medium at 44°. The experiments recorded in this paper show that ability to multiply at 44° depends not only on the strain of *Bact. coli* but also on the nutritive materials supplied.

METHODS

The defined medium used throughout the work was Bigger's (1950) modification of Gale's (1947) medium which contains only organic salts and glucose, the sole nitrogen source being ammonia. Broth, where used, contained 1 % Lab-Lemco and 1 % peptone. Experiments were carried out with 5 ml. volumes of medium in small glass bottles covered with loosely fitting aluminium caps. When other substances were required in the medium, additions were made either in bulk or by adding to the individual bottles a sufficient number of drops (Dreyer pipette) of a suitable dilution of the substance. Serial dilutions, to halve the concentration of a substance, were made by transferring 5 ml. amounts from bottle to bottle. *Bact. coli* strain no. 18 was used as test organism, except in certain experiments designed to determine whether the phenomenon described occurred with other strains of *Bact. coli*. The cultural characteristics of *Bact. coli* strain no. 18, which was isolated from faeces, are given in Table 4. Inoculation was, in all cases, carried out by adding to 5 ml. of the medium, one drop (Dreyer pipette) of a 1/10,000 dilution in buffered saline of the test organism grown in defined medium at 37°. Cultures were examined every 24 hr. during incubation. One 5 mm. loopful of each culture which had remained clear was spread over the surface of an agar slope, which was examined after 24 hr. incubation, to determine the colonies resulting from the viable organisms in the original culture.

RESULTS

Growth curves were obtained from viable counts (2 hr. intervals) on *Bact. coli* growing in defined medium or in broth at 37 and 44°. It was found that whereas the organism multiplied freely in both media at 37° and in broth at 44°, it died when exposed to 44° in defined medium. The effect of adding certain

of the broth constituents to defined medium was then examined. A concentration of 25 mg. Lab-Lemco/100 ml. defined medium was sufficient to allow full multiplication of *Bact. coli* at 44°; whilst as little as 1 part in 128,000 allowed some organisms to survive; at lower concentrations of Lab-Lemco the bacteria died. The protective effect of peptone was similar to that of Lab-Lemco but slightly less marked. Lab-Lemco and peptone were not the only substances which showed the 'protective effect'; various meat and vegetable extracts and gelatin acted similarly. A mixture containing nineteen pure substances some of which, it was thought, might be responsible for the effect was tested. By a series of grouping experiments, in which inactive constituents of the mixture were eliminated one by one, it was found that full protection was afforded by adding to defined medium glutamic acid at 1/4000 and asparagine, nicotinamide, or nicotinic acid. In the presence of glutamic acid (1/500) a concentration of 1/500 asparagine was required for growth. Nicotinamide was much more active, a dilution of 1/81,000,000 allowing visible turbidity to develop in 24 hr.; nicotinic acid had the same activity.

Table 1. *The effect of incubation at 52° on a suspension of Bact. coli strain no. 18 in defined medium with and without glutamic acid + nicotinamide*

(Samples (loopful) of the various media were taken at intervals and plated.)

Period of incubation (min.)	Surviving organisms per loopful	
	Defined medium	Defined medium + glutamic acid (1/1000) + nicotinamide (1/10 ⁷)
0	+++	+++
10	+++	+++
20	+	+
30	216	200
40	57	87
50	43	44
60	40	28

Where colonies were too numerous to count the results were recorded:

+ Uncountable number of discrete colonies.
+++ Homogeneous confluent growth.

Table 1 shows the result of an experiment to determine the thermal death times for *Bact. coli* in defined medium and in defined medium with added glutamic acid (1/1000) and nicotinamide (1/10⁷) at 52°. No significant difference between the thermal death times in the two media was found.

The rates of growth of *Bact. coli* strain no. 18 in defined medium with various additions were examined, and are summarized in Table 2. A slight increase was noticeable in the rate of multiplication at 37° where glutamic acid and nicotinamide were both present, but not with nicotinamide alone, where there was slight retardation at both temperatures.

To determine whether substances capable of sustaining growth at 44° were liberated into the medium during growth at 37°, defined medium (50 ml.) was inoculated with *Bact. coli* strain no. 18 and incubated for 24 hr. at 37°. The culture was then filtered through a Seitz EK filter, boiled briefly and the

Table 2. *Viable counts of Bact. coli strain no. 18 after different periods of incubation (at 37° or 44°) in four defined media*

(Media: A=basal defined medium alone;
 B=basal defined medium+glutamic acid (1/1000);
 C=basal defined medium+nicotinamide (1/10⁷);
 D=basal defined medium+glutamic acid (1/1000)+nicotinamide (1/10⁷).

Medium Period of incubation (hr.)	Incubated at 37°				Incubated at 44°			
	A	B	C	D	A	B	C	D
	Plate counts (colonies/loopful)							
0	34	34	34	34	34	34	34	34
2	61	42	38	55	24	31	36	42
4	120	81	80	193	23	23	9	58
6	+	+	193	++	30	18	8	180
8	++	++	+	+++	26	17	11	+
10	+++	+++	++	+++	22	14	8	+++
12	+++	+++	+++	+++	13	8	0	+++

Where colonies were too numerous to count the results were recorded:

- + Uncountable number of discrete colonies.
 ++ Confluent growth with individual colonies discernible.
 +++ Homogeneous confluent growth.

pH adjusted to 7.5. Halving dilutions of this filtrate plus glutamic acid (1/1000) or nicotinamide (1/10⁷) were inoculated with *Bact. coli* no. 18 and incubated at 44°. Growth occurred only in the series to which glutamic acid had been added, indicating that material had been produced during growth at 37° which, when present with glutamic acid, allowed growth at 44°. This material may well have contained nicotinic acid or a related substance, synthesized by the *Bact. coli* grown at 37°.

Table 3. *Protective effect of nicotinamide and glutamic acid on five strains of Bact. coli grown in defined medium for 24 hr. at various temperatures*

(Media: A=defined medium alone;
 B=defined medium+1/1000 glutamic acid;
 C=defined medium+1/1000 glutamic acid+1/10⁷ nicotinamide.)

Growth temp (° C.)	Strain of <i>Bact. coli</i>														
	No. 2			No. 7			No. 12			No. 13			No. 18		
	Medium														
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	Relative degree of growth														
37	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
41	C	T	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
43	C	T	TT	C	TT	TT	C	TT	TT	C	TT	TT	C	T	TT
44	C	C	TT	C	TT	TT	C	TT	TT	C	T	TT	C	C	TT
45	C	C	C	C	C	T	C	C	TT	C	C	C	C	C	T

Naked eye appearance of culture recorded: C, clear; T, slight turbidity; TT, marked to full turbidity.

Nicotinamide and glutamic acid are both required for growth at 44°, but at 42–43°, although still unable to grow in the basal defined medium, *Bact. coli* strain no. 18 grew when only glutamic acid was added to the medium. Table 3 shows the effects of various temperatures on five strains of *Bact. coli* in the different media. Although the temperature at which a given additional nutrient is required differs slightly for the different strains, no organism grew at 44° without some addition to the medium. The addition of glutamic acid increased the temperature at which a given strain could grow, whilst the further addition of nicotinamide increased still further the tolerated temperature. Of the strains of *Bact. coli* tested, all were able to grow in the basal defined medium at 37° and none grew in that medium at 44°; but these were able to grow at 44° after the addition of glutamic acid and nicotinamide. The characters of the strains of *Bact. coli* tested are summarized in Table 4.

Table 4. *The characters shown in the five strains tested*

Strain no.	Fermentation of		Growth on Koser's medium	Indole production	Voges- Proskauer reaction	Motility
	Dulcitol	Saccharose				
2	AG	—	—	+	—	—
7	AG	—	—	+	—	—
12	—	AG	+	—	+	—
13	A	—	—	—	—	+
18	—	AG	—	+	—	+

A = acid; AG = acid and gas.

DISCUSSION

The mechanism of this protective action is probably not directly physical; if it were it would be accompanied, at higher temperatures, by an increase in the thermal death time of the organisms tested under the same test conditions. Table 2 shows that death of a culture was not sudden, although often complete within 24 hr. It may be suggested that death was the result of an inability of the organisms in the inoculum to multiply, the organisms dying slowly as they usually do under conditions unfavourable to normal division. The slightly greater rate of growth at 37°, when both glutamic acid and nicotinamide were present, was probably due to a nutrient being present in a more completely prepared form, thus requiring less synthetic activity on the part of the organism. This slight stimulating effect by itself appears inadequate to explain the phenomenon of growth at 44°. For the production of essential metabolites required by the organism from the inorganic salts, ammonia and glucose of the defined medium, numerous series of enzymic syntheses are required. The breaking of any step in the biosynthesis of the essential metabolite nicotinamide would prevent growth. It seems, therefore, possible that at 44° an enzyme responsible for one stage in the biosynthesis of nicotinamide is inactivated and, in consequence, the organism becomes dependent upon an external supply of nicotinamide or nicotinic acid.

Glutamic acid could be regarded as playing a similar role in other biosyntheses. Table 3 shows that glutamic acid was required in general at a lower

temperature than nicotinamide, but that the actual temperature at which it became an essential nutrient was a function of the particular strain, as it was with nicotinamide.

Certain practical aspects of these findings may be considered as they effect the investigations usually employed to distinguish faecal strains of *Bact. coli*, which are described as able to grow at 44°, from non-faecal strains which cannot grow at this temperature. As ability to grow at a specified temperature may be a function not only of the strain of organism, but also of the constituents of the medium in which it is grown, it is evident that a medium deficient in nicotinamide might lead to the identification of a faecal *Bact. coli* as a non-faecal type. Although glutamic acid and nicotinamide produce a protective effect against an otherwise adverse temperature, it cannot be asserted that these substances are the only ones which are responsible for the effect with complex preparations such as Lab-Lemco.

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