INFLUENCE OF GLYCERIN IN DIFFERENTIATING CERTAIN BACTERIA.*

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In 1895, when working in the Hygienic Laboratory of the Marine-Hospital Service, the writer was engaged in making a study of the bacteria isolated from the water supply of Washington. In the course of this investigation some experiments were made with acid fuchsin as an agent for differentiating the intestinal forms. The results obtained with this agent were published in the same year.[1] In the course of this study it was observed that the addition of a certain amount of glycerin to the nutrient media increased the acid-producing power of a number of these intestinal forms. The amount of acid or change in the index of either acidity or alkalinity, was minutely shown when a small amount of acid fuchsin solution neutralized by caustic potash was added. An aqueous solution of acid fuchsin (Grübler's) loses its color by the addition of an alkali, and recovers it on adding an acid.

The solution of this salt found best suited for the indicator was prepared in the following manner: A solution of acid fuchsin, one-half of one per cent in strength, was made in distilled water and titrated with normal caustic potash solution until only a very slight pinkish color remained, that is to say, until it was exactly neutral. It was found that .0001 of a gram of caustic potash unites with .005 of a gram of acid fuchsin. This salt is an extremely sensitive indicator for acids; as little as .00003 of a gram will respond to .001 of a gram of pure hydrochloric acid.

The addition of solutions of this salt in a proportion of one-half cubic centimeter to ten cubic centimeters of nutrient media does not appear to influence the growth of the bacteria in any way whatsoever, it matters not the kind or character

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of the media. There were no differences observed in the growth of bacteria on media containing this solution and the growth on similar media not containing it.

After numerous trials it was found that the solution most sensitive was one containing one part of the neutral salt to twenty-five thousand parts of the media, and for those rendered slightly alkaline one part of the salt to thirty-three thousand parts of the media. In preparing the neutral fuchsin care must be taken in exactly titrating it to the neutral point. When this point is reached the solution will have a slightly pinkish color. After standing for several hours the pinkish color disappears and it becomes slightly yellow. Solutions of the fuchsin salt were added to the several media, such as are ordinarily employed in the laboratory. Cultures of Bacillus acidi lactici, Bacillus lactis aerogenes, Bacillus coli communis, developed a considerable amount of acid, the acid curd beginning within a few hours. The acid rapidly increased in amount until about the end of twenty-four hours, when it began to disappear, and after forty-eight hours the cultures were distinctly alkaline.

Proteus vulgaris did not develop an acid, but on the other hand began to produce an alkaline reaction soon after inoculation. This was beautifully demonstrated in a medium containing the solution of fuchsin not exactly neutralized. Bacillus typhosus developed acid, but the acid production was observed later than in any of the above mentioned bacteria. Acid commenced to show after twelve hours and continued to show for seven to ten days, sometimes even longer, then the acid began to disappear gradually, and usually the medium was alkaline after the twelfth day.

The results obtained with the usual nutrient media containing the fuchsin salt were not altogether constant, as occasionally it would happen that media prepared exactly as others would only show a partial reaction. This was found to be due to the breaking up of the sugar-like bodies in the media by sterilization. Recourse was then had to Dunham's peptone solution to which was added glycerin in definite proportions. After a few trials it was shown that the amount

of glycerin best suited for acid production lay between five and six per cent. Glycerin is essential for the development of acid in Dunham's solution. This medium gave constant results.

Solutions of the fuchsin salt can be added to any of the nutrient media except milk. It does not cause a cloudiness or a precipitate in any of these, but it is contraindicated in milk because the amount of alkali present in the indicator will prevent coagulation. Another advantage of this salt is that it is not easily decomposed by heat. It is unchanged even when heated for a considerable time to 100° C. or over, as for example in fractional sterilization or in the autoclave.

The best method of employing this agent as a differential test is to inoculate tubes containing Dunham's peptone glycerin solution and the fuchsin salt with a loop of a fresh twenty-four hours' old agar culture. The optimum temperature for the acid or alkali development is about 37° C. It will take place at ordinary temperature, but requires longer to complete. Observations should be made at stated intervals for a period of several weeks if it is desired to have full data regarding the reaction.

Since publishing my results in 1895 this method has been used almost constantly in differentiating the intestinal bacteria found in water supplies. During the past year an opportunity afforded itself to apply this test to a number of other bacteria not included in my previous paper. The results obtained in this study are believed to further demonstrate the utility and value of the method. The following organisms were studied and the approximate time and amount of the acid or alkali production is shown in the table:

DYSENTERY GROUP.

	Days.							Weeks.			
	1	2	3	4	5	6	7	1	2	3	4
B. dysenteriæ, Shiga	o	o	o	o	x	xx	xx	xx	x	o	o
B. " Kruse	o	o	o	x	xx	xxx	xx	xx	xx	xx	xx
B. " Flexner	o	o	o	x	xx	xxx	xx	xx	xx	xx	xx
B. " New Haven	o	o	o	x	xxx	xxx	xxx	xxx	xxx	xxx	xxx
B. "Y," Hiss and Russell	o	o	o	o	o	o	o	a	a	a	a

HOG CHOLERA GROUP.

B. choleræ suis	o	o	o	o	o	o	o	o	a	a	a
B. icteroides, Sanarelli	o	o	x	x	xx	xx	xx	xx	xx	xx	xx

PARACOLON GROUP.

B. paracolon, Strong	o	o	o	o	o	o	o	a	a	a	a
B. " Kurth	o	o	o	o	o	o	o	o	a	a	a
B. " Badash	o	o	o	x	xx	xx	xx	xx	xx	xx	xx
B. " Gwynn	o	o	x	xx	xx	xx	xxx	xxx	xxx	xxx	xxx
B. " Muller	o	o	o	x	x	xx	xx	xx	xxx	xxx	xxx
B. " Buxton	o	o	o	o	x	x	xx	xxx	xxx	xxx	xxx
B. " Cushing	o	o	o	o	x	x	x	x	x	x	x

o: negative; x: slight acid production; xx: considerable acid production; xxx: large acid production; a: alkali production.

By reference to the above table it will be seen that the acid-producing properties of this group of bacteria present marked differences, both with regard to the time that the change of reaction occurs and the amount of acid produced. The amount of color set free varies with the amount of acid produced, so it permits one to follow the reaction very closely.

Certain differences are noted in regard to the dysentery group. Bacillus dysenteriæ Shiga produces acid, the change of reaction occurring from the fifth to the eighth day. In some cultures the amount of acid is slight and disappears after two or three weeks. The old laboratory cultures of this

organism produce but very little acid; freshly isolated cultures, however, produce more. The dysentery bacilli of Flexner, New Haven, and Kruse produce variable amounts of acid, the one of New Haven origin producing a greater amount than the others. The change of reaction begins from the fourth to the sixth day until the maximum is reached. Bacillus "Y" of Hiss and Russell does not produce acid. After ten days or two weeks the medium becomes alkaline, assuming a yellow color. The paracolon group shows considerable variation. Paracolon Kurth and Paracolon Strong do not produce acid at any stage of their growth, and behave like Bacillus "Y" of Hiss and Russell, producing alkali after ten days or two weeks. Of the acid producers the Paracolon of Gwynn produces the largest amount, and that of Cushing the least. The others vary considerably. The Paracolon of Gwynn commences to produce acid between the third and the fourth day and all others somewhat later. Of the Hog-Cholera group, Bacillus choleræ suis does not produce any acid and acts very much like Bacillus "Y." Bacillus icteroides Sanarelli shows a marked acid reaction beginning on the fourth and fifth days. The Hog Cholera Bacillus, the Bacillus "Y" of Hiss and Russell, the Paracolon of Strong, and the Paracolon of Kurth do not act upon the glycerin and seem to form a group apart. Bacillus coli communis and Bacillus typhosus were tested along with the above groups, using cultures from several sources, the results exactly coinciding with those of the writer's experiments of 1895.

The media containing the fuchsin salt differ from most of the other media in the composition of which anilin colors form a part. The acid fuchsin is used only as an indicator of the change in reaction brought about by the action of certain bacilli on glycerin. The chemistry of the change is probably the following: The lactic acid (?) produced by the decomposition of the glycerin combines with the colorless rosanilin set free from the fuchsin solution and brings about the red color. Ramond[2] has more recently suggested a modification of the fuchsin salt by decolorizing the acid fuchsin solution with sodium carbonate. Endo[3] in 1903

proposed an agar medium containing milk-sugar and an alcoholic solution of fuchsin decolorized by sodium sulphite. This he claims as particularly useful in differentiating the colon from the typhoid bacillus. The writer has compared this modification with the media prepared according to the original formula and observed no advantage. In fact the medium of Endo is quite unstable and not so sensitive as the medium here described.

REFERENCES.

1. Acid fuchsin as an agent for the differentiation of bacteria. Abstract of Sanitary Reports, x, pp. 679–682, 1895.
2. Soc. de Biologie, 1896, No. 28.
3. Centralbl. f. Bakt., etc., Abt. I., xxxv, p. 109, 1903.