HYDROGEN ION CONCENTRATION AND TITRATABLE ACIDITY IN RELATION TO BACTERIOLOGICAL MEDIA.

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It has long been recognized that the reaction of media is a very important consideration in the cultivation of bacteria. The bacteriologist is confronted with the problem of determining or measuring this reaction in two instances. First, in the adjustment of the original reaction of the media and secondly in the measurement of acid production by bacteria.

In the past two methods of measurement have been used, based on two different chemical phenomena. The older method of the two is the titration of the media with a standard acid or basic solution, using phenolphthalein as an indicator. In using this method media was almost universally made +1 to phenolphthalein, or 1% of normal acid was added after the neutrality point was reached. This method was based on a measurement of the total acid or base in the solution. The newer method is a measure of the concentration of the free hydrogen ions in the solution. This may be accomplished by either the electrolytic or the colorimetric method. The electrolytic method is the more accurate of the two, but requires more time and complicated apparatus, to which the bacteriologist rarely has access. The colorimetric method, since the introduction by Clark and Lubs of a series of phthalein indicators whose sensitive ranges have been accurately determined, is accurate enough for bacteriological work, is applicable to the solutions with which the bacteriologist is working and is quick and simple in operation.

Since it is the concentration of free or disassociated hydrogen ions and not the total amount of acid present that affects the bacterial growth, it is readily seen that a method which measures hydrogen ion concentration is preferable to one which measures titratable acidity. Also it should be clearly understood that a determination of the titratable acidity gives no indication of the hydrogen ion concentration. A single example using two common acids will illustrate this point. Normal acetic acid has ten times the titratable acidity of tenth normal hydrochloric acid, yet tenth normal hydrochloric acid has about 22.4 times the hydrogen ion concentration of normal acetic acid.

Another very serious source of error in the determination of the reaction of media by the old method of titrating with a standard solution lies in the buffer effect of various ingredients of the media. By the buffer effect of a substance we mean its ability to combine with an acid or base in the unionized condition. Peptone, mainly due to the proteoses and phosphates which it contains, has a marked buffer effect. Thus the addition of an acid or a base to a peptone solution may change the hydrogen ion concentration but very slightly. Also the extent of the buffer effect of a peptone solution is dependent upon the brand of the peptone and the technic followed in making up the solution. As mentioned above, the buffer effect of peptone is largely due to its content of proteoses and phosphates, according to Kligler's work

the proteoses will be in a large measure precipitated out if the acidity becomes greater than Ph 5.4, while the precipitating point for the phosphates is aromnd Ph 8.8.

In an effort to determine just what the variations in the final result might be, between the use of the two methods, a number of samples of media were made up according to the American Public Health Association standards and the reaction determined by both methods. The procedure they laid down was used in making the media +1, and the colorimetric method, using the indicators and standards of Clark and Lubs, was followed in determining the hydrogen ion concentration.

From the accompanying table it may be seen that the actual amount of base necessary to make the media neutral was less than that indicated by the phenolphthalein titration in all cases except the bouillon made up with Wittes Peptone. Also the results of this experiment show that the media made up with Difco Peptone was practically neutral as made, however due to the buffer effect of the peptone the phenolphthalein titration indicated that it was necessary to add base. In the case of the agar media where two per cent peptone was used, the difference between the actual amount of base necessary to bring the media to neutrality and the amount indicated by the phenolphthalein titration is greater than in the bouillon. In the gelatin media the bouillon was neutralized before the gelatin was added and as a result there is no difference indicated between the two peptones, however, there is a great difference shown between the two methods of determining the reaction.

The results of these tests show that the method of making media +1 to phenolphthalein can not be depended upon to bring it to neutrality and that in the case of all careful work the reaction must be determined by measurement of the hydrogen ion concentration.

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A COMPARISON OF THE TWO METHODS OF ADJUSTING THE REACTION OF BACTERIOLOGICAL MEDIA

Sample No.	Media	Witte Peptone cc of N/1 Base to make		Difco Peptone cc of N/1 Base to make	
		+1	Ph 7	+1	Ph 7
1	Bouillon	1.0	0.8	3.0	0.3
2	.....	1.0	1.0	1.0	0.0
3	.....	1.0	1.0	1.0	0.0
4	.....	2.0	2.0	2.0	0.0
Ave.	.....	1.25	1.2	1.75	0.08
1	Agar.....	7.0	1.3	6.0	0.0
2	.....	5.0	1.2	6.0	0.0
3	.....	4.0	1.2	6.0	0.0
4	.....	7.0	1.3	5.0	0.0
Ave.	.....	5.75	1.25	5.75	0.0
1	Gelatin ...	13.0	5.0	15.0	5.2
2	.....	15.00	5.4	13.0	5.3
3	.....	14.0	5.3	13.0	5.0
4	.....	14.0	5.2	14.0	5.1
Ave.	.....	14.0	5.22	13.75	5.15