THE COLORIMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION AND ITS APPLICATIONS IN BACTERIOLOGY

PART III

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SECTION XIV. ELECTROMETRIC AND COLORIMETRIC COMPARISONS

As indicated in a previous section, the solutions which must be dealt with in bacteriological investigations are diverse indeed and are not subject to a classification which facilitates systematic study of the indicator errors. We have considered it wise, therefore, to omit, for the present, detailed consideration of particular media, and have studied a considerable variety of solutions upon which we have made more than 400 electrometric measurements and many more colorimetric comparisons. These comparisons between the colorimetric and electrometric measurements are given in tables 4 to 19.

In these comparisons we shall consider the hydrogen electrode measurements as the standard, since they were made with an equipment and in a manner which has proved accurate and reliable. The details of the special electrometric methods used will be found in previous papers by Clark (1915 d) and Clark and Lubs (1916). Occasionally an electrometric determinator was made upon a solution not well adapted to hydrogen electrode measurements. Those familiar with the subject will recognize such a solution in Dorset's egg medium, for instance. In one or two other cases electrometric determinations with solutions in a P_H region where their buffer effect is small have been somewhat uncertain. In general, however, the electrometric measurements may be trusted to the second decimal of P_H if it be granted that the correct order of magnitude of liquid contact potentials has been properly determined (Clark and Lubs 1916). Where

the measurements are not to be so trusted the second decimal place will be omitted.

In the colorimetric comparisons we have purposely avoided, in this preliminary survey, some of the best colorimetric methods which we might have applied, such as the use of optical instruments of correct design, and we have used the somewhat wide interval of 0.2 P_H in the standard comparison solutions between which to interpolate. In these interpolations we have estimated only to the nearest 0.1. We have, furthermore, given our attention to colored and turbid solutions rather than to clear, colorless ones. In bringing various media within the range of the several indicators NaOH or HCl has been added, which generally produces a precipitate. This we have not filtered out but have studied the solutions with color and turbidity present. In short, our procedure while carried out with care and with exactly prepared standard comparison solutions has been such as we may reasonably expect the equipment of any laboratory to permit, and upon such material as that to which we must expect it to be applied. The electrometric measurements were made by one of us, the colorimetric measurements by the other and without any exchange of information which could influence the results. Except in two or three cases where obvious mistakes were involved and in two cases in which it was realized that no accurate measurements could be made, no data have been omitted because of disagreement between the values obtained with the two methods. The brackets found in the tables enclose values found for the solutions indicated at the left. Each value represents a determination on a separate sample.

In some instances a sufficient number of determinations were made with one kind of solution to justify a summary. Such summaries are given in tables 4, 5, and 6. In these tables we mean by "average deviation" the value obtained when the electrometric value was subtracted from the colorimetric value in each case and the arithmetical average of the + and − deviations was taken. Thus, in table 4 the average deviation for phenol red, comparator method, is + 0.03, and with dilution method, − 0.01, which means that if the electrometric determi-

nation were $P_H = 7.00$, the average colorimetric value in one case would be 7.03 and in the other 6.99. Since such averages might agree with the electrometric value, although the individual positive and negative deviations were large, we have given the "mean deviation," which is the mean of all deviations neglecting their sign. A ± following this shows that the P_H values obtained colorimetrically were greater in some cases than the electrometric value and in other cases less. When only + for instance, follows, it shows that the colorimetric P_H in every instance was greater than the electrometric P_H. The maximum and minimum deviations need no explanation except as to sign which follows the order given above.

In tables 8 to 9 we have included under "Remarks" only a rough indication of the depth of color in the solutions studied. As already mentioned, NaOH or HCl was added in many instances and this produced more or less turbidity. Only in the case of extreme turbidity, however, is this mentioned in "Remarks." In order to simplify the tables further, mention of the addition of acid or alkali will be omitted. *It will, therefore, be understood that to bring the* P_H *of certain solutions within the range of the indicators studied HCl or NaOH has been added although not mentioned in the tables.*

In the columns headed "Dilution" the values given were determined after dilution of 2 cc. of the solution to 10 cc. with distilled water. In all but a few cases such determinations were made without a comparator. In the columns headed "Comparator" the values given were determined without dilution and with the aid of a comparator according to Walpole's compensation method (see Journal of Bacteriology, ii, p.118 and 136). When determinations were made with a comparator after dilution of the solution the values given are placed *between* the other columns, unless dilution is mentioned under "Remarks".

There are contained in these tables numerous points which may be emphasized to better advantage in a discussion. A few of the more important will now be considered.

All the indicators studied are reliable for approximate determinations upon a wide variety of solutions, and, if good judgment

is used, they may be depended upon for approximate determinations even when the solution studied is highly colored and turbid. For precise determinations, however, each indicator must be used with caution in particular cases and some of them can not be depended upon.

TABLE 4

Deviations of colorimetric determinations from electrometric determinations of P_H in Dunham's solutions

NUMBER OF DETERMINATIONS	INDICATOR	COLORIMETRIC METHOD	DEVIATIONS OF P_H			
			Average	Mean	Maximum	Minimum
12	Brom phenol blue	Comparator	+0.10	0.10±	+0.16	+0.01
10		Dilution	+0.09	0.11±	+0.24	−0.02
17	Methyl red	Comparator	+0.21	0.21+	+0.35	+0.02
19		Dilution	+0.27	0.27+	+0.45	+0.13
3	Propyl red	Comparator	+0.09	0.10±	+0.15	−0.02
4		Dilution	+0.10	0.11±	+0.15	−0.02
10	Brom cresol purple	Comparator	±0.00	0.04±	−0.10	0.00
4	Brom thymol blue	Comparator	+0.08	0.12±	+0.14	+0.08
5		Dilution	+0.01	0.06±	−0.10	−0.02
14	Phenol red	Comparator	+0.03	0.06±	+0.14	−0.01
8		Dilution	−0.01	0.04±	+0.10	−0.01
8	Cresol red	Comparator	+0.07	0.07+	+0.13	+0.01
10	α-napthol phthalein	Comparator	−0.06	0.06±	−0.14	+0.02
5		Dilution	−0.05	0.05±	−0.11	+0.02
9	Thymol blue	Comparator	+0.07	0.07+	+0.11	+0.05
		Dilution	−0.05	0.06±	−0.15	−0.01

Let us consider each indicator in the order of the range of P_H which it covers.

Thymol blue in its *acid range* seems to be fairly reliable according to the limited number of determinations made with it (see table 8). It is especially gratifying to observe this because hitherto the zone of P_H covered has been without a brilliant and

at the same time reliable indicator. If our few determinations with thymol blue (acid range) are a good indication of its reliability it should be of great use in the study of yeasts,

TABLE 5

Deviations of colorimetric from electrometric values in P_H determinations of beef infusion, 1 per cent peptone broths

NUMBER OF DETERMINATIONS	INDICATOR	METHOD	DEVIATIONS			
			Average	Mean	Maximum	Minimum
7	Brom phenol blue	Comparator	−0.05	0.16±	−0.38	−0.01
6	Methyl red	Comparator	+0.10	0.11±	+0.28	0.00
4		Dilution	+0.08	0.08+	+0.18	0.00
4	Propyl red	Comparator	+0.08	0.08+	+0.18	0.00
2		Dilution	±0.00	0.00	0.00	0.00
10	Brom cresol purple	Comparator	−0.01	0.04±	±0.07	0.00
5		Dilution	−0.03	0.05±	−0.14	−0.01
14	Brom thymol blue	Comparator	−0.10	0.15±	−0.25	+0.03
5		Dilution	−0.10	0.12±	−0.26	+0.01
12	Phenol red	Comparator	−0.04	0.04±	±0.07	−0.01
8		Dilution	−0.06	0.06−	−0.12	−0.02
6	Cresol red	Comparator	−0.03	0.03−	−0.07	−0.01
3		Dilution	−0.06	0.06−	−0.11	−0.02
3	*a*-napthol phthalein	Comparator	−0.06	0.06−	−0.12	−0.02
5	Thymol blue	Comparator	−0.04	0.09±	+0.14	−0.01
3		Dilution	−0.01	0.03±	−0.06	−0.01
3	Phenol phthalein	Comparator	+0.03	0.07±	+0.14	−0.01
3	O-cresol phthalein	Comparator	+0.03	0.07±	+0.14	−0.01

molds, and acidophilic bacteria as well as for special purposes such as the study of the gastric contents.

Brom phenol blue has given considerable trouble because almost all the solutions tested with it have been turbid. This turbidity has introduced the error of dichromatic indicator solu-

TABLE 6

Deviations of colorimetric from electrometric determinations

NUMBER OF DETERMINATIONS	METHOD	DEVIATIONS IN P_H			
		Average	Mean	Maximum	Minimum
		Methyl red			
Turbid cultures of *B. coli* in 1 per cent Witte peptone, 0.5 per cent K_2HPO_4+1 per cent various sugars					
36	Comparator	+0.03	0.04±	+0.13	±0.01
34	Dilution	+0.08	0.08±	+0.18	±0.01
Turbid cultures of *B. coli* in 0.5 per cent Witte peptone, 0.5 per cent K_2HPO_4, 1 per cent to 3 per cent various sugars					
6	Comparator	+0.05	0.05+	+0.08	+0.01
11	Dilution	+0.10	0.10+	+0.28	+0.03

TABLE 7

Colorimetric and electrometric determination of the P_H of urines. Comparator used in all cases and screen with brom cresol purple in most cases

INDICATOR	P_H COLORIMETRIC	P_H ELECTROMETRIC
Methyl red	5.5	5.54
	5.3	5.38
	5.5	5.55
	5.3	5.33
Propyl red	5.7	5.77
	5.6	5.62
	6.0	6.01
Bromcre sol purple	6.4	6.39
	5.9	5.88
	6.5	6.67
	6.1	6.01
	6.0	6.04
	6.4	6.36
	5.7	5.62
	5.7	5.77
	5.5	5.54
Brom thymol blue	6.8	6.80
	6.5	6.67
	6.5	6.43
	6.4	6.38
Phenol red	6.8	6.80

tions which has been discussed in a previous section. Table 9, showing determinations with brom phenol blue does not, unfortunately, show any determinations made with the screened electric light. Some of these determinations are included in the summarized results of tables 4 and 5. A few measurements made with the mercury arc are shown in table 10.

TABLE 8

Comparison of P_H determinations made with thymol blue (acid range) and with hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent K_2HPO_4	Moderate color	2.4 2.0	2.4	2.37 1.97
10 per cent gelatine, 0.3 per cent Liebig's extract	Moderate color		1.9	1.77
0.2 per cent $NH_4H_2PO_4$, 0.02 per cent KCl, 0.02 per cent $MgSO_4$, 5 per cent sucrose, *Aspergillus* culture	Clear		1.8	1.76
0.1 per cent $(NH_4)_2HPO_4$, 0.02 per cent KCl, 0.01 per cent $MgSO_4$, 5 per cent sucrose, *Aspergillus* culture	Clear		1.9	1.86
Whey	Very turbid		2.7 1.9	2.56 1.64
"White vinegar"	Clear		2.3	2.36

Methyl red has given some puzzling results. Table 6 shows that it may be depended upon in the study of colon cultures on the media indicated and in the range of $P_H = 4.5 - 5.5$. On the other hand, methyl red has given more or less consistently high P_H values for various Dunham's solutions (table 4) and in many other cases (table 11). Superficially this appears to be more of a so-called "salt error" than a "protein error." Some of this inaccuracy with methyl red is doubtless due to the turbidity of media acidified to such a range of P_H.

TABLE 9

Comparison of P_H determinations made with brom phenol blue and with the hydrogen electrode. (Determinations made in daylight)

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
0.2 per cent $NH_4H_2PO_4$, 0.02 per cent KCl, 0.02 per cent $MgSO_4$, 5 per cent sucrose	Clear		4.3	4.3
1 per cent peptone, 1 per cent dried yeast	Moderate color, turbid	4.0 3.9 3.8		4.13 3.69 3.68
1 per cent peptone, 1 per cent dried yeast, 1 per cent K_2HPO_4	Moderate color, turbid	4.2		4.14
1 per cent peptone, 0.3 per cent Liebig's extract	Moderate color, turbid	4.1 3.9		3.95 3.41
Beef infusion, 1 per cent peptone, 0.5 per cent K_2HPO_4	Moderate color, turbid	4.3 4.1 3.8		4.59 4.13 3.77
10 per cent gelatine, 0.3 per cent Liebig's extract	Moderate color, turbid	3.9		3.71
1 per cent peptone, 0.5 per cent K_2HPO_4 (culture + HCl)	Very turbid		4.1 4.1	4.55
Cow feces extract (unfiltered)	Extremely colored and turbid		4.3	4.0
Silage juice	Green and very turbid.		4.0 4.0	3.91 3.82
2 per cent egg white, pepsin digest	Turbid		3.9	3.59
Cider vinegar	Moderate color, not turbid		3.1	3.21
Apple juice	Moderate color, not turbid		3.8	3.76
Prune juice	Very dark, not turbid		4.2	4.12

Propyl red on the whole has given somewhat better results than methyl red in spite of the fact that it is chemically so similar and in spite of the fact that propyl red is comparatively so much less soluble that it precipitates on standing in the comparison solutions. We have used propyl red chiefly to cover a narrow range of P_H between those zones covered by methyl red and brom thymol blue, a zone about $P_H = 6.0$. Recently we have discovered that this same zone may be covered much more satisfactorily by the next indicator we shall discuss.

TABLE 10

Comparison of P_H determinations made with brom phenol blue and the hydrogen electrode. (Determinations in mercury are light)

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.3 per cent Liebig's extract	Moderate color, turbid	4.3	4.4	4.45
1 per cent peptone, 1 per cent dried yeast	Moderate color, turbid	4.0	4.0	3.98
		3.8		3.72
		3.7		3.65
		3.9		3.80
		3.9		3.84
Liver infusion, 1 per cent peptone	Extremely dark, and turbid	3.7	3.8	3.70
1 per cent peptone, 1 per cent dried yeast, 1 per cent glucose, culture	Very turbid	4.0	4.0	3.98

Brom cresol purple exhibits the dichromatism discussed in a previous section and may produce the same confusion which has rendered brom phenol blue so hard to deal with. This indicator on the other hand lends itself beautifully to determinations made in the screened electric light. So used, its indications in Dunham's solutions (table 4), beef infusion media (table 5), urine (table 7) and in several other cases (table 13) are very satisfactory. This is important, for the range of P_H which may be studied with this indicator is one of great bacteriological importance. The brilliant red color of brom cresol purple in the screened

TABLE 11

Comparison of P_H determinations made with methyl red and hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent NaCl	Turbid	4.8	4.7	4.49
1 per cent yeast, 1 per cent peptone	Turbid	4.8	4.8	4.55
			5.3	5.32
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent sugar (cultures)	Very turbid	5.1	4.9	4.94
			5.2	5.18
			5.2	5.15
Beef infusion, 1 per cent peptone, 0.5 per cent K_2HPO_4	Very turbid	5.5	5.4	5.25
Veal infusion, 1 per cent peptone	Very turbid	4.9		4.59
Liver infusion, 1 per cent peptone	Very dark, 5–10 dilution	5.4	5.2	5.31
			5.3	5.31
		4.8		4.67
			4.8	
Beer wort	Very dark	5.1	4.9	4.91
Carrot juice	Moderate color	5.5	5.4	5.21
Cucumber juice			5.1	5.08
Apple juice	Dark color		5.1	5.02
String bean juice	Moderate color	5.5	5.3	5.23
Prune juice	Dark color	4.8	4.5	4.41
		5.1	4.7	4.80
		5.3	5.1	5.08
Banana juice	Extremely turbid, diluted 2–10		4.7	4.62
Whey	Very turbid, diluted 2–10		5.0	4.69
Cow feces extract (unfiltered)	Extremely turbid		4.3	4.1
2 per cent egg white partial pepsin digest	Very turbid		5.0	4.9
2 per cent egg white full pepsin digest	Turbid		4.7	4.49

light has an advantage over the blues and greens of the indicator next in order, and should, when possible, be used in preference to it. The screened light is chiefly useful in the more acid region.

TABLE 12

Comparison of P_H determinations made with propyl red and hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.5 per cent K_2HPO_4, 3 per cent various sugars (cultures)	Very turbid	6.2	6.0	6.07
		5.6	5.5	5.51
		5.9	5.8	5.81
			5.8	5.59
		6.0	5.8	5.84
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent NaCl	Moderate color	6.1	6.0	5.91
1 per cent yeast, 1 per cent peptone	Moderate color	5.3		5.32
		5.7		5.60
1 per cent yeast, 1 per cent peptone, 1 per cent K_2HPO_4	Moderate color	6.3		6.12
1 per cent yeast, 0.3 per cent Liebig's extract	Moderate color	5.9		5.80
Beef infusion, 1 per cent peptone, 0.5 per cent K_2HPO_4	Moderate color	6.2		5.97
		5.5		5.25
Beer wort	Very dark	6.1	6.0	5.92
10 per cent gelatine, 0.5 per cent Liebig's extract	Moderate color		6.0	6.04
Hay infusion	Moderate color	5.9	5.7	5.81
Irish potato juice	Black	6.1	6.2	6.06
Apple juice	Dark		5.7	5.65
Sweet potato juice	Dark	5.8	5.7	5.80

Brom thymol blue. In the conduct of this indicator, whose solutions exhibit no appreciable dichromatism, may be discerned some evidence that a blue indicator is not particularly well adapted to colorimetric determinations with colored solutions. The data in table 14 indicate that while very good determinations have been made with this indicator, the results are somewhat uncertain. This has been especially noted in the

study of beef infusion media (table 5). There is no difficulty in obtaining good approximate results with this indicator, but its color changes, especially in the greens, are deceptive and must be judged with caution if accurate data are desired. As already suggested the absorption bands of colored media themselves may

TABLE 13

Comparison of P_H determinations with brom cresol purple and hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 1 per cent yeast	Moderate color	6.7		6.73
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent K_2HPO_4	Moderate color	6.5	6.5	6.43
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent sugar	Turbid cultures		5.6	5.63
		6.4	6.5	6.58
		6.4	6.5	6.61
Cow feces extract	Unfiltered, extremely turbid		6.8	6.9
			6.8	6.7
			6.8	6.7
Hay infusion	Moderately dark		5.9	5.81
			5.9	5.99
Liver infusion, 1 per cent peptone	Very dark, 5–10 dilution, 2–10 dilution		6.2	6.19
			6.2	
Veal infusion, 1 per cent peptone	Moderate color		6.3	6.21
			5.3	5.26
10 per cent gelatine, 0.5 per cent Liebig's extract	Moderate color	6.0	6.0	6.04

cause confusion in the judgment of a blue indicator even when the compensation method is used. At present we believe that brom cresol purple and phenol red may be used in sufficiently extended ranges so as to make unnecessary any extensive use of brom thymol blue.

Phenol red, which we propose as an abbreviated name for phenol sulfon phthalein, we have found to be very reliable. In

TABLE 14

Comparison of P_H determinations made with brom thymol blue and hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent glycerine	Turbid cultures	6.7	6.6	6.74
		6.7	6.5	6.69
		6.3	6.2	6.28
		6.1	6.0	6.09
		6.5	6.3	6.53
		6.4	6.3	6.27
		6.3	6.2	6.32
1 per cent yeast	Moderate color	6.3		6.80
1 per cent yeast, 1 per cent peptone	Moderate color	6.6		6.77
			6.5	6.73
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent NaCl, 0.1 per cent K_2HPO_4	Moderate color	6.7	6.7	6.63
			6.2	6.43
			6.9	7.05
1 per cent peptone, 1 per cent yeast, 1 per cent K_2HPO_4	Moderate color	7.2		7.15
		6.9		6.91
		6.7		6.68
		6.5	6.4	6.42
		6.2		6.12
Beef infusion (double strength), 2 per cent peptone	Dark color		6.8	6.14
		6.0	6.0	6.00
			6.0	6.39
			6.3	6.62
		7.0	6.9	7.07
Liver infusion, 1 per cent peptone	Very dark, 5–10 dilution		6.7	6.89
Beer wort	Very dark	6.9	6.7	6.84
Hay infusion	Moderate color	6.9	6.9	6.97
10 per cent gelatine, 0.3 per cent Liebig's extract	Moderate color		6.0	6.04
Vermont maple sirup	Very dark and thick	6.8	6.9	6.75
Extract cow feces (unfiltered)	Extremely turbid and colored		7.1	7.1
			6.8	6.9
			6.6	6.7
			6.8	6.6
			6.6	6.7

TABLE 14—*Continued*

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
Whey	Very turbid, 2–10 dilution		6.4 6.4	6.55 6.44
2 per cent egg white (partial pepsin digest)	Turbid		6.8	7.1
Carrot juice	Moderate color	6.6	6.5	6.43
String bean juice	Moderate color	6.9	7.0	7.15
Prune juice	Dark color	6.6	6.6	6.46
Sweet potato juice	Moderate color	6.7	6.6	6.80
Beet juice	Very dark	6.2	6.2	6.07

very few instances has it given results which could be called inaccurate. The data found in tables 4, 5, 7, and 15 need no detailed discussion. This indicator is invaluable because it is useful in the region of true neutrality. Although its praise has been sung by others, we believe that the data we here present are the first which show in any extensive manner the agreement between colorimetric determinations made with this indicator and electrometric determinations.

Cresol red. What has been said of phenol red appears to hold true for cresol red, although we have not studied it so extensively. It is very important to note that cresol red extends the range which may be accurately studied colorimetrically appreciably further into the alkaline region than phenol red, and that its color changes, from clear yellow to brilliant red, are preferable to those of the previously used α-naptholphthalein whose intermediate colors are rather muddy. Data obtained with cresol red will be found in tables 4, 5, and 16.

α-naptholphthalein, which we studied before we realized the usefulness of cresol red in the same region of P_H, has proved reliable but not so satisfactory as cresol red. Several determinations with α-naptholphthalein will be found in tables 4, 5, and 7.

Thymol blue (alkaline range) is one of those indicators with which we again encounter difficulties that appear to attend the use of a yellow-blue color change. On the whole, however, thymol blue has proved more satisfactory than its brom deriva-

TABLE 15

Comparison of P_H determinations made with phenol red and hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent glucose	Moderate color	7.1	7.0	6.95
1 per cent peptone, 1 per cent K_2HPO_4, 1 per cent yeast	Moderate color	7.5		7.58
		7.2		7.15
		6.9		6.91
		7.7		7.68
1 per cent peptone, 1 per cent yeast	Moderate color	7.4		7.45
		8.1		8.24
			6.7	6.73
1 per cent peptone, 0.3 per cent Liebig's extract	Moderate color	7.1		7.18
		7.4		7.57
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent glycerine	Very turbid cultures	7.2		7.20
		7.6		7.62
		7.2	7.2	7.18
		7.3	7.3	7.22
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent sugars	Very turbid cultures		7.1	7.10
			7.7	7.77
			7.7	7.78
			7.7	7.70
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent sugars	Moderate color	7.1	7.1	7.10
			7.6	7.65
			7.7	7.67
			7.4	7.45
			7.0	7.07
			7.5	7.51
1 per cent peptone, 0.5 per cent Liebig's extract, 0.5 per cent K_2HPO_4	Moderate color	7.4		7.39
		8.3		8.30
		6.9		7.05
McConkey's bile salt medium	Moderate color	7.3	7.3	7.45
			8.4	8.43
Beef infusion, 1 per cent peptone, 0.5 per cent NaCl, 1 per cent glucose (overheated)	Deep color		7.0	6.83
			7.0	6.72

TABLE 15—Continued

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
0.3 per cent Liebig's extract, 1 per cent peptone, 0.5 per cent NaCl, 0.1 per cent K_2HPO_4	Moderate color	7.1 7.0	7.1 7.0	7.06 6.99
Beef infusion, 1 per cent peptone, 0.5 per cent K_2HPO_4	Moderate color	7.1		7.17
Liver infusion, 1 per cent peptone	Very dark, dilution 5–10		6.9 7.3 8.1	6.89 7.32 8.13
Veal infusion, 1 per cent peptone	Moderate color	7.0 7.7	7.0 7.5	6.98 7.66
10 per cent gelatine, 0.5 per cent Liebig's extract	Moderate color	7.3 7.5	7.3 7.4	7.41 7.58
Dorset's egg medium before coagulation	See text	7.2-7.6		7.4
Hay infusion	Moderate color	6.9	6.9 8.1	6.97 8.13
Beer wort	Very dark		7.8	7.79
Cow urine	Moderate color		7.4	7.4
Cow feces extract (unfiltered)	Extremely turbid		7.3	7.1
Sweet potato juice	Moderate color	7.4	7.4	7.38
Carrot juice	Moderate color	7.8	8.1	7.85
White potato juice	Black!	7.7	7.9	7.71
String bean juice	Moderate color	7.7	8.1	8.08
Prune juice	Moderate color	7.7	7.8	7.80
Beet juice	Black!	7.7		7.92
0.1 per cent $(NH_4)H_2PO_4$, 0.02 per cent KCl, 0.01 per cent $MgSO_4$, 5 per cent sucrose; after sterilization	Clear		7.05	7.08

tive, brom thymol blue, and we place as much confidence in it as we do in phenolphthalein which covers most of the same P_H zone. Determinations with thymol blue are given in tables 4, 5, and 18. We may call particular attention to a point frequently observed. When making determinations with thymol blue and

simultaneously with phenolphthalein or o-cresol phthalein, which are one-color indicators, we have found, when the solution under examination is colored, that the two-color indicator, thymol blue, is preferable. The approximate P_H, at least, may be discerned with much greater ease than when the one-color indicators are used. This has appealed to us forcibly.

TABLE 16

Comparison of P_H determinations made with cresol red and with the hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent K_2HPO_4	Moderate color	7.4	7.4	7.39
		8.3	8.3	8.30
1 per cent peptone, 0.5 per cent K_2HPO_4 (cultures)	Very turbid		7.7	7.77
		7.7	7.7	7.78
		7.7	7.6	7.70
		7.7	7.7	7.70
Veal infusion, 1 per cent peptone	Moderate color, very turbid	8.4	8.6	8.49
			7.0	6.98
			7.7	7.66
Liver infusion, 1 per cent peptone	Very dark, turbid	7.3		7.32
		8.1	8.1	8.13
Hay infusion	Moderate color	8.0	8.1	8.13
		8.2	8.3	8.40
McConkey's bile salt medium	Moderate color		7.3	7.45
		8.3	8.4	8.43
10 per cent gelatine, 0.3 per cent Liebig's extract	Moderate color	7.4	7.4	7.58
2 per cent egg white, partial tryptic digest			8.5	8.4

Ortho cresol phthalein is useful as a supplement to thymol blue. Determinations made with it will be found in tables 5 and 19.

Phenolphthalein in some dozen tests made with it gave values close to those indicated by ortho cresol phthalein but with the advantage in favor of the cresol compound. We have aban-

doned phenolphthalein almost entirely for P_H determinations and whenever there has been occasion to use it, whether in determinations of P_H or in titrations, we have preferred its homologue, the more brilliant ortho cresol phthalein.

A few particular observations deserve notice. Determinations with whey taken from a cheese vat, with banana juice, cow feces, silage juice, and Dorset's egg medium, are examples of

TABLE 17

Comparison of P_H determinations made with α-napthol phthalein and with the hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC
		Dilution P_H	Comparator P_H	P_H
1 per cent dried yeast, 1 per cent peptone	Moderate color	8.1		8.24
1 per cent dried yeast, 1 per cent peptone, 1 per cent K_2HPO_4	Moderate color	8.4		8.44
		7.5		7.48
		7.7		7.68
		7.5		7.58
		8.0		7.99
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent NaCl	Moderate color	8.1	8.2	8.08
1 per cent peptone, 0.3 per cent Liebig's extract	Moderate color	8.1		8.47
Beer wort	Very dark	7.6–7.7		7.79
String bean juice	Dark		8.0	8.08
Prune juice	Dark		8.0 8.1	8.14
			8.1 8.1	8.24
Irish potato juice	Black		8.4 8.4	8.57

solutions which have been studied which were so turbid that any colorimetric determination appeared hopeless. By the use of dilution, or a comparator, or both, and by the use of the brilliant indicators we have described, reasonably good determinations were made with these solutions. Dorset's egg medium we could only place between 7.2 and 7.6 (see table 15). Accurate electrometric determinations with this medium are extremely difficult. Two determinations with freshly platinized electrodes gave 7.38 and 7.41, and the average of five deter-

minations with old and new electrodes was 7.36. If the rounded value 7.4 is taken for the electrometric determination the colorimetric limits 7.2 to 7.6 which we found are in agreement to a surprising degree, when the dense turbidity and color of this medium is considered.

TABLE 18

Comparison of P_H determinations made with thymol blue (alkaline range) and the hydrogen electrode

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
1 per cent peptone, 1 per cent yeast, 1 per cent K_2HPO_4.....	Moderate color	8.9 8.4		8.91 8.44
1 per cent peptone, 0.3 per cent Liebig's extract, 0.5 per cent NaCl.....	Moderate color	9.4	9.5	9.60
1 per cent peptone, 0.5 per cent K_2HPO_4, 1 per cent glucose (culture).....	Extremely dark and turbid	8.5		8.54
10 per cent gelatine, 0.3 per cent Liebig's extract.....	Moderate color	8.4	8.4	8.60
Hay infusion.....	Dark		9.2	9.40
Veal infusion, 1 per cent peptone.....	Moderate color		9.2	9.24
2 per cent egg albumin, partial tryptic digest.....			8.5	8.4
Irish potato juice.....	Black		8.9 9.0 9.4 9.4	8.97 9.44
Carrot juice.....	Moderate color		8.6 8.9 8.9 9.2	8.81 9.27
Beet juice.....	Very dark		8.4 8.7 8.8	8.57 8.75
Sweet potato juice.....	Moderate color		8.7 8.8	8.75
Prune juice.....	Very dark		9.5	9.44

Much less difficult were determinations made with such solutions as oxidized potato extract, liver infusion, deep red overheated sugar bouillons and green silage juice, the coloration of which made these solutions look almost black when in bulk. In fact, we have experienced much less difficulty with color than with turbidity.

We feel certain that had we given more attention to clear and colorless solutions the colorimetric and electrometric comparisons would have shown better agreement in many cases. In other cases we might have been able to show with greater clarity consistent discrepancies such as have appeared in tests of Dunham's solutions with methyl red. We believe, however, that a systematic investigation of "protein" and "salt" errors had

TABLE 19

Comparison of P_H *determinations made with O-cresol phthalein and with hydrogen electrode*

SOLUTION	REMARKS	COLORIMETRIC		ELECTROMETRIC P_H
		Dilution P_H	Comparator P_H	
Dunham's solution	Moderate color		8.6	8.65
			9.5	9.40
1 per cent dried yeast	Moderate color		8.8	8.93
1 per cent dried yeast, 1 per cent peptone	Moderate color		8.8	8.92
			9.1	9.27
1 per cent dried yeast, 1 per cent peptone, 1 per cent K_2HPO_4	Moderate color		8.3	8.44
			8.9	8.91
Beer wort	Very dark	8.4		8.55
Prune juice	Dark		9.3	9.44
Sweet potato juice	Moderate color		8.7	8.75
Beet juice	Black	8.4		8.57
String bean juice	Moderate color		8.5	8.63
Hay infusion	Moderate color		9.2	9.4
10 per cent gelatine, 1 per cent peptone			8.4	8.60

best be left to more intensive investigation of particular solutions. Our present purpose has been to subject the indicators and the simple crude methods which we have described to as severe trials as could reasonably be imposed, and it may again be emphasized that we have *not selected* solutions which are particularly well adapted to colorimetric determinations, we have not adjusted the salt content of the solutions, we have not filtered off any precipitates which were found or produced by the

addition of acid or alkali, and we have sought only to attain such accuracy as could be attained in less time than that required for the electrometric determinations. The results in certain instances may be regarded as unsatisfactory by those who seek to make the colorimetric method precise, but in our opinion they are, with few exceptions, quite close enough for all bacteriological work which does not require the precision of electrometric determinations.

SECTION XV. ADJUSTMENT OF CULTURE MEDIA

The adjustment of the P_H of culture media is one of the most obvious applications of the colorimetric method as was suggested by one of us in a former paper (Clark (1915 e)). It has taken us some time to complete the investigations which were promised in this former paper, and, in the meantime, Hurwitz, Meyer and Ostenberg (1915, 1916) have to some extent anticipated our treatment of this particular subject.[19] Attention, however, may be called to the fact that these authors did not make comparisons between colorimetric and electrometric measurements upon which judgment of the accuracy of colorimetric measurements should at present be based.

The procedures suggested by Hurwitz, Meyer and Ostenberg, it seems to us, have been unnecessarily complicated. In the first place they appear to have avoided by all possible means dilution of the solutions in testing for P_H, not realizing how inappreciable is the change in P_H on moderate dilution of the solutions studied. They have suggested the use of Standardized

[19] Anthony and Ekroth (1916), discussing the adjustment of culture media in a recent paper, have made several statements concerning the theory involved with which we must disagree. We are not aware that the hydrogen electrode has ever been used in *conductivity* measurements of hydrogen ion concentration. Points of inflexion in titration curves certainly do not indicate the true neutral point, nor does the nearness of an indicator's "point" of change to this point of inflexion determine its suitability for a particular medium. We regret that the interesting data presented in this paper by Anthony and Ekroth are discussed in so many details from points of view with which ours are at variance, and that some of the work of this and other laboratories has been erroneously interpreted.

N/20 and N/1 alkali, which is of course necessary in adjustments to Fuller's scale; they have proposed a solution which cannot be accepted without more investigation than they record, namely the indicator mixed with the alkali solution used in titrating and they have burdened the subject with what we believe to be too elaborate and to some extent inaccurate stoichometrical relations. It may also be noted that these authors restricted their attention to adjustments which could be made with phenol sulfon phthalein (phenol red). It is undoubtedly true that the requirements of many bacteria, especially those which live in the blood stream, are met when media are adjusted to the blood reaction or to neutrality. But such a consideration has little weight with the plant pathologist or with one who must study the organisms of cheese, silage, wines, beers, soils, sewage, the acid loving yeasts and molds, the alkali enduring organisms or the metabolism of organisms under different environments. We consider it unwise therefore to confine adjustments to any particular range of P_H and we shall refrain from suggesting any "standard" P_H whatsoever, believing that this is a subject which should be left to separate researches and that the adoption of a standard in any particular line of investigation should be based upon experimental data rather than upon general considerations. We may however again emphasize the wide range of usefulness of the set of indicators we are describing.

The procedure we recommend may be illustrated as follows.

Having the colorimetric equipment described in section XII there is needed in addition a *roughly* standardized N/I NaOH solution relatively free from carbonate and a more dilute solution made by an *exact* 1–10 dilution of the first. This may be called the N/10 NaOH solution.

A standard beef infusion medium was to be given an initial reaction of P_H 7.4. After the original infusion had been properly diluted, heated and filtered, one per cent peptone was added. The mixture was kept warm for fifteen to twenty minutes with occasional stirring and then filtered. When cooled under a tap to room temperature the solution was made up to a known volume, less than the final volumes, and the reaction was adjusted

roughly after titrating 5 cc. with the N/10 NaOH solution in the presence of phenol red until it was judged that P_H 7.4 was reached. There were required for this 0.85 cc. N/10 NaOH. Hence to one litre of the solution were added 17 cc. of the N/1 NaOH solution. The medium was now ready for the *preliminary heating, at or near the final reaction.* Heating for fifteen minutes in the autoclave at 15 pounds pressure brought down a precipitate which was filtered out. The reaction as then determined colorimetrically (comparator) was somewhat more than 7.3. For most purposes this would have been sufficiently close to the value desired, but an attempt was made to adjust the reaction more precisely.

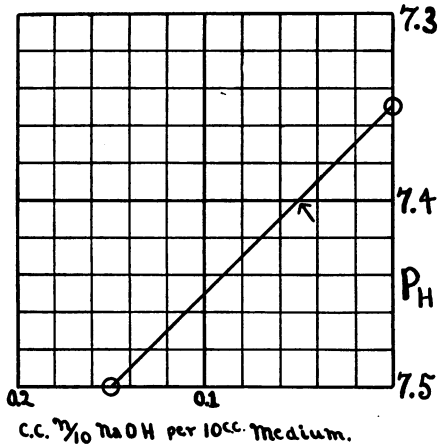

FIG. 8. PORTION OF TITRATION CURVE USED IN ADJUSTMENT OF A BEEF INFUSION MEDIUM

For a test 10 cc. were taken and readings were made with the aid of a comparator. On the addition of 0.15 cc. N/10 NaOH $P_H = 7.5$ was reached. This and the initial value, which was judged to be 7.35, were plotted as in figure 8. Interpolating, it was found that 7.4 would have been reached on the addition of 0.05 cc. N/10 NaOH to the 10 cc. of medium. Hence there were added to a litre of the solution 0.5 cc. N/1 NaOH. A colorimetric check showed that the desired reaction of 7.4 P_H had been

given to the medium. An electrometric determination however showed that the true reaction was $P_H = 7.47$.[20] The solution was now contaminated so it was resterilized after its volume had been brought to the desired final point. After this the reaction found colorimetrically was 7.3 and electrometrically 7.36.

This procedure may serve to illustrate *in outline* what we may suggest as a provisional method. It will perhaps need modification with special media, and in special cases. Hand in hand with the adjustment of the reaction of media must go a consideration of the substances precipitated by change in P_H, a consideration of the manipulation which is permissible, a consideration of the amount of reheating which is permissible, and a consideration of the change in P_H during sterilization. These constitute a few items of a very large subject which, we cannot treat in this paper in any adequate manner. Some of the diffi-

[20] These are about the maximum errors which we have observed with this indicator in beef infusions. We quote this particular example, however, to indicate that great accuracy cannot always be assured. Although the point attained before sterilization was 7.4, exactly that desired, when the colorimetric method alone was used, as in the studies of Hurwitz Meyer and Ostenberg, the error of 0.07 P_H was discovered when the electrometric measurement was considered as the true value.

It may be illuminating to those who are more familiar with "percentage titratable acidities" to note that in this particular solution this error amounts to about 0.07 per cent in "titratable acidity." In less strongly buffered solutions a like error in P_H would correspond to a much smaller error in "titratable acidity." In 0.3 per cent Liebig's extract, 1 per cent Witte peptone it would amount to about 0.03 per cent in "titratable acidity."

It cannot be emphasized too strongly that the reference point in determining "titratable acidities" of culture media is in reality but a P_H point indicated by the tint of phenol phthalein, that the particular tint (or particular P_H tacitly assumed) adopted by different workers varies, that the particular tint which is sometimes assumed is that at which phenol phthalein is least sensitive, and that without a proper standard comparison solution of known P_H value the same worker can hardly avoid errors of 0.1 P_H in establishing his *reference point* for "titratable acidity." Consequently "titratable acidities" which are apparently identical may vary much more widely when translated into an accurate titration curve than will the corresponding P_H values when determined by even crude methods. *Different* media adjusted to the same degree of "titratable acidity" may vary enormously in P_H. Since the publication of the paper "The 'Reaction' of Bacteriologic Culture Media" numerous instances have come to our notice of the errors, the inadequacy, and the confusion of the titrimetric method of adjusting media.

culties involved may be partly obviated by adjustments *after* sterilization such as Deelman used (see also Hurwitz, Meyer and Ostenberg (1916)). It may be suggested, however, that in a great many cases adjustment by the most direct and simple procedure will insure a medium more constant in composition and more constant in true reaction than that obtained by complex manipulation or adjustment on the old scale. We do urge that the most simple and direct procedures be given a trial before more elaborate ones creep into "standard methods." A reason for this will be discussed in section XVIII.

For the adjustment of the common solid media the following considerations may be offered. Gelatine media may be kept liquid at moderate temperatures and adjusted like liquid media. Pure agar has practically no buffer effect in the P_H ranges usually employed. Its addition to a medium should not appreciably affect the P_H of a solution, and consequently agar media may be adjusted before the addition of the agar. (High temperatures, over 40°C. should be avoided in using indicators since we have no data for these temperatures.) What effect will ensue when gel formation takes place in gelatine or agar media we may not venture to guess. If marked changes in *apparent* P_H as shown by indicators are observed, it may be found better by empirical tests to adjust to the *apparent* P_H desired as observed in the solidified medium. What interpretation other than a purely empirical one may then be put upon the indication we do not know. We are certain however that it will be quite as reliable if taken at face value as any "titratable degree of reaction" the determination of which involves implicitly the determination of a reference P_H point by means of an indicator.

Reduced to its lowest terms the colorimetric method of adjusting the "reaction" of culture media is a return to the older simple method of adjusting to a given tint of an indicator as with litmus, but with more reliable indicators, a wider choice of indicators, a logical scale of "reaction" and a clearer conception of the ends desired in adjustment. The method contains no sources of error not inherent in the titrimetric method and it avoids many sources of error which are inherent in the titrimetric method.

SECTION XVI. CONDUCT OF INDICATORS IN ACTIVE CULTURE MEDIA

It is well known that many compounds are reduced in the presence of active growths of bacteria. Reduction of colored compounds is generally accompanied by a color change. This has no *direct* connection with the color change of indicators due to alteration of P_H. Furthermore some indicators are not only reduced but fundamentally altered or destroyed by bacterial action. Therefore the conduct of an indicator when left exposed to bacterial action may be complex.

It is not to be denied that such complex conduct may furnish valuable information, just as the complex picture of numerous growth characteristics is significant to a trained eye. Nevertheless the use of indicators as indicators of P_H should be kept distinct from their other uses; and, when used as indicators of P_H, they should be introduced only at the time of the test unless used to indicate roughly the reaction. We emphasize this distinction because some have imagined that in the colorimetric measurements we are discussing, the indicators are made a part of the medium.

We have confirmed with cultures of *B. coli* Fred's (1912) observation with denitrifying organisms, that methyl red may be destroyed by bacterial action. Indeed this indicator should not be allowed to stand for more than a few minutes in active cultures. The sulfon phthalein indicators are of a very much more resistant nature. Some preliminary tests indicate that these indicators may be used to advantage in replacing other indicators which are now used in making indicator media.

We may emphasize particularly the promising value of brom cresol purple to replace the inconstant and often impure litmus or azolitmin widely used for "litmus milk." It imparts to milk a bluish grey-green color which changes on sterilization of the milk only to the extent that might be expected from the consequent change in P_H. As acid fermentations take place the color becomes yellow in easily distinguished gradations, and when alkali formation occurs a distinct blue color is imparted to the milk.

SECTION XVII. THE REACTION OF IMPORTANT SOLUTIONS

When attention is transferred from the analytically determined acid or alkali content or the so called titratable acid or alkali of solutions to the hydrogen ion concentration, some confusion is inevitable unless one has some idea of the position on the P_H scale of familiar solutions. In figure 9 are shown some of these relations. The figure is supplemented by tables 20 to 24 in which are given compilations of the P_H values of body fluids, fruit and plant juices, miscellaneous solutions and some optimal agglutination points and limiting reactions for bacteria. These compilations are given merely to help orient the true reactions of important and familiar solutions and for their suggestive value. No attempt has been made to cover the voluminous literature in detail or to present "weighted values."

SECTION XVIII. GENERAL DISCUSSION

It would be an injustice to the subject, if, after discussing its importance, its limitations and its advantages in bacteriology, we failed to indicate how the true field of usefulness of the colorimetric method is revealed by a consideration of certain broad aspects of the influence of hydrogen ion concentration.

Without fear of contradiction we may state that the hydrogen ion concentration influences the condition in solution of every substance with acidic or basic properties—native proteins and their products of hydrolysis, amines and amids, carboxyl, sulfonic and phenolic compounds, even alcoholic compounds, as well as very many of the inorganic compounds. Largely as a consequence of this the hydrogen ion concentration of a solution has a part in determining effective solubilities and the dispersion of colloids, in determining tautomeric equilibria, and in one way or another in governing the activity of catalists such as the hydrolytic enzymes and the oxidases. One or another of these effects, induced directly or perhaps very indirectly by the hydrogen ion concentration of a medium, must impress bacterial life.

Such a generalization, however, would be entirely misleading if not tempered by a proper appreciation of proportion. Rarely

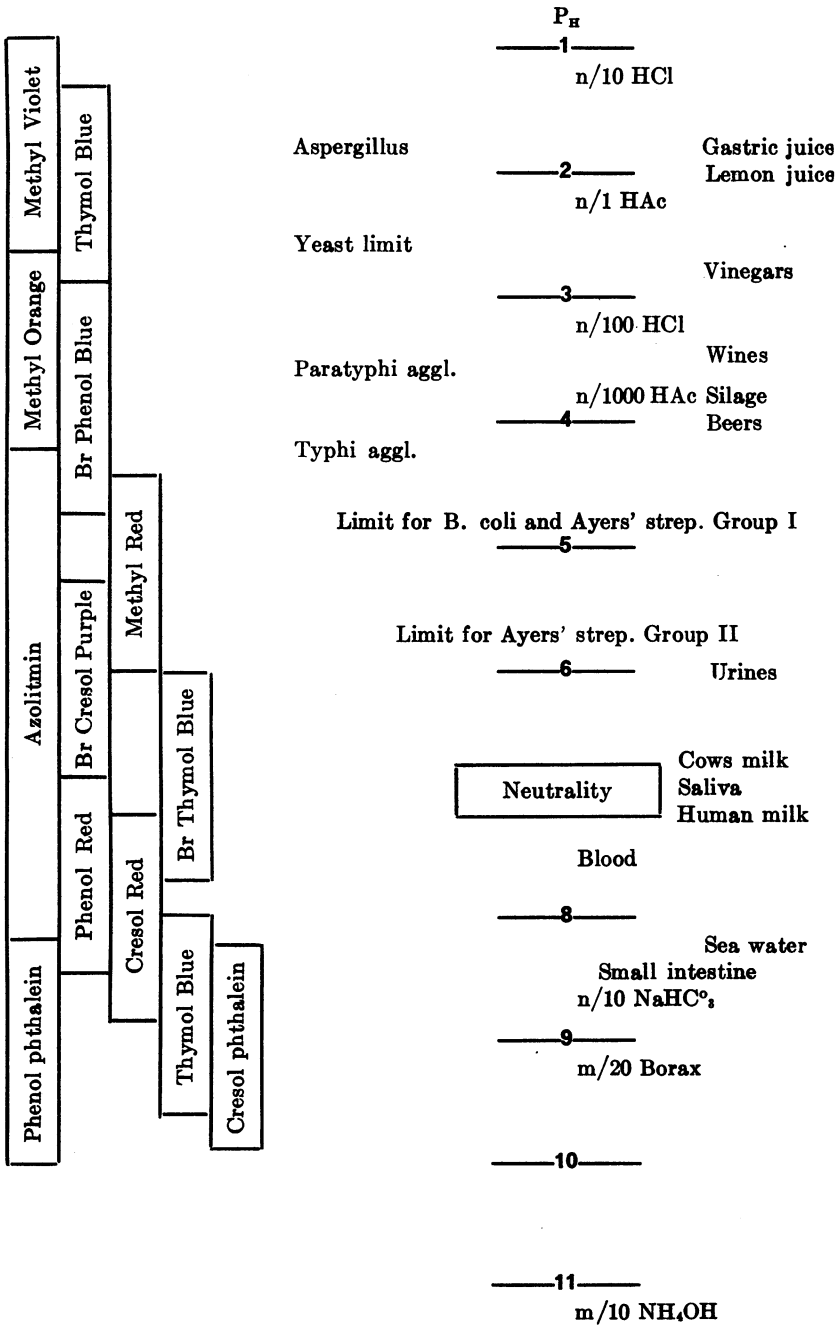

FIG. 9. THE P SCALE SHOWING THE POSITION OF IMPORTANT SOLUTIONS AND INDICATOR RANGES.

TABLE 20

True reactions of body fluids

FLUID	P_H	AUTHORITY
Blood	7.4	(See Michaelis (1914 b)
Urine	6.0[21]	(See Henderson and Palmer (1912) and Blatherwick (1914))
Saliva	6.9[22]	Michaelis and Pechstein (1914)
Gastric juice (adult)	0.9–1.6	Menton (1915)
Gastric juice (infant)	5.0	Hess (1915)
Pancreatic juice (dog)	8.3	Auerbach and Pick (1912)
Small intestinal contents	8.3	Auerback and Pick (1912)
Small intestinal contents (infant)	3.1	McClendon (1915)
Bile from liver	7.8	Okada (1915)
Bile from gall bladder	5.3–7.4	Okada (1915)
Perspiration	7.1	Foà (1906)
Perspiration	4.5[23]	Clark and Lubs
Tears	7.2	Foà (1906)
Muscle juice (fresh)	6.8	Michaelis and Kramsztyk (1914)
Muscle juice (autolyzed)	Variable	Michaelis and Kramsztyk (1914) (see Morse (1916))
Pancreas extract	5.6	Long and Fenger (1916)
Peritoneal fluid	7.4	Foà (1906)
Pericardial fluid	7.4	Foà (1906)
Aqueous humor	7.1	Foà (1906)
Vitreous humor	7.0	Foà (1906)
Cerebrospinal fluid	7.2	Foà (1906)
Cerebrospinal fluid	8.3	Hurwurtz & Tranter (1916)
Amniotic fluid	7.1	Foà (1906)
Amniotic fluid	8.1	Löb and Higuchi (1910)
Milk (human)	7.0–7.2	(See Clark (1915 b))
Milk (cow)	6.6–6.8	(See Clark (1915 b))
Milk (goat)	6.6	Foà (1906)
Milk (ass)	7.6	Foà (1906)

[21] This is a purely "general average." The reaction of urine varies considerably.

[22] The true reaction of saliva is worthy of more extensive investigation from the bacteriological point of view. The value $P_H = 6.9$, which is given by Michaelis and Pechstein, is almost exactly that of absolute neutrality. If applied at 37°C. it is more alkaline than acid, since the neutral point at 37° is about $P_H = 6.8$. Several salivas which we have tested colorimetrically were found to be 6.8–6.9, yet these were distinctly acid to both red and blue litmus paper, and would therefore be called "acid" salivas. It is not to be denied that, so far as the action upon the constituents of the teeth is concerned, the relation of this reaction to absolute neutrality is of little consequence. Rather must it be

TABLE 21

True reactions of miscellaneous solutions

SOLUTION	H	AUTHORITY
Soil extracts	4.5–8.7[24]	Gillespie (1916)
Sea water	8.0–8.4	Palitzsch (1911 a)
Mineral waters	6.5–7.0	Michaelis (1914 b)
Flour extract	6.0–6.5	Jessen-Hansen (1911)
Tan liquors	3.5–4.7	Wood Sand and Low (1911)
Vermont maple sirup	6.8	Clark and Lubs
Beers	3.9–4.7	Emslander (1914)
Wines	2.8–3.8	Paul (1914)
Cider vinegar	3.1	Clark and Lubs
"White vinegar"	2.6	Clark and Lubs
Corn silage juice	3.7[25]	Clark and Lubs
Human feces (adults')	7.1–8.8	Howe and Hawk (1912)
Human feces (infants')	6.0–7.0	(See Michaelis (1914 b))
Cow feces	6.6–7.2	Clark and Lubs
Snail lymph	9.0	Foà (1906)

related to the solubilities of the constituents of the teeth at different P_H. Nevertheless this neutral reaction of so-called "acid" salivas is a good illustration of the confusing connotations which have been attached to the term acidity.

[23] The value of $P_H = 7.1$ was obtained by Foà (1906) on perspiration whose flow was stimulated by a hot air bath and from skin which had been washed with alkali to remove the fatty acids. These fatty acids, it has been claimed, are not normal constituents of the perspiration but are excreted by the sebaceous glands. Whatever may be the value of Foà's determination for the student of the mechanisms by which the body reactions are regulated it is of little interest to the bacteriologist when compared with the importance of the reaction of the perspiration as it normally occurs. It has been very commonly held that the reaction of the perspiration is variable but generally acid. Park and Williams (1910) state "The secretions of the sebaceous glands appear to be little, if at all, bactericidal, but the perspiration, on account of its acidity, is slightly so."

Three samples which we collected from clean normal skin had a reaction of about $P_H = 4.5$. Whether this is extreme and exceptional or not remains for others to determine. We may however note that such a reaction will doubtless tend to keep dormant many bacteria even if it does not exert a distinct bactericidal action.

[24] This wide variation in the acidities of soil extracts cannot fail to indicate some wide differences in the effective acidities of the soils themselves. These observations by Dr. Gillespie, which are the most enlightening data on the mooted question of soil acidity, are of profound importance to soil bacteriology.

[25] This acidity of silage juice is considerably higher than that attained in cultures of any of the streptococci we have yet studied and is practically the P_H that some of our unpublished researches have shown the bacteria of the Bul-

TABLE 22

True reaction of fruit and plant juices

SOLUTION	P_H	AUTHORITY
Lime juice	1.7	Patten and Mains[26]
Lemon juice	2.2	Patten and Mains[26]
Cherry juice	2.5	Patten and Mains[26]
Grapefruit juice	3.0–3.3	Patten and Mains[26]
Orange juice	3.1–4.1	Patten and Mains[26]
Rhubarb juice	3.1	Patten and Mains[26]
Strawberry juice	3.4	Patten and Mains[26]
Pineapple juice	3.4–4.1	Patten and Mains[26]
Tomato juice	4.2	Patten and Mains[26]
Pear (nearly ripe)	4.2	Foà (1906)
Grape juice	4.5	Foà (1906)
"Normal plant cell sap"	5.3–5.8	Wagner (1916)
Fiscus elastica milk	5.7	Foà (1906)
Prune juice (autoclaved)	4.3	Clark and Lubs
Apple juice (autoclaved)	3.8	Clark and Lubs
Banana juice (autoclaved)	4.6	Clark and Lubs
String bean juice (autoclaved)	5.2	Clark and Lubs
Carrot juice (autoclaved)	5.2	Clark and Lubs
Cucumber juice (autoclaved)	5.1	Clark and Lubs
Beet juice (autoclaved)	6.1	Clark and Lubs
Potato juice (autoclaved)	6.1	Clark and Lubs

is it necessary, for instance, to consider the dissociation of sugars, since their acid dissociation constants are so small that these substances are generally found only in a practically molecular state in neutral or acid solutions. Likewise there are zones within which any given acidic or basic group will be found in a practically totally dissociated or practically undissociated state. Perhaps there is no more vivid way of illustrating this than by a contemplation of indicators. Above a certain zone of hydrogen ion concentration phenolphthalein solutions are colorless. Below this zone (until intense alkalinity is reached) only the colored form is observed. Within the zone the color change

garicus type to reach. This observation is of very interesting significance now that Hunter and Bushnell (1916) and Sherman (1916) have shown the dominant organisms in silage to be of the Bulgaricus type.

[26] These values were obtained by Dr. Patton and Mr. Mains of The Bureau of Chemistry. By the courtesy of Dr. Patton and Mr. Mains and of the Bureau of Chemistry we are permitted to publish these average values in advance.

with change in hydrogen ion concentration is great. The conduct of phenolphthalein, which happens to be visible because of tautomeric changes which accompany dissociation, is a prototype of the conduct of all acids. Just as we may suppress the dissociation of phenolphthalein by raising the hydrogen ion concentration of the solution, so we may suppress the dissociation of any acid. A similar illustration for the conduct of bases is obtained if we regard methyl red as a base. The general

TABLE 23

Acid agglutination optima

ORGANISM	P_H	AUTHORITY
B. paratyphi	3.7	Michaelis (1911)
B. typhi	4.4	Michaelis (1911)
Pneumococci	5.0	Gillespie (1914)
Plague bacillus	5.0	Markl (1915)

TABLE 24

Limiting reactions

ORGANISM	P_H	AUTHORITY
B. coli	5.0	Michaelis and Marcora (1912)
B. coli (low gas ratio group)	4.3–5.3	Clark (1915c)
Streptococci, Group I	4.6–4.8	Ayers (1916)
Streptococci, Group II	5.5–6.0	Ayers (1916)
Streptococcus erysipelatos	4.8	Itano (1916)
B. subtilis, acid limit	4.2	Itano (1916)
B. subtilis, alkaline limit	9.4	Itano (1916)
Yeast	2.5–2.7	Lüers (1914)
Yeast	2.3	Högglund (1915a)

relations between the strength of acids as expressed by their dissociation constants and the percentage dissociation at different hydrogen ion concentrations will be found graphically illustrated in figure 5 (Journal of Bacteriology, ii, 110).

The point we wish to emphasize is, that within certain zones of hydrogen ion concentration slight changes may have an enormous effect just as we observe them to have in the case of indicators; while in other regions no appreciable effect is observed although the hydrogen ion concentration is varied enormously.

An important instance where such a consideration is very suggestive has been noted by Michaelis (1914 b). Michaelis (1914 a) and his collaborators (e.g., Michaelis and Davidsohn (1911)) have shown that enzyme solutions conduct themselves as if the enzyme were an electrolyte. Invertase, for instance, gives an activity curve for different hydrogen ion concentrations which has the form and many of the characteristics of the dissociation residue curve of an amphoteric electrolyte. In such curves there is seldom a sharp optimum point as has sometimes been inferred, but rather an optimum *zone*. Within and beyond this zone slight changes in hydrogen ion concentration produce no great change in the activity of the enzyme, but upon the borders of the zone a small change in P_H may make a very large difference. Michaelis suggests that there may be maintained in the organs of the body hydrogen ion concentrations which lie upon the border of the zone of optimal activity of a given enzyme and that this permits the rate or the direction of enzymatic action to be controlled by slight changes in P_H.

Considerations such as these, which will become more apparent if one studies the beautiful exposition of the dissociation curves of acids, bases and amphoteric electrolytes given by Sörensen (1912) and Michaelis (1914), would lead one to believe that the methods of determining hydrogen ion concentrations should be developed to meet two general classes of problems. In the first place, when the phenomenon under investigation is connected with an electrolytic equilibrium occurring in a P_H region where the equilibrium is seriously disturbed by slight changes in P_H the method of determining P_H values should be the most reliable available. In the second case, when the equilibrium is held practically constant within a certain wide zone of hydrogen ion concentration, it will do for most purposes to determine P_H by some approximate method. Neglecting the question of availability and arguing only from the basis of scientific efficiency the electrometric method should be applied in the first case because of its greater accuracy and the colorimetric method in the second case because of its simplicity. When the nature of the process investigated is not known and we are there-

fore unable to tell *a priori* which method on the above basis should be used, then a preliminary survey may be made with the colorimetric method. This it seems to us furnishes a rational basis for judging the true usefulness of the two methods.

Exception will be taken to this point of view as a comprehensive basis since there are cases in which the electrometric method is inapplicable and the colorimetric method must be used in preference, or *vice versa*. Nevertheless, we believe that in general the true utility of the colorimetric method lies, first in its availability where approximate determinations are needed but where exact determinations are useless, and second in its value for reconnaisance.

In some instances the mere fact that a phenomenon will occur over a considerable range of P_H should be clearly distinguished from the significance of an optimum point. A case in point is the acid agglutination of bacteria. An analogy which is imperfect, but which may serve to illustrate this, is found in the conduct of indicators. Phenol red and cresol red are distinct compounds of the same series which we may compare for present purposes with two distinct strains of bacteria of the same species. The two indicators conduct themselves in a very similar way. To a casual observer their colors would appear very much alike and their color changes would appear to occur in the same range of P_H. If we introduce these indicators into standard buffer mixtures a red color will be observed with each at P_H 7.4, 7.6, 7.8, etc., just as acid agglutination of bacteria may be observed at, say P_H 4.8, 5.0, 5.2, etc. But the *half transformation point* of each indicator is *characteristic* and may be used to *identify* the compounds. Likewise it is the *optimum* agglutination point which is *characteristic* of bacteria or of their protein.

It may not be out of place to prolong this discussion, keeping in mind the general point of view we have presented, and to indicate more concretely whese the colorimetric method may be applied in bacteriology.

In regard to the "reaction" of culture media several facts have become apparent. In the first place, adjustments by the old titrimetric method gave widely divergent hydrogen ion con-

centrations in media of different composition and not very close agreement in different samples of the same medium. In spite of this, media so adjusted were successful in many cases. This would indicate that rough adjustment of the hydrogen ion concentration is all that is necessary. Such adjustments may be adequately controlled by the colorimetric method. If they are, we shall not again encounter situations like that which occurred when the composition of a standard official medium was changed without revision of the "standard degree of reaction," the P_H of the revised medium being at about the limit endured by the organism the medium was intended to nourish.

But although the rough adjustment of the P_H of culture media may serve in most cases, we should not overlook the fact that many organisms have been described as very sensitive to "reaction." As one of us pointed out in a former paper we do not know whether this sensitivity has been overestimated because buffer poor media were used or because of the inaccuracies and uncertainties of the titrimetric method, or whether it is a true sensitivity. For a reconnoisance of this problem the colorimetric method should prove valuable.

As media become simplified and approach closer to the ideal of synthetic composition they can be made up with greater assurance of a constant initial P_H. The establishment of particular reactions is especially easy when there are present phosphates or similar well defined substances which exert strong buffer action at certain zones of P_H to the advantage of bacterial growth as Sörenson has pointed out. Henderson has repeatedly emphasized the value of mixtures of basic and acid phosphates in regulating solutions at or near the neutral point, and Henderson and Webster (1907) have specifically recommended such a mixture for prolonging the activity of "lactic bacteria." It may be noted however that the *addition of mixtures* of the phosphates to media is not always necessary. Phosphates have relatively little buffer action at hydrogen ion concentrations lower than that of blood. Consequently, if a secondary phosphate is added to a medium which is relatively rich in base binding compounds, the secondary phosphate cannot greatly lower the hydrogen ion

concentration. On the other hand at and above neutrality the phosphate will exert a strong buffer action and tend to keep the medium near the neutral point. Consequently the addition of a proper quantity of K_2HPO_4 to an acid medium is an effective way in which approximately to adjust the medium to neutrality. The use of di ammonium phosphate is even safer because it cannot retain an excess of base, especially after heating, and as ordinarily obtained is a mixture of the mono with an excess of the diammonium phosphate.

Synthetic media present some special problems. Not a few of those which have been proposed have so little buffer effect that they are practically useless and although "synthetic" are physiologically variable because of the enormous difference in P_H which very slight impurities may produce. With the proper set of indicators this may be easily discovered. Media have also been proposed which, in the language of the druggist, contain "incompatibles." The original Czapek (1901) medium for molds contained magnesium and a phosphate, but the magnesium was kept from precipitation as magnesium phosphate by using an acid phosphate. Copyists have neglected to specify this and, in some cases, have even specified the use of a basic phosphate. The resulting P_H permits the precipitation of the magnesium. Doubtless there remains even in such a solution a sufficient quantity of magnesium to supply the minimum requirement, but since magnesium is one of the essential elements for mold growth it is better technique, at least, to keep in solution that which is supplied.

Certain similar aspects of synthetic media will be discussed more fully in a subsequent paper, but before leaving the topic we may call attention to the well known fact that the reaction of a medium has a considerable influence upon the decompositions which occur during sterilization. Pure glucose and pure "microcosmic salt" form an excellent medium for many purposes, but after sterilization such a solution may hardly be called a synthetic medium in the strict sense of the term. Likewise the more complex media suffer changes on sterilization which in some instances especially on overheating, are of serious impor-

tance. Among many items of special interest to media makers may be mentioned the formation of complexes between sugars and amino acids, treated by Maillard (1913), the decomposition of asparagine, an important constituent of many synthetic culture media, which has been studied by Ehrlich and Lange (1913), innumerable researches on the cleavages of sugars in alkaline solutions, and the reports of Lobry de Bruyn and Alberta van Ekenstein (1895–1900) on the transformations of sugars into one another in alkaline solutions. These last are of such grave importance to bacteriologists that most careful confirmation may be demanded. It may not be too speculative to suggest at this point that in some instances the baneful effect of overheating media may be due to the destruction of special compounds which in small quantities are essential to the best activity of fastidious organisms. It is well known that certain so-called "vitamines," which are essential to the health of higher organisms and without which disease and death ensue, are destroyed upon heating, especially when the reaction of the solution is slightly alkaline or even neutral. It is not at all impossible that analogous compounds necessary for the continued growth of highly specialized bacteria may be destroyed by sterilization at the reactions usually employed. The later work on "vitamines" recalls the earlier work on Wildier's (1901) "bios," a substance which he describes as "indispensable to the development of yeasts" (see also Amand (1903–04)). Devloo's (1906) description of this sounds like a modern paper on a vitamine. Of particular interest to the present discussion is the statement that it is stable in acid solutions but unstable in alkaline solutions (like the vitamines).

Now Michaelis and Rona (1906 b) have emphasized, more particularly with reference to the alleged decomposition of sugar at the temperature and alkalinity of the blood, that we should not transfer indiscriminately to the conditions of physiological solutions the conclusions of those who have worked with molecular concentrations of alkali or acid. The effective alkalinity or acidity as measured by the hydrogen ion concentration may be thousands of times greater in the one case than in the other.

Therefore much of the data on the decompositions which occur in bacteriological culture media and the influence thereon of "reaction" had best be studied with at least such control as the indicator method will afford.

When a correlation is observed between P_H and some effect, the mere determination of P_H alone will of course throw but little light upon the real nature of the phenomenon except in rare instances. Determination of the hydrogen ion concentration will not even distinguish whether a given effect is influenced by the hydrogen or the hydroxyl ions, nor will it always reveal whether the influence observed is direct or indirect. It is true however that even when the hydrogen ion concentration is effective through remote channels it may be very important. Therefore advantage should be taken of the comparative ease with which the concentration of hydrogen ions may be determined or controlled and their influence known or made a constant during the study of any other factor which may influence a process. From this point of view methods of determining hydrogen ion concentration take their place beside thermometers, and buffer mixtures beside thermostats. In some instances automatic regulation of a hydrogen ion concentration may be provided, to take its place beside the automatic thermo regulator. For instance, Dr. Jones of this laboratory provided a very good automatic regulation of the P_H of a medium at a desired point by keeping the medium saturated with CO_2. Since anaerobiosis was also desired the procedure in this case was one of unique beauty. For alkaline solutions a modified use of the ammonia vapor tension of ammonia solutions might do.

The influence of hydrogen ion concentrations upon solubilities has been mentioned. It extends quite naturally to many of those substances which exist in solution in aggregates which bring them within the classification of colloids. Whether by reason of its influence on dissociation or because of other phenomena the hydrogen ion concentration of a solution may influence the dispersion of colloids. Among such substances of interest to the bacteriologist are bacterial enzymes, certain toxins, and the components of bacteria and viruses which may

react as if the bacteria themselves were dispersible, and several of the membranes used for the separation of enzymes, toxins bacteria and viruses from their media. Whether certain of these enzymes toxins, etc., would have appeared in the filtrates obtained by certain workers, had the medium been given the proper reaction, it is difficult to say; but it is worth while to call attention to the remarkable observations of Maurice Holderer (1911) which have been confirmed in many details by others. He not only controlled the filterability of enzymes[27] by adjusting the reaction of their solutions with the aid of indicators, but he also obtained new enzymes from certain organisms by extractions at certain reactions. Aubel and Colin (1915) have confirmed Holderer's conclusions with some bacterial toxins.

In the study of all of these problems, in the testing of acid and alkali fermentations, in the investigation of such influences as the reaction of the medium is reported to exert on the formation of toxin by the diphtheria bacillus [see Park and Williams (1910)) and Ficker (1913)], and especially in the study of that neglected but profoundly important subject,—the relative *rates* of different bacterial enzymatic processes, the indicator method may be used with advantage, at least for reconnoissance.

There is always danger that so important a subject will be either overrated or underrated before its position has been clearly defined by extensive investigation. Let us therefore recall the view point established by Sörensen in his classic paper "Enzymstudien II." As he said, the influence of hydrogen ion concentration is analogous to that of temperature. In each case there are limits beyond which the action of enzymes practically ceases. In each case there is a broad optimal zone. In each case there is at least upon one side of this zone a region where slight changes in the condition produce great changes in the rate of action. May we not extend this analogy to bacterial activity in general, and maintain that, just as we pay attention to the temperature of the incubator, so should we pay attention

[27] Since writing the manuscript of this paper one of us (W. M. C.) has found that the passage through Berkfeld filters of the gelatine liquefying enzyme of B. proteus can be controlled by adjusting the P_H of the solution.

to the hydrogen ion concentration of the medium. For general work precise thermometry, as the physicist knows it, has no place in bacteriological technique; for general work precise hydrogen electrode measurements of P_H are unnecessary. But a consideration of the facts already established is making it evident that we must have some means of determining hydrogen ion concentration which is comparable with the use of an ordinary thermometer for determining temperature and that it is advisable to use it with the same frequency. Experience with temperature effects enables one to judge of the accuracy required in any particular case; only experience will furnish a true measure of the limitations as well as the advantages of the indicator method. Our present judgment is that certain bacteriological problems are worthy of the instrumental accuracy of the very best hydrogen electrode equipment and of the most careful and skilled technique that can be applied to that instrument, but that to discover the cases where this may be necessary as well as to deal with numerous problems where this is surely not necessary the indicator method is adequate.[28]

[28] It is perfectly obvious that the indicators and the methods which we have described in this paper with particular reference to the needs of bacteriology may prove to be useful in other fields of investigation. The excellence of some of the indicators for the determination of the P_H values of urines is shown in table 12. Dr. Gillespie (1915) has used some of the new indicators in his soil work, and Dr. Patton and Mr. Mains have used them in studies on the acidities of fruit juices. Many other special uses might be suggested.

It is perhaps less obvious that many of the considerations which have led us to abandon the titrimetric method of adjusting the reaction of culture media apply to a great many other tests. Extracts of natural products very frequently give titration curves which are similar in many respects to the curves of culture media already described. One important characteristic is the strong buffer effect exhibited in the P_H region where phenol phthalein changes. It can easily be shown that such solutions are not subject to the application of the principles which have been developed for the titration of pure solutions of strong and moderately weak acids. In the great majority of cases the titration of such solutions reduces to a method of showing mere differences in different samples without showing the analytical content of the acids or bases.

According to Trillat (1916) the ancient Romans titrated the alkalinity of natural waters with drops of red wine. Although modern standards of concentration are more exact than the wine standard used by the Romans the modern

SECTION XIX. SUMMARY

Recent developments in the study of the influence of the hydrogen ion upon various processes of physiological importance have made it evident that these studies must be extended to bacteriological problems. We have given a very brief sketch of work already accomplished in other fields and have noted some instances where the influence of hydrogen ion concentrations upon the activities of bacteria, yeasts and molds has already been demonstrated.

With due consideration for certain superiorities in the electrometric method of determining hydrogen ion concentrations we have pointed out that for general work the colorimetric method promises to be most useful.

Certain major and minor hindrances to its general application in bacteriology we have materially lessened or removed. A new series of indicators of exceptional brilliancy has been assembled from among those already described or from the several new indicators which we have synthetized for the first time. The selection is composed of the following individual compounds with which are listed the useful ranges.

choice of indicators is oftentimes no more logical than the Roman choice of the coloring matter of red wine.

The frank admission that the analytical content of acid or base in a given complex solution cannot be determined by titration to a given tint of some arbitrarily selected indicator does not necessarily destroy the empirical value of such a titration. There can be no doubt, for instance, that the titration of milk furnishes information of great value to the cheese maker or the creameryman, but it is nonsense to elaborate the simple straightforward procedure of titration so that the results even in the case of perfectly fresh milk must be stated as *percentage lactic acid!*

In the titration of the various extracts which have to be dealt with by some empirical method in food or inspection laboratories it may frequently happen that significant differences can be detected better by a simple rapid colorimetric test of the original P_H values of different samples. In other cases it may be better to titrate to some point other than the customary phenol phthalein point or rather region. In either case the brilliancy and what we now know of the reliability of the indicators we have had the pleasure of describing will, we are sure, appeal to others.

	Range P_H
Thymol blue (acid range)	1.2–2.8
Thymol blue (alkaline range)	8.0–9.6
Brom phenol blue	2.8–4.6
Methyl red	4.4–6.0
Propyl red	4.8–6.4
Brom cresol purple	5.2–6.8
Brom thymol blue	6.0–7.6
Phenol red	6.8–8.4
Cresol red	7.2–8.8
Cresol phthalein	8.2–9.8

These indicators have been tested in a wide variety of solutions such as are used for the cultivation of bacteria, and especially in colored and turbid media, and their indications compared with electrometric measurements. The correlation reveals a general agreement which may be considered satisfactory for most purposes. In certain instances discrepancies which may be attributed to "protein" or "salt" errors and in some instances discrepancies which were traced to optical effects have shown that the indicators are not always reliable for accurate measurements.

In the colorimetric determinations the Walpole compensation method for colored and turbid media has been extensively studied with the simple device of Hurwitz, Meyer and Ostenberg. The dilution method has also been elaborated. Approximate and systematized procedures have been described. Special apparatus of simple design including a special light source for use with the two dichromatic indicators have been devised.

A new set of standard comparison solutions especially designed for accuracy and ease of preparation has been described, and, as reported elsewhere, carefully studied.

Brief sketches have been given of the more important theoretical aspects of the use of indicators, and general considerations have been presented to show the proper field of usefulness of the method.

It is concluded that, with the improvements presented, the colorimetric method is available for routine as well as research purposes in bacteriology.

Applications already made have been mentioned and new uses have been suggested.

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