## DIFFERENTIA OF B. COLI\*

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N the 1917 edition of the American Public Health Association's "Standard Methods of Water Analysis" it is recommended that the B. Coli group be considered as including all non-spore forming bacteria which ferment lactose with gas formation and grow aerobically on standard solid media.

It is the purpose of this paper to consider briefly the two sub-groups of the B. Coli group which are of the most importance to the water bacteriologist, namely, B. Coli Communis and B. Lactis Aerogenes.

In 1893 Theobald Smith contributed his well-known paper, "The Fermentation Tube With Special Reference to Anaerobiosis and Gas Production Among Bacteria," in which he described the fermentation tube originated by him. In this paper he gave the results of his studies of gas production by certain bacteria, and suggested that the B. Coli group might be divided into two distinct sub-groups by reason of the action of the organisms upon saccharose.

## Varied Conclusions

In the years intervening between the presentation of that paper and the present, we find a number of investigators reporting work done on gas formation and gas ratios. In the light of our present knowledge of the subject we must attribute their varied conclusions to the method and degree of accuracy used in their work.

In 1914-1915 Rogers, Clark, and Davis and Rogers, Clark and Evans found that the accurate determination of gas volumes and gas ratios produced in the anaerobic fermentation of glucose furnished most valuable data.

The work of Rogers, Clark, and Lubs, 1917, on "The Characteristics of Bacteria of the Colon Type Occurring in Human Feces" agrees with these former conclusions, and gives a new working basis for the study of the B. Coli group.

Later in the same year, Rogers, in his paper "The Occurrence of Different Types of the Colon-Aerogenes Group in Water," draws the conclusion that there are two types of the Colon-Aerogenes group which occur in fecal matter in large numbers.

By determining the ratio of the carbon dioxide gas to the hydrogen gas produced in anaerobic fermentation of glucose by the B. Coli group we can divide this group into two distinct sub-groups. The one, having a ratio of about unity, is designated B. Coli Communis, or low ratio sub-group; while the other, having a ratio of one and five-tenths, or higher, is designated B. Aerogenes, or high ratio sub-group.

It has been shown by these more recent investigations that the final hydrogen iron concentration of a given media correlates perfectly with the gas ratios and by the use of methyl red as an indicator we can readily make a colorimetric differentiation between the low and high ratio sub-groups, (low ratio—B. Coli methyl red, positive; high ratio—B. Aerogenes methyl red, negative.)

## Determining Nature and Source of Pollution

In routine work we deal with colonies fished from Endos media which has been streaked from lactose broth tubes after 24 or 48 hours incubation at 37 degrees C. growth on Agar slants made from these colonies is inoculated into the methyl red media and back into lactose broth. The gelatin liquefaction test may also be made to eliminate cloacae. (The standard methods of water analysis with the corrections as suggested by Hasseltine are followed by the

It is then generally assumed that the organisms of the methyl red positive sub-group are of fecal origin, while those of the methyl red negative may be from either fecal or other origin.

If the low ratio or B. Coli Communis. alone. is shown to be present it fixes definitely the source of the pollution.

If the high ratio or B. Lactis Aerogenes alone is shown to be present the pollution may come from either fecal or non-fecal origin.

Rogers, Clark, and Lubs. suggested means of differentiating Lactis Aerogenes of fecal and non-fecal origin. Whether or not this differentiation will prove satisfactory in routine work can only be decided after repeated trials.

In some recent work the writer collected water samples from sources exposed to varying degrees of pollution and isolated typical and atypical colon colonies on Endos media. Inoculating tubes of methyl red media with one hundred and thirty-three colonies it was found that eight atypical colonies gave the methyl red positive result, while seven typical colonies gave a negative result. This one example shows the failure of Endos media to differentiate the colon-aerogenes group. The opinion is held that many bacteriologists attribute to Endos media a differential action for which it was not intended and does not perform.

It is believed that wherever possible the water analyst should differentiate between B. Coli Communis and B. Lactis Aerogenes by use of the methyl red test. A sufficient number of colonies should be isolated from each sample tested to determine the relative quantity of the organisms present.

This procedure will bring us one step nearer to our ultimate aim: the determination in the laboratory of the exact nature and source of the polluting organisms with which we have to deal in order that laboratory work may supplement and substantiate the field survey and enable the executive authorities whose duty it is to eliminate the source of pollution to act with intelligence and confidence.

## Bibliography

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A bill has been introduced in the House of Commons authorizing the reclamation of Dominion lands by drainage, and also providing for a Director of Reclamation Services. It is proposed to reclaim at first about 1,200 acres. Representations have been made to the government regarding the advisability of draining Lake Manitoba.

An amendment to the Ontario Highways Act has been introduced in the legislature by the Hon. Finlay G. Macdiarmid, Minister of Public Works, which will enable the Ontario government to give greater financial assistance in the construction of suburban roads. Previously forty per cent. of the cost of construction has been paid on county roads, but the act has limited the assistance for highways within suburban areas around cities and large towns to \$4,000 a mile. This limit is now being removed, and the full forty per cent. will be paid on all suburban roads. Another amendment permits the provincial government to designate as a provincial county road, any part of a highway in a suburban area. In the past, provincial county roads ended at the suburban boundaries, but hereafter roads within such areas may be so designated.

<sup>\*</sup>Paper read before the Illinois section of the American Water Works Association.