DECOMPOSITION OF CARBOHYDRATES AND ALCOHOLS WITH PRODUCTION OF GAS AT 46°C. BY MEMBERS OF THE GENUS ESCHERICHIA

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Although it has been known for many years that *Escherichia coli* grows rapidly and luxuriantly at a temperature well above that of the body, only a few workers (Leiter, 1929; Williams, Weaver, and Scherago, 1933; Perry and Hajna, 1933 and 1935; Hajna and Perry, 1935) have utilized higher temperatures either in the isolation of this organism or in the study of its fermentation of various carbohydrates or alcohols. As far as the author is aware, only glucose (Eijkman, 1904; Perry and Hajna, 1933, Hajna and Perry, 1935; Skinner and Brown, 1934), mannitol (Bulir, 1907; Minkewitsch, 1929; Skinner and Brown, 1934), and lactose (Levine, Epstein and Vaughn, 1934) have been utilized in the investigation of the ability of *Escherichia coli* to ferment at, or around, the temperature of 46°C.

The following investigation was therefore undertaken to determine whether the members of the genus *Escherichia* can decompose carbohydrates and alcohols other than those referred to, at this temperature.

METHODS AND MATERIALS

Cultures. Cultures isolated from human feces, were grouped on the basis of (1) fermentation of sucrose, sorbitol, dulcitol, adonitol, and salicin at 37°C.; and (2) the degree of roughness and smoothness as indicated by their colony characteristics on eosin methylene-blue agar plates. (See table 1.)

Inoculation of cultures. Inoculations were made at first by a

loop from 24-hour broth cultures and subsequently (as a check method) by a straight needle.

Temperature and duration of incubation. Duplicate tubes of the media were inoculated at room temperature. One series of tubes was incubated at 46°C. while the other series was incubated at 37°C. Wire test-tube racks were used to permit free circulation of air about the tubes with the result that the temperature

TABLE 1

Cultural characteristics of strains

Total strains 129

REACTIONS AND TESTS	NUMBER OF STRAINS USED											
	18	15	2	17	3	3	6	4	7	11	25	18
	A	B	C	D	E	F	G	H	I	J	K	L
Sucrose	–	–	–	–	–	–	–	AG	AG	AG	AG	AG
Sorbitol	–	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Dulcitol	–	–	AG	AG	–	–	–	–	–	AG	AG	AG
Adonitol	AG	AG	–	–	–	–	–	–	–	–	–	–
Salicin	–	–	–	AG	AG	AG	–	–	AG	–	AG	AG
Type of colony on E.M.B. plate	S	S	S	S	S	R	S	S	S	S	S	R

Sugars: – = no reaction in 72 hours; AG = acid and gas, at 37°C.

Plate: S = smooth phase; R = rough phase.

All of the 129 strains ferment glucose, levulose, d-mannose, d-galactose, xylose, rhamnose, lactose, maltose, trehalose, and d-mannitol with the production of acid and gas; do not utilize citrate as source of carbon; produce indol; do not hydrolyze hippurate;* are methyl-red positive; do not produce acetylmethylcarbinol.

Composition of carbohydrate broth: Meat-extract, 0.3 per cent; peptone, 1 per cent; NaCl, 0.5 per cent; carbohydrate, 0.5 per cent; brom thymol blue indicator (1.6 per cent alcohol solution), 0.4 cc. per 100 cc. of medium.

* Synthetic medium of Hajna and Damon, Amer. Jour. Hyg., 1934, **19**, 545.

of the medium in the tubes reached 46°C. in approximately an hour.

Gas production in the media was noted at 24, 48 and 72 hours. The final readings as given in the tables are those made at 72 hours.

Type of incubator used. A Castle precision incubator as described in a previous paper (Perry and Hajna, 1933) was used

throughout the investigation. The thermoregulator was of the capsule type. The temperature fluctuation from 46°C. was at most ±0.5°.

A tube temperature of 46°C. was selected for use in this study basing on the results obtained by Perry and Hajna[1] in the recent (unpublished) study of influence of various factors in the Eijkman test. The tube temperatures, used in that study, ranged from 43° to 49°C. It was found that, in a favorable medium, bacteria of the genus *Escherichia* (human origin) outgrow other members of the colon group. *Citrobacter* organisms, *Aerobacter cloacae*, and *Aerobacter aerogenes* (of water origin) failed to produce gas from glucose at 46°C. whereas all of the organisms of the *Escheri-*

TABLE 2

Composition of various basic media

INGREDIENTS	MEDIUM I	MEDIUM II	MEDIUM III	MEDIUM IV†	MEDIUM V	MEDIUM VI	MEDIUM VII	MEDIUM VIII
	grams per liter	*grams per liter*	*grams per liter*	*grams per liter*	*grams per liter*	*grams per liter*	*grams per liter*	*grams per liter*
Peptone (Bacto).....	10	10	10	15	10	10	10	10
NaCl.....	5	5	5	5	5	5	5	5
Beef extract (Bacto).....	—	3	3	—	—	—	—	—
K_2HPO_4.....	—	—	4	4	—	4	—	4
KH_2PO_4.....	—	—	1.5	1.5	—	1.5	—	1.5
Sugar-freed beef infusion*.	—	—	—	—	1 liter	1 liter	—	—
Beef infusion (not sugar-freed).....	—	—	—	—	—	—	1 liter	1 liter

* After the method of Skinner and Brown (1934).

† Basic medium of Perry and Hajna (1933 and 1935).

Note: Final pH of all media 7.0.

chia group and a few of the *Aerobacter aerogenes* (of human origin) produced gas.

Basic media used. It is well known that the amount and type of protein and the buffering qualities of the media employed influence the amounts of acid and gas produced from carbohydrates. Various brands of peptone (Bacto-peptone, Bacto-neopeptone, Bacto-proteose peptone, and Bacto-tryptone) were tested by Perry and Hajna for use in the Eijkman test. Bacto-

[1] Unpublished work, 1936, "Further Studies on the Eijkman Test."

peptone was eventually selected for use in this study as consistent results were obtained by the use of this brand of peptone.

The concentration of buffer, as stated in table 2, was likewise determined beforehand.

Eight media varying in regard to these factors were, therefore, used. (See table 2.) No acidity was observed in any of the sterilized media except that containing fructose.

EXPERIMENTAL RESULTS

All of the strains described grew abundantly in all eight basic media containing the fifteen carbohydrates and alcohols, at both 37° and 46°C. Fermentation was decided solely on the basis of gas production. (See table 3.)

MONOSACCHARIDES

Xylose and arabinose. Xylose and arabinose were easily decomposed with evolution of a moderate amount of gas in all of the nutrient media at 46°C. However, a larger volume of gas was evolved in media with buffers and in non-sugar-free beef infusion. Observations at 37°C. were identical with those at 46°C. with both of these sugars and in all media.

Rhamnose. Although rhamnose was decomposed, the amount of gas was small both at 37° and at 46°C. in all media.

Glucose, mannose and fructose. A considerable amount of gas was produced by all strains from d-glucose, d-mannose and fructose and did not vary widely in any of the media employed. More gas was, however, produced in buffered media than in unbuffered, and in beef infusion not sugar-free than in sugar-free beef infusion.

Galactose. D-galactose was decomposed with difficulty at 46°C. if the medium contained beef extract and no buffers or if the medium contained beef infusion free of muscle sugar. In buffered peptone broth, the reactions were identical with those of d-mannose at 46°C.

DISACCHARIDES

Trehalose. Trehalose is generally thought to be more resistant to hydrolysis than sucrose. Contrary to expectation, trehalose

TABLE 3

Production of gas from carbohydrates and alcohols at 37° and 46°C. by members of the genus Escherichia

CARBOHYDRATE OR ALCOHOL	MEDIUM	CULTURES																					
		A		B		C		D		E		F		G		H		I		J		K		L	
		37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°	37°	46°
Sucrose	All	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	−	+	−	+	−
Maltose	I	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
	II, V	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v
	Rest of media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	I, II, III	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v
	Rest of media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	All	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
Adonitol	All	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sorbitol	I, II	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
	Rest of media	−	−	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v	+	v
*Rest of carbohydrates	All	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = gas produced; − = gas not produced; v = variable gas production.

* All of the strains fermented arabinose, xylose, rhamnose, glucose, mannose, fructose, lactose, galactose, and mannitol with the production of gas both at 37° and 46°C.

was readily decomposed in buffered media by the bacteria of the genus *Escherichia* at 46°C. In unbuffered beef extract broth or in sugar-free beef infusion, it was slowly decomposed. From 30 to 70 per cent of gas was produced in buffered media and in beef infusion medium not sugar-free.

Sucrose. The sucrose-fermenting strains decomposed sucrose readily at 37°C. in all the basic media. However, at 46°C. only one group (H) fermented sucrose. Gas occurred in all media both at 37° and 46°C.

Maltose and lactose. Maltose and lactose offer contrasting pictures. At 46°C. all strains failed to produce gas from maltose in plain peptone broth; they were able to attack this sugar slightly when beef extract or sugar-free infusion was added to the peptone and salt (media II and V). A small amount of gas was formed when buffers were added to a peptone-beef-extract medium or when beef infusion (not sugar-free) was added to peptone broth (media III and VII). In the three remaining media (IV, VI and VIII), maltose was decomposed slowly but certainly with the evolution of a large amount of gas in 72 hours. These media were well-buffered.

Lactose was fairly well decomposed with the production of gas in 24 hours in a medium containing only peptone and sodium chloride. The addition of beef extract or sugar-free beef infusion did not result in a material increase in gas production from this sugar but with the addition of buffers, more gas was evolved. The same reaction was observed in beef infusion not freed of muscle sugar without added buffers.

At the temperature of 37°C., both carbohydrates were readily decomposed.

ALCOHOLS

Although d-mannitol, d-sorbitol, and d-dulcitol are stereoisomers, d-mannitol, the alcohol of d-mannose, is more readily decomposed than d-sorbitol, the alcohol of d-glucose. The alcohols offer, in the order given, increasing difficulties to microbic decomposition with gas production.

Mannitol. In all of the media employed, d-mannitol was

regularly attacked by all the strains studied. Maximum gas production occurred, however, in buffered media. The average production of gas in 24 hours was 50 to 90 per cent in the inner tube, both at 37° and 46°C.

Sorbitol. Variable results were obtained with d-sorbitol at 46°C. The sorbitol-fermenting types produced more gas from sorbitol in buffered media.

Dulcitol and adonitol. In no instance was gas production from dulcitol or adonitol obtained at 46°C., although at 37°C. the dulcitol-fermenters and the adonitol-fermenters were able to produce gas respectively from dulcitol and adonitol in all of the media without difficulty. These two alcohols, however, were best decomposed with gas in buffered media.

INFLUENCE OF PROTEIN COMPOSITION ON GAS PRODUCTION

Although Eijkman (1904) used plain peptone broth plus glucose in his work, in which he distinguished the coli-form strains of warm-blooded animal origin from those of cold-blooded animal origin, this broth is the poorest culture fluid medium for production of gas from carbohydrates by bacteria of the genus *Escherichia* at 46°C.

Perry and Hajna and de Graaf (1928) have demonstrated that different results might be obtained when various brands of peptone are utilized in the Eijkman test. This observation has to be borne in mind when interpreting results and formulating conclusions as to the value of the temperature of 46°C. in the isolation and study of bacteria of the genus *Escherichia.* In this study, Bacto-peptone was used throughout.

The presence of meat extract in media seems to interfere with the production of gas although growth was heavy at both 37° and 46°C.

In beef-infusion broth, free of muscle sugar and with the carbohydrates under investigation added, many of the *Escherichia* strains produced only a small amount of gas. With the addition of buffers to the infusion broth, gas production was equivalent to that in media made from unfermented broth. The addition of buffers to the unfermented broth, however, made it superior

to that of the buffered sugar free infusion broth. This observation is in agreement with that of Brown (1921). It is well known that beef infusion broths vary greatly in their carbohydrate content and other growth stimulating factors, and those broths that are freed from muscle sugar are comparatively less nutritive than untreated broths.

BUFFERING EFFECTS

Isolation of cultures from unbuffered media was almost impossible in contrast to the ease of isolation from the buffered media even after 72 hours of continuous incubation at 46°C. The presence of buffer in the medium aids in holding the concentration of hydrogen-ions below the toxic limit of acidity, thus permitting a larger amount of sugar or alcohol to be decomposed with a larger amount of gas evolved. This confirms another observation made by Brown in regard to the value of buffers in media.

SUMMARY

A study has been made of the action of bacteria of the genus *Escherichia* on seven monosaccharides, four disaccharides, and four alcohols at 37° and 46°C. Eight different basic media were used in order to observe the effect of buffers, peptone, meat infusion, meat extract on gas production. In all, 129 strains were studied.

Buffers were found to enhance gas production at both 37° and 46°C. In a well-buffered basic medium, all the sugars and alcohols tested, except dulcitol and adonitol which were never fermented with gas at 46°C., were readily decomposed at both temperatures. In an adequately buffered medium, all the bacteria used remained viable after an incubation period of 72 hours.

Gas production is likewise influenced greatly by the type of protein in the medium. Meat extract was found to restrain gas production. Gas production tended to be less in meat infusion freed of muscle sugar.

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