

Influence of Bile and Bile Salts on *Aërobacter aërogenes*.*

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Horwood and Webster¹ recently asked the question: "Is *Escherichia coli* a derivative of *Aërobacter aërogenes* in the human intestine?" The question was prompted by their study of Gram-negative lactose-fermenting bacteria from the ileostomy and fecal discharges of a patient. They found that 87.5% of the cultures from the ileostomy discharges were *Aërobacter aërogenes*, while those from the rectal discharges were all *Escherichia coli*.

The possibility that all members of the coliform group² of bacteria may be variants of only a few species of the group had been suggested to the writer by his experience with a group of 259 strains of coliform bacteria isolated differently from specimens of water, fish fillets, and feces. These strains had been carefully purified by approved methods. They were carried for 3 years on nutrient-agar slants, being transferred once each month to fresh media. Once each 6 months differential tests were made, the tests employed being the Voges-Proskauer, methyl-red, sodium-citrate, uric-acid, indol, and sodium-malonate. It was noted that many of the strains classified as *Escherichia coli* and as *Aërobacter aërogenes* remained constant in their reactions; but there was a pronounced tendency on the part of many strains, especially the intermediates, to vary in their reactions over the 3-year period.

The experience of Horwood and Webster, following as it did upon the author's experience, prompted the study reported here. The study was based upon the following assumption: If the intestinal environment is capable of inducing marked alterations in the biochemical behavior of *Aërobacter aërogenes*, it seemed that bile and its constituents would be likely factors in inducing these alterations. Therefore, it was the purpose of this study to determine the influence of prolonged propagation of *Aërobacter aërogenes* in media containing bile and bile salts, on the biochemical reactions of the organism.

Thirty strains were chosen for the study. They had been isolated

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¹ Horwood, M. P., and Webster, R. A., *J. Bact.*, 1937, **33**, 21.

² Jordan, H. E., *J. Am. Water Works Assn.*, 1937, **29**, 1999.

from water, carefully purified, and carried for 3 years on nutrient-agar slants. Transfers to fresh medium had been made monthly. The identification had been made on the basis of the Voges-Proskauer, methyl-red, sodium-citrate, and uric-acid tests. In addition, all of the strains were indol-negative, and were positive to the sodium-malonate tests. These strains were chosen because of the constancy of their reactions to the tests mentioned, for the period elapsed since their isolation.

The medium employed was prepared as follows:

Bacto peptone	10 g
Bacto lactose	10 g
K ₂ HPO ₄	10 g
Water	1000 ml

To this formula bile, or a bile salt, was added in the desired quantity. Bacto oxgall was employed for the bile, and sodium glycocholate and sodium taurocholate (both Pfanstiehl) were the bile salts used. The media were tubed in 10 ml quantities and sterilized at 15 lb pressure for 15 minutes.

Two series of cultures were set up, one series containing 1% and the other 5%, of the bile and the bile salts respectively. All cultures were made in duplicate, and were incubated continuously at 37°C until transferred. Cultures were transferred once each week to fresh media, and at the end of 5 months they were transferred to solid media of the same formulæ, with 1.5% agar added, to facilitate their inoculation into the differential media. This treatment was decided upon because it would have been difficult to make transfers from the fluid media without also transferring traces of the bile or the bile salts to the differential test media, and thereby possibly influencing the reactions in some of the tests.

The differential tests were those previously mentioned. In addition, lactose-fermentation, the reaction on Endo's medium, and the morphology of the organisms were rechecked. These tests were run several times during the 5 months' duration, and at the end of the experiment. All tests were carried out as recommended in the Standard Methods of Water Analysis (8th ed.), except the sodium-malonate test which is not included in Standard Methods. For this test Liefson's³ technic was employed.

No variations were observed in any of the physiological reactions employed during the course of the experiments. The morphology of some strains was somewhat altered, the organisms appearing as long curved filaments. After transfer to ordinary nutrient-agar

³ Liefson, E., *J. Bact.*, 1933, **26**, 329.

slants the rods regained their normal shape and size after 2 or 3 transfers at the most.

At the time of the weekly transfers it appeared that the sliminess of the culture media was much increased, particularly after the experiment had been under way for several weeks. Sliminess was especially marked in the bile and the sodium-glycocholate cultures. At the end of the experiment transfers were made into tubes of sterile milk and of lactose broth. It was found that most of the cultures gave markedly slimy (ropy) growth in both media, while only 3 of the strains were able originally to give this type of growth. However, when the strains were transferred back from the bile and the bile-salt media to nutrient-agar slants, the tendency to produce slimy growth in milk and in lactose broth was lost in all but 3 cultures originally giving that type of growth.

While the primary objective of the experiment was not to study the effect of the concentrations of bile employed on the rate of growth, it seemed desirable to determine that the growth was not interfered with to such an extent as to invalidate the results obtained. The extent of growth was checked by transfers from week-old cultures in ox-bile and bile salts into lactose broth. Serial dilutions were employed as in setting up water samples. This was repeated several times during the course of the study. The growth of all strains was vigorous enough to indicate that the bile or bile salts did not inhibit their rate of multiplication materially, if at all.

The quantity of bile employed was based on the statement of Coles and Levine⁴ that a 2% concentration of bile accelerated growth of "colon bacilli", while 5% inhibited growth in an acid medium and was a stimulant in an alkaline medium. There appears to be no reference in the literature to the influence of bile on *Aërobacter aërogenes*, but since the organism usually will grow well under the same conditions as will *Escherichia coli*, the statement of Coles and Levine was used as a guide in setting up the experiments. It is known, also, that *Aërobacter aërogenes* grows well in the brilliant-green lactose-bile broth medium (Standard Methods of Water Analysis, 8th ed., 1936), which contains 2% bile.

Sufficient dipotassium phosphate was added to the media to maintain the pH definitely on the alkaline side. This was done because of the statement of Coles and Levine that 5% bile inhibited growth of the "colon bacillus" in an acid medium, and was stimulating in an alkaline medium, and in view of the fact that the recommended

⁴ Coles, H., and Levine, M., *Proc. Iowa Acad. Sci.*, 1927, **24**, 92.

reaction for the brilliant green lactose bile medium is between pH 7.1 and 7.4.

It cannot be stated definitely that the characteristics of the strains employed in this study might not have been changed if the experiment had been carried on for a considerably longer time, or if greater concentrations of bile had been employed. However, in spite of these factors, it seems that the results of the experiments here reported should argue in favor of the stability of the culture reactions of pure established cultures of *Aërobacter aërogenes*, so far as the influence of bile or bile salts alone is concerned.

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Radioactive Iodine as an Indicator in the Study of Thyroid Physiology.*

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The known facts of thyroid physiology indicate that iodine is selectively taken up by the thyroid gland, and that in some measure that gland's function is regulated by its iodine content. Artificial radioactivity may be induced in a variety of elements by means of neutron bombardment. It seemed that the possibility of using "tagged" (radioactive) iodine as a physiologic indicator was one which demanded investigation.

Ethyl iodide (600-1000 cc) was irradiated in a paraffin-surrounded bottle by immersing in it a neutron source consisting of 110 mg of radium mixed with beryllium in a sealed tube. The radioactive iodine thus obtained was concentrated by a method which has been described elsewhere.¹ This method gave a precipitate of radioactive silver iodide, which was dissolved in a solution of 0.5-1.0 g of sodium thiosulphate, and then diluted to 10-15 cc for intravenous injection. In a series of 48 rabbits, no toxic effects from the acute administration of such quantities were experienced. Aliquot portions of the solution of radioactive iodine used for injection were withheld for measurement of radioactivity.

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¹ Roberts and Irvine, *Phys. Rev.*, 1938, **53**, 609.