THE PRODUCTION OF CO₂ BY THE TYPHOID BACILLUS AND THE MECHANISM OF THE RUSSELL DOUBLE SUGAR TUBE

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In the course of some work on the nature of the changes in the Russell double sugar medium during the growth of the typhoid bacillus, a fact of considerable theoretical interest was observed, namely, that the typhoid bacillus produces CO₂ in appreciable amounts. This fact has apparently escaped previous observation, but seems definite in spite of the absence of any visible evidence of gas.

The explanation of the appearance of the double sugar tube as given in the original description and repeated elsewhere, is as follows:¹

"The entire point of the medium rests upon the difference in changes produced by the growth of the typhoid bacillus under aerobic and under the imperfect anaerobic conditions found in the butt of the tube, where the bacillus obtains its oxygen by breaking down the glucose with the liberation of considerable acid; on the surface, however, in the presence of free oxygen, no acid is formed."

In this explanation no place is found for the fact that the typhoid bacillus ferments glucose with acid end-products under both aerobic and anaerobic conditions. It occurred to the writer that the glucose might be fermented in the slant as well as in the butt and that the small amount of acid formed from the 0.1% glucose might volatilize and leave the medium apparently unaffected. In order to test this hypothesis several experiments were made with results which support this explanation as one important factor in the mechanism of the tube.

When a double sugar tube inoculated with the typhoid bacillus (Rawlings), is coupled up² in an anaerobic system of pyrogalllic acid and KOH, the appearance of the slant is the same as usual. In other words, in an atmosphere free of oxygen, no acid is apparently produced, as should be the case according to the original explanation. If a volatile acid is assumed, however, the neutral slant can be readily explained by absorption of the acid by the KOH.

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¹ Russell, F. F.: Jour. Med. Research, 1911, 25, p. 227.

² Nichols, H. J., and Schmitter, F.: Ibid., 1906, 15, p. 113.

In an inoculated double sugar tube covered with oil, the slant becomes strongly acid. This result, in the light of our knowledge of oil seals,³ can be explained by the retention of acid better than by the exclusion of oxygen.

In an inoculated double sugar tube sealed by heat or paraffin the slant becomes acid. This result also can be explained better by assuming a retention of a volatile acid rather than an exhaustion of oxygen.

When an inoculated double sugar tube is coupled up in a tight system with an uninoculated tube, the slant of the second tube becomes acid as well as the first; in other words, some volatile acid passes over to the uninoculated tube.

Finally a set of glucose agar Andrade tubes of various strengths from 0.01% to 1% by tenths, inoculated with the typhoid bacillus, gives these results:

TABLE 1
RESULTS OF INOCULATION OF GLUCOSE ANDRADE TUBES WITH THE TYPHOID BACILLUS

Slant Acid			Slant Acid	
			24 Hours	48 Hours
			(+—)	(—)
0.01	—	0.2	+	—
0.02	—	0.3	+	—
0.03	—	0.4	+	—
0.04	—	0.5	+	—
0.05	—	0.6	+	—
0.06	—	0.7	+	—
0.07	—	0.8	+	—
0.08	—	0.9	+	—
0.09	—	1.0	+	—
0.10	—			

These results indicate that the typhoid bacillus ferments glucose in the slant under aerobic conditions. The acid products of fermentation are evident above 0.3% and were undoubtedly present in small amounts in the lower percentages, but leave no evidence of fermentation on account of their escape and neutralization. This latter additional factor is indicated by the appearance of the 0.2% glucose tube which shows some acid on the slant in 24 hours, but none in 48 hours. It is common knowledge among users of the double sugar tube that a distinct alkaline reversion occurs after several days. It has recently been shown by Ayers and Ruff⁴ that simultaneous acid and alkaline fermentations occur with members of the colon-aerogenes group.

It is seen that the volatile acid, of which evidence has been presented, acts very much like CO₂. In fact, the appearance of a litmus double

³ Gates, F. L., and Olitsky, P. H.: Jour. Exper. Med., 1921, 33, p. 51.

⁴ Jour. Infect. Dis., 1918, 23, p. 188.

sugar tube, under the varying conditions given above, can be exactly duplicated by saturating sterile medium with CO_2 . But the typhoid bacillus does not produce CO_2 according to any authority. The most complete work on the acid products of the growth of the typhoid bacillus in glucose is that of Harden⁵ who found about 40% of lactic acid, 17% of acetic acid, 17.5% of formic acid, a trace of succinic acid and 10% alcohol. "He points out that with forms like *B. typhosus*, formic acid must be produced from the fractions of the molecule which with *B. coli* yield CO_2 and H_2 ."⁶ As acetic and formic acids are volatile, it was at first thought that they might be responsible for the appearance of the tube. About this time, however, the writer became acquainted with the work of Ayers, now in press, on the production of CO_2 by streptococci. Ayers used the little known but practical Eldredge tube,⁷ and showed that CO_2 is produced not only from sugars but also from proteins.

TABLE 2
RESULTS OF EXPERIMENTS WITH ELDREDGE TUBE

	Gm. of CO_2
Control, uninoculated, 1% glucose extract broth.....	0.0
Uninoculated broth + 1 c c formic acid.....	0.0066
Uninoculated broth + 1 c c acetic acid.....	0.0035
Uninoculated broth + 1 c c sodium bicarbonate.....	0.0158
Plain extract broth (average of 2 tests).....	0.011
Glucose extract broth (average of 5 tests).....	0.0178
1% Witte peptone water.....	0.0019
1% peptone + 1% glucose.....	0.0056
0.25% beef extract.....	0.0082
Sugar free broth.....	0.0044
Sugar free broth + peptone.....	0.0059
Sugar free broth + peptone + glucose.....	0.0127

THE PRODUCTION OF CO_2 BY THE TYPHOID BACILLUS

Some experiments with the Eldredge tube yielded the following significant results. (In all the experiments 50 c c of medium and the Rawlings typhoid strain were used, 25 c c of 0.1 N $\text{Ba}(\text{OH})_2$ were placed in the absorbing tube. After 24 hours' growth, this was titrated with 0.1 N HCl to a loss of color with phenolphthalein and then to a pink color with methyl orange. The difference between 25 and the first figure [for example 17.4], such as 7.6 gives a result in terms of 0.1 N, H_2CO_3 . This figure was in turn multiplied by 0.0022 to give gm., of CO_2).

This table shows that CO_2 is produced by the typhoid bacillus both from glucose and from proteins. It also shows that the decomposition of formic acid is not the source of much of the CO_2 . The relative

⁵ Jour. Chem. Soc., 1901, 79, n. 610.

⁶ Winslow, Kligler and Rothberg: Jour. Bacteriol., 1919, 4, p. 429.

⁷ Eldredge, E. E., and Rogers, L. A.: Centralbl. f. Bakteriologie, 1914, 40, p. 5.

amount of CO₂ to other acids is of importance and incidentally has a direct bearing on the explanation of the double sugar tube. In terms of 0.1 N acid and alkali, the ratio was found to be 7.6 to 9.5 or about 45% of the total acid produced is CO₂.

CO₂ is soluble in about 60% its own volume in water at 760 mm. of Hg and 35 C. and the fallacy of the ordinary CO₂ determination has been pointed out by Keyes⁸ and Clark.⁹ It is evidently necessary to use more exact methods such as those of Harden, Keyes, Rogers, Clark and Davis,¹⁰ and Eldredge and Rogers. Just why the CO₂ was missed in Harden's work on the typhoid bacillus, and by other observers, the writer is unable to say. Nor can he discuss the physical chemistry of CO₂. But if the Eldredge is reliable, as it seems to be, there is no doubt about the production of CO₂ in important amounts. Several other organisms, such as paratyphoid A and B, the dysentery bacilli and *B. subtilis*, affect the double sugar mediums in somewhat the same way as the typhoid bacillus. All these organisms yield considerable amounts of CO₂ in the Eldredge tube from dextrose broth.

The explanation of the double sugar tube is largely an academic question and does not affect the value of this medium, which with slight variations in indicators or sugar content has come into routine use in the diagnosis of organisms of this group. But, of course, the most truthful explanation will link up best with other observations and will be least confusing to students.

SUMMARY

CO₂ is produced by the typhoid bacillus in significant amounts both from sugars and from proteins.

The appearance of the Russell double sugar tube during the growth of the typhoid bacillus is not due to direct oxygen requirements. It is due (a) to the retention of CO₂ in the butt of the tube and its escape from the slant and (b) to alkaline reversion of other acids.

⁸ Jour. Med. Res., 1909, 21, p. 69.

⁹ Science, 1913, 38, p. 669.

¹⁰ Jour. Infect. Dis., 1914, 14, p. 411.