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Carbohydrate fermentation by bacteria as influenced by the composition of the medium.

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The value of the fermentation test among the methods at our disposal for identification of bacteria is generally accepted. And yet, every bacteriologist must have encountered in his experience a number of instances of apparently inexplicable inconsistencies in the results obtained by this test. Not only does it often happen that a given strain producing a large amount of acid or gas will occasionally produce very little, but at times indeed it produces none at all. In fact, the amount of gas produced by a bacterium at different times varies so widely, that at present it is suggested by some bacteriologists that the amount of gas produced by a given culture has no diagnostic significance. This point of view owes its existence merely to the fact that the amount of gas produced by a given culture has no diagnostic significance. This point of view owes its existence merely to the fact that the amount of gas produced by bacteria depends on too many factors to attempt to control them. In our work we came across inconsistencies in the amount of acid and gas production, but discovered that these inconsistencies were very often due to variations in the composition of the media. The study is indeed not finished, but even in its present stage it is quite convincing. Omitting the details of the experiment, which will be published in full later, we shall state here merely the general plan and the results obtained.

The experiment consisted in growing a strain of *B. coli*, which, in the original culture, produced very little acid or gas, upon a medium consisting of peptone-phosphate-lactose-water with the addition of an indicator permitting direct reading of hydrogen ion concentration developed in the growing culture.[1] The composition of this medium was varied in every possible direction.

[1] This indicator, consisting of China blue and rosolic acid, was described by us in the September issue of the *Journal of Medical Research.*

Thus, in all, there were 294 combinations, including variable amounts of peptone, lactose and phosphate from zero to and slightly above that used in ordinary media. Both the hydrogen ion concentration and the amount of gas produced were recorded daily for three weeks, thus giving us 294 curves.[1]

The analysis of these curves brought out the following facts:

Increase in the concentration of lactose generally increased the rate of acid production (given constant concentration of peptone and buffer-salts).

Thus, when the amount of buffer-salts is not sufficient, the increase in concentration of the carbohydrate inhibits the growth.

With any concentration of carbohydrate, the amount of free acid indicated depends of course on the concentration of the buffer-salts.

Concentration of peptone affects the amount of free acid indicated partly in the same manner as does the concentration of neutral salts, but in addition the amount of sugar attacked is smaller as the concentration of peptone in the medium is increased. Thus, when 1 per cent. or more of peptone is used in the medium, the amount of carbohydrate has to be increased in proportion in order to obtain sufficient amount of free acid (amount of neutral salts being constant).

The amount of gas produced changes inversely with variations in the hydrogen ion concentration, other conditions being equal.

Therefore, other conditions being equal, the amount of gas produced increases directly with concentration of buffer.

Given constant concentration of carbohydrate and buffer salts, the amount of gas produced changes directly with the concentration of peptone.

These findings suggest that the number of discrepancies in results of the fermentation experiments are largely due to the fact that media are not usually compared with sufficient attention to factors above mentioned. The variations in sugar content and especially in buffer content of the media are not guarded against sufficiently in routine procedure of making media. Moreover,

[1] While in this paper we refer only to the part of the experiment which dealt with the production of acid and gas, the complete records will cover also the data on nitrogen and carbohydrate metabolism, including the gas analysis and the curves of multiplication.

it has been the custom to have the same concentration of the different ingredients in carbohydrate media. The usefulness of media could be greatly increased if the composition of each medium were more closely adapted to the purpose in hand. Thus, for isolating bacteria on a plate by the process of excluding all the lactose-fermenting bacteria, one should have the initial reaction as nearly neutral as possible; one should incorporate as high an amount of lactose as consistant with the concentration of peptone in the medium; one should buffer the medium only enough to prevent excessive penetration of the acid into the surrounding agar, but not so much as to delay the appearance of free acid within and immediately around the colony. On the other hand, when preparing a liquid medium in which one desires to follow gas formation in addition to that of acid, one should be careful to have a fair excess of buffer. Even a small amount of free acid produced can be easily distinguished in a comparatively thick layer of a tube of liquid culture; thus the excess of buffer will not interfere materially in diagnosing the acid production, but it will greatly improve the growth by keeping down the hydrogen ion concentration and will facilitate the production of gas. Again, if one desires to follow the growth of bacteria through a longer period of time in order to determine the character of later stages of metabolism (late alkalinity for instance) one should correlate the respective amounts of carbohydrate and peptone. If one desires to determine the power of reduction, one must use a different indicator from that which ought to be used if the free acidity alone is to be determined, for in that case one should select an indicator which is not easily affected by the reduction.[1]

[1] This work is a part of the investigation of food poisoning, conducted under the direction of Dr. M. J. Rosenau, professor of preventive medicine and hygiene, Harvard Medical School. The investigations are done under the auspices of the Advisory Committee on the Toxicity of Preserved Foods of the National Research Council, and under a grant of the National Canners Association.