NOTES ON BACT. COLI AND BACT. AEROGENES

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Accurate information on the relative incidence of Bact. coli and Bact. aërogenes in nature would oid materially in the interpretation of the colon test in water analysis. Professor Levine suggests the lines along which selective media, for the isolation of these organisms, may be devised.

IN studies on the distribution of *B. coli* and *B. aërogenes,* the author has given preference to the plate method of isolation. This, also, seems to be the view of most other investigators who have concerned themselves with similar studies. In water analysis, on the other hand, the plate method of direct isolation is inconvenient and practically impossible when dealing with large samples (10 to 100 cc.), and preliminary enrichment, therefore, is resorted to. What happens in the preliminary enrichment tube as to the relative abundance of *B. coli* and *B. aërogenes,* is not definitely known as far as the author is aware. Overgrowths of one or the other of these organisms in the preliminary enrichment tube make it extremely difficult, if not impossible, to correlate the water results with the findings that have been reported (with the plate method) on the distribution of *B. coli* and *B. aërogenes* in nature.

The author has felt for some time that before much light will be thrown on the true relative incidence of *B. coli* and *B. aërogenes* in water and in feces, etc., it will be necessary first, so to modify our preliminary enrichment media, or other conditions, as to enable the investigator to isolate or suppress either *B. coli* or *B. aërogenes* at will.

With this in mind, studies were begun to determine the influence of various factors such as dyes, bile-salts, concentration of peptone, etc., on the rate of multiplication of *B. coli.* In general, it was found—

(1) That *B. coli* would not grow in ½ percent peptone with crystal violent in a dilution of 1-200,000, or brilliant green in a dilution of 1-1,000,000.

(2) That bile-salts stimulated the growth of *B. coli* when the concentration was less than 0.5 percent, but showed a marked inhibitory action if the concentration were raised to 0.7 or 1.0 percent. It was intended to continue this work with *B. aërogenes,* but the outbreak of the war interfered with the plans. The following factors are now being studied as to their influence on the growth of *B. coli* and *B. aërogenes.*

1. Temperature.
2. Boric acid.
3. Crystal violet.
4. Brilliant green.

Temperature.—That *B. coli* and *B. aërogenes* have different optimum growth temperatures may be inferred from the literature. Rogers and his associates have often mentioned the necessity for using a relatively low temperature (30° C.) for growth of some strains of *B. aërogenes* isolated from grains. Similarly Rettger reports that in studying the distribution of the colon group in unpolluted soils a temperature of 30° C.

was desirable for isolation of the *B. aërogenes* types.

As to *B. coli,* a temperature of 40° C. has often been recommended for its isolation and in the Eijkman test 46° C. is employed for the isolation of the organism from water. In fact, it has been observed that the maximum rate of multiplication of *B. coli* is at about 45° C.

The author observed that in peptone lactose media at 43° C. (in a water bath) all the cultures of *B. coli* (16.) grew luxuriantly as evidenced by strong turbidity in 24 hours, but 69 percent showed no gas or only a bubble in 24 hours. Of 20 cultures of *B. aërogenes,* on the other hand, 16 showed no growth, 2 slight, and 2 grew luxuriantly.

Boric Acid.—In agar of the following composition: peptone 1.0 percent, agar 1.5 percent, dipotassium phosphate 0.3 percent, and glucose .05 percent with 0.63 percent of boric acid, *B. aërogenes* failed to grow, whereas *B. coli* grew luxuriantly. In liquid media, 1.0 percent peptone with 0.63 percent boric acid, *B. coli* multiplied slowly, while *B. aërogenes* died off as evidenced by the following figures:

Culture 19b, *B. coli* increased from 65,000 per c.c. to 1,500,000 per c.c. in 48 hours, while *B. aërogenes* was reduced from 2,300 per cc. to 20 in 24 hours and to 0 in 48 hours. It was found in subsequent studies, however, that the difference in concentration of boric acid, which did not inhibit *B. coli* and which did inhibit *B. aërogenes,* was so close that it could not be safely employed as a selective agent.

Crystal Violet.—One percent peptone water containing ½ percent lactose and varying concentrations of crystal violet were inoculated from 48-hour peptone cultures of *B. coli* and *B. aërogenes.* Five different strains of each species were employed. A concentration of 1-100,000 of crystal violet prevented the growth of all the cultures of *B. coli,* whereas all of the *B. aërogenes* grew heavily. One culture of *B. coli* failed to grow in a dilution of 1-250,000 of crystal violet.

Decreasing the concentration of peptone to ½ percent increased markedly the inhibitory action of the dye. Thus in ½ percent peptone lactose solution none of the *B. aërogenes* grew with a dye concentration of 1-100,000, but all grew luxuriantly in 1-250,000 crystal violet. Among the *B. coli* cultures, all were inhibited in 1-250,000 dilution of the dye and two failed to grow in a dilution of 1-500,000.

Brilliant Green.—Some time ago, the author was informed that growths of *B. coli* are rarely encountered in the isolation of *B. typhosus* from stools by the use of eosine brilliant green agar, and that if a growth other than *B. typhosus* was present, it was very likely to be *B. aërogenes.* This suggested that the inhibitory action of brilliant green was much greater for *B. coli* than for *B. aërogenes.*

In a medium consisting of 1.0 percent peptone and 0.5 percent lactose with various concentrations of brilliant green, four cultures of *B. aërogenes* grew very luxuriantly in a concentration of 1-750,000, whereas one failed to grow in this concentration, but grew very well in 1-1,000,000 dilution of the dye. The 5 cultures of *B. coli,* on the other hand, all failed to grow in 1-750,000 of the dye, 3 did not grow in a dilution of 1-1,000,000 and 2 failed to grow even in a dilution of 1-1,500,000.

Reducing the concentration of peptone to ½ percent increased very markedly the antiseptic action of brilliant green. The five *B. coli* cultures now failed to grow even in a dilution of 1-3,000,000. The *B. aërogenes* cultures grew luxuriantly in a dilution of 1-2,000,000. Four grew in a dilution of 1-1,500,000, but only 1 grew in more concentrated solutions of the dye.

The selective action of brilliant green was even more strikingly shown by the use of a plate medium consisting of the simplified eosine methylene blue agar

with various concentrations of the brilliant green. Four cultures of *B. aërogenes* and five of *B. coli* were employed with the following results:

With a dilution of 1-100,000 of brilliant green, none of the *B. coli* grew at all. All of the *B. aërogenes* grew, but the colonies were only half as large as the controls indicating a marked inhibition. With 1-200,000 dilution, *B. coli* still failed to grow whereas *B. aërogenes* grew reasonably well, but not as luxuriantly as the controls. With 1-300,000 of the dye, three of the *B. coli* still failed to show any evidence of growth and two others grew very poorly. All the aërogenes showed a very heavy growth. With 1-400,000 brilliant green, the growths of *B. aërogenes* were as luxuriant as the controls, whereas 2 cultures of *B. coli* failed to grow and the three others showed very small, stunted non-characteristic colonies.

In conclusion, it may be said that these preliminary studies indicate that the concentration of peptone exerts a marked influence on the inhibitory action of dyes in culture media, and that it appears feasible to devise both liquid and solid media which will inhibit *B. coli,* but not *B. aërogenes.* The most promising inhibitory agent which we have as yet encountered for this purpose is brilliant green.

MAINTAINING STANDARDS OF ANIMAL FOODS

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I will take advantage of this opportunity to present one or two matters, which to me are fundamental to the application to every-day life, of the results of scientific nutritional studies. Such data have little value if they cannot be successfully and practically used in rational feeding, whether for man or other animals.

We are all interested in foods, some in drugs, but at all events we are all interested in the maintenance of high standards of purity of foods and drugs. It is to the maintenance of such standards with special reference to the stock feeds that I wish to devote a few moments. The food supply of man is, thanks to the efforts of the national and state bureaus of food and drugs, quite satisfactorily safeguarded. Cattle and poultry foods, however, require more attention and will continue to do so until the subject is as well covered as that of human foods.

At present our best authority for standards for cattle and poultry feeds are the definitions adopted by the Association of Feed Control Officials of the United States. These were originally adopted nine years ago, but changes or modifications have been made at every succeeding annual convention.

We should encourage and welcome any and every addition, change, or modification which would tend to raise standards; but should, on the other hand, deprecate in no unmeasured terms modifications of any definitions which would directly or indirectly allow the marketing of a lower grade product than that called for by the original definition. Let me offer two examples illustrating both phases of the subject. The present definition for alfalfa meal reads:

"Alfalfa meal is the entire alfalfa hay ground, and does not contain an admix-