THE IMPORTANCE OF UNIFORM CULTURE MEDIA IN THE BACTERIOLOGICAL EXAMINATION OF DISINFECTANTS

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Neither of the two methods for the bacteriological standardization of disinfectants which are in common use today has proven entirely satisfactory. Even in the hands of the most careful workers wide discrepancies in results have occurred, both with the Hygienic Laboratory and Rideal-Walker tests. While it is probably true that a few individuals have succeeded in so controlling their work as to obtain generally uniform and consistent results, a method worthy of general adoption must be capable of yielding satisfactory results in the hands of any well trained bacteriologist. It is hoped that the present investigation will throw some light upon the fundamental principles involved and stimulate such further investigations as may be necessary for the development of a universally satisfactory procedure.

In the past the general tendency has been to lay the blame for discordant results upon the so-called "personal equation." In our experience the "personal equation," in so far as the actual manipulation of the test is concerned, does not enter into the problem at all. Any two workers, provided they are sufficiently experienced and dexterous with their hands to perform the necessary manipulations with accuracy and despatch, will obtain comparable results provided they are working with uniform materials throughout.

After watching the results of a large number of tests extending over a long period of time our attention was drawn to the fact that duplicate tests made with the same batch of culture

medium always gave uniform results, and that when discrepancies did occur, it was almost invariably when different batches of culture medium had been used for growing the test cultures or the subcultures. This led to the keeping of detailed records of all media used in this laboratory for the testing of disinfectants, including both the Rideal-Walker and Hygienic Laboratory broths. These records, which now cover a period of more than

TABLE 1

Variation in coefficient with different batches of media

Hygienic Laboratory method

DATE OF TEST	MEDIA FOR TEST CULTURES		SUB-CULTURE MEDIA, DATE MADE	COEFFICIENT	
	Date made	Amount of growth		Disinfectant A	Disinfectant C
1915	*1915*		*1915*		
July 2	June 28	Medium	July 1	15.00	5.25
July 3	June 28	Medium	July 1	15.25	5.50
July 5	June 28	Medium	July 2	15.00	5.47
July 6	June 28	Medium	July 2	15.25	5.47
July 7	June 28	Medium	July 2	15.06	5.50
July 8	July 2	Medium	July 6	15.12	5.37
July 10	July 2	Medium	July 10	15.50	5.25
July 12	July 3	Medium	July 10	15.00	5.50
July 13	July 10	Light	July 10	16.87	6.37
July 14	July 10	Light	July 10	16.62	6.41
July 15	July 10	Light	July 10	17.12	6.56
July 16	July 10	Very light	July 16	17.31	6.41
July 19	July 16	Medium	July 16	15.87	5.62
July 20	July 16	Medium	July 19	15.25	5.25
July 22	July 16	Medium	July 19	15.00	5.37
July 23	July 19	Light	July 20	17.12	6.67
July 24	July 19	Light	July 20	17.50	6.75
July 26	July 23	Medium	July 23	15.25	5.50

two years, include the following points: the serial number of each lot of media, the particular jar of meat extract and peptone used in its preparation, the batch of media used each day for growing the test cultures, the relative amount of growth upon test cultures, and the number of each lot of media used for subcultures. These records, which now include more than 1500 separate tests by both methods and more than 200 different

lots of culture media, have proven of the utmost value and have led to the firm conviction that a great majority of discordant results are due to variations in culture media. Never during this time has there been the slightest difficulty in checking our coefficients within very narrow limits when the same lot of culture medium was used. When on the other hand two or more lots of media were employed for check tests, wide variations have frequently occurred.

TABLE 2

Variation in coefficient with different batches of media

Rideal-Walker method

DATE OF TEST	MEDIA FOR TEST CULTURES		SUB-CULTURE MEDIA, DATE MADE	COEFFICIENT	
	Date made	Amount of growth		Disinfectant A	Disinfectant C
1915	*1915*		*1915*		
July 20	July 18	Heavy	July 19	18.00	6.50
July 21	July 19	Heavy	July 19	18.88	6.50
July 22	July 19	Heavy	July 19	18.00	6.25
July 24	July 19	Heavy	July 22	18.00	6.00
July 26	July 19	Heavy	July 22	18.00	6.00
July 27	July 22	Medium	July 22	20.00	7.50
July 29	July 22	Medium	July 22	21.10	7.00
July 30	July 22	Medium	July 29	19.90	7.50
August 2	July 22	Medium	July 29	20.00	7.25
August 3	July 29	Heavy	July 29	18.00	6.50
August 4	July 29	Heavy	August 3	18.00	6.50
August 6	July 29	Heavy	August 3	18.00	6.75
August 7	August 3	Medium	August 3	21.00	7.50
August 9	August 3	Medium	August 3	21.00	8.00
August 10	August 3	Medium	August 10	20.00	7.50
August 11	August 3	Medium	August 10	21.00	7.75
August 12	August 12	Heavy	August 12	18.88	6.50

Tables 1 and 2 show the record of a series of tests by both the Hygienic Laboratory and Rideal-Walker methods on each of two disinfectants. The tables speak for themselves and show clearly the variations which may be anticipated when different batches of media are used for check tests. Extreme care is used in the preparation, adjustment and sterilization of all media, and every precaution taken to have the procedure uniform in

every detail. Yet, in spite of the utmost care, different lots of media are at times sufficiently unlike to cause serious deviations in coefficients, this condition being evident with either the Hygienic Laboratory or Rideal-Walker method.

A careful study of the above tables will bring out the exceedingly important fact that, while slight differences in media used for growing the test organism previous to its employment in a test may cause serious discrepancies, the same differences in subculture media are of practically no significance. This has held true throughout all of our tests, so that it seems well demonstrated that, provided one has a uniform medium for growing the test culture, normal variations in the subculture medium are of little importance. It is true that differences in the subculture medium may cause a profound difference in the character of the chart obtained; that is, in the actual number of positive tubes shown in the subcultures from a given dilution. This influence, however, appears to be fairly uniform both for coal tar disinfectants and for the phenol control, so that there is no material change in the resulting coefficient.

In an effort to ascertain the cause of these variations in culture media the first consideration was, of course, the uniformity of the materials used in preparing the media. The salt used was in all cases the highest grade of C. P. product obtainable, so that it seemed safe to eliminate this factor from the problem. This leaves the water, beef extract, and Witte's peptone open to suspicion.

The Rideal-Walker method specifies the use of distilled water for the preparation of culture media, while the Hygienic Laboratory method specifies the use of tap water. Although it is claimed by some that tap water gives a medium more suited to the growth of bacteria, the writer, in view of the wide differences in the composition of various city water supplies as well as the seasonal and other variations occurring in a single supply, is convinced that its use is not desirable. A series of experiments performed by him extending over a period of several years indicated that media prepared with tap water were not in all cases comparable with those prepared with distilled water;

furthermore, the seasonal variations in the composition of the New York City water supply seemed to be sufficiently great to affect the uniformity of our phenol coefficient determinations. The only possible advantage in the use of tap water lies in its inorganic salt content. That a certain inorganic salt content is essential to any culture medium cannot be denied. This may, however, well be supplied by the use of sodium chloride in conjunction with the inorganic salts of the peptone and meat extract. Thus there seems to be no good reason for complicating the situation by employing tap water of unknown and variable composition. Throughout the work here reported distilled water was used in the preparation of all culture media. This factor could not, therefore, be concerned in any possible variation in the results obtained.

A careful study of the records described above has failed to show any indication that lack of uniformity in Witte's peptone was in any way responsible for the unreliable results often obtained with different lots of media. These results, which now include upwards of twenty-five different lots of peptone, together with the experiments to be described later, seem to show with reasonable certainty that for purposes of standardization Witte's peptone is a reliable and uniform product.

Liebig's Extract of Meat, on the other hand, does not appear to be nearly so satisfactory. Several jars have been found which invariably produced a culture medium giving very high coefficients. The proportions of salts and other ingredients of meat extract undoubtedly vary from time to time and are possibly responsible for some of the variations in culture media. In the writer's opinion, however, the greater part of the difficulty is caused by the presence in some jars of a considerable amount of fat. Chemical examinations have shown that those jars giving high coefficients contained over 50 per cent more ether extractive than the average for Liebig's extract of meat. The usual process of boiling culture media with caustic soda would convert this fat, partially at least, into soap. The powerful antiseptic action of soap, even in very high dilutions, is well known and is probably sufficient to explain the high results

obtained with these jars of extract. If, as will be recommended in this paper, the media be left with acidity unadjusted the presence of fat will have no effect; furthermore, it appears to be only an occasional lot of extract which proves unsatisfactory, so that if each new lot be checked against a disinfectant of known strength this source of error may be eliminated.

When we come to the actual manipulations used in the preparation of culture media, an extremely complex and involved problem is presented. This question is largely one of the influence of the kind and amount of acidity upon the growth of the test organism as well as upon the composition of the medium itself during the various stages of its preparation. Clark, in a recent article in the Journal of Infectious Diseases, July, 1915, clearly shows the fallacy of our present titrimetric methods. He points out the important influence of the hydrogen ion concentration upon the viability of bacteria, as well as upon the chemical composition of media, and shows conclusively that our present methods of adjusting the acidity of culture media by phenolphthalein give absolutely no information upon this all-important point.

The Rideal-Walker and Hygienic Laboratory broths form excellent examples of the differences in actual acidity (hydrogen ion concentration) which may be expected when media of different composition are brought to the same reaction with phenolphthalein by our present methods. The Rideal-Walker broth contains 20 grams of meat extract, 20 grams of peptone, and 10 grams of salt; while the Hygienic Laboratory broth contains 3 grams of meat extract, 10 grams of peptone and 5 grams of salt. The titration curves of these two solutions are shown below in Chart I.

The curves show the progressive changes in the hydrogen ion concentration of these solutions caused by successive additions of acid or alkali. Assuming the value $P_H = 8.5$ as the neutral point of phenolphthalein and calculating both from the point at which the curves cross this line we find that when the reaction has been brought to + 1.5 by the usual method, the Rideal-Walker broth would have a hydrogen ion concentration of ap-

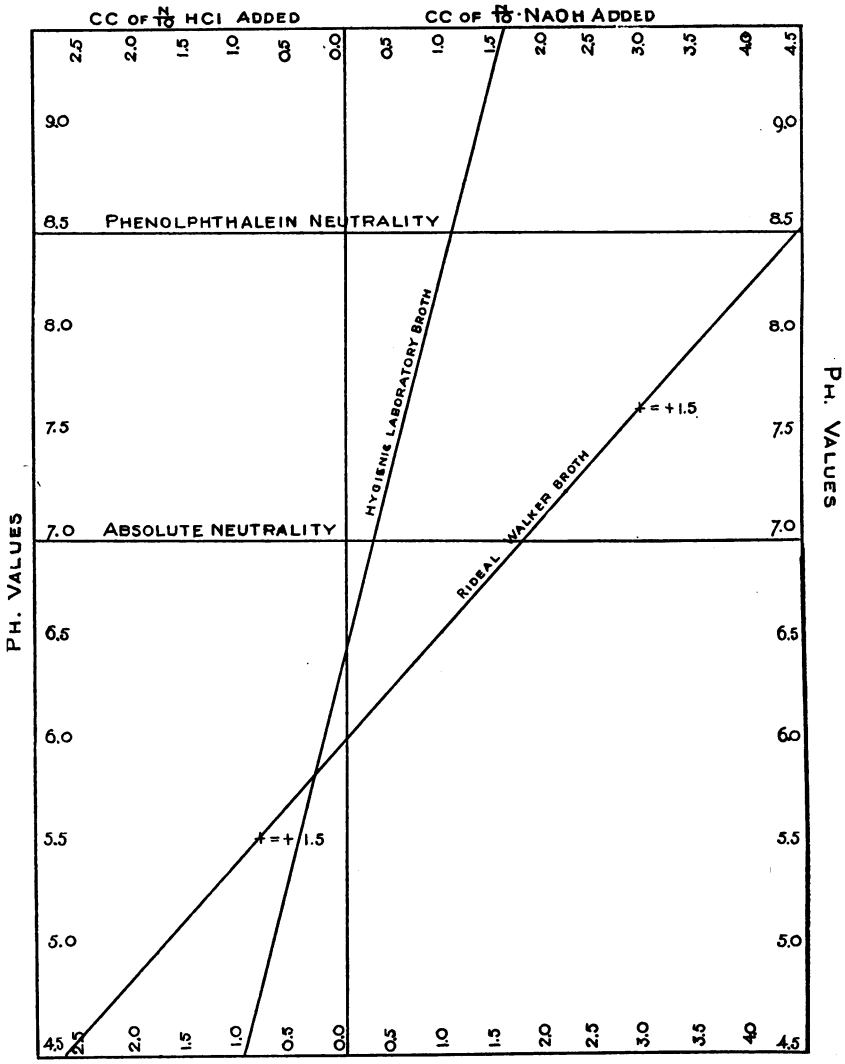

CHART 1. TITRATION CURVES OF 10 CC. OF HYGIENIC LABORATORY AND 10 CC. OF RIDEAL-WALKER BROTHS

The hydrogen ion concentrations given in this paper are expressed in the P_H values of Sörensen, (Compt. rend. du Lab. Carlsberg, 1909, **8**, p. 1.) The hydrogen electrode and a Leeds-Northrup potentiometer were used in the majority of the determinations. In some cases, however, the values given were obtained colorimetrically by means of the indicators, and essentially the same technique as described by Clark and Lubs (this Journal, vol. **2**, nos. 1, 2, and 3). We have made some rather extensive comparisons between this colorimetric procedure and the electrometric method as applied to the various media described in this paper and including the entire series of indicators recommended by Clark and Lubs. With all the sulphonaphthalein indicators the agreement has always been within 0.1 P_H. With methyl red, however, the results have been uniformly about 0.2 P_H more acid than those obtained with the hydrogen electrode. We believe, however, that for the majority of bacteriological purposes such an error is without significance and that the colorimetric method, carefully applied, constitutes a reliable and exceedingly convenient means for controlling the acidity of most types of culture media.

proximately $P_H = 7.65$ while that of the Hygienic Laboratory broth would be about 5.55. Due to the fact that few people judge the end point of phenolphthalein at exactly $P_H = 8.5$ the hydrogen ion concentration of media as ordinarily prepared varies somewhat from the above figures. This is particularly true of the Hygienic Laboratory broth in which the P_H value is usually very close to 5.0. Two media with such a wide difference in hydrogen ion concentration surely could not be expected to give comparable coefficients.

While the matter will be taken up in detail later, it may be well to mention here that the high results of the Rideal-Walker test as compared with the results of the Hygienic Laboratory test are not due to the difference in hydrogen ion concentration. The Rideal-Walker broth, with its 10 grams of NaCl in addition to the large amount introduced with 20 grams of meat extract, contains sufficient inorganic material to inhibit the growth of a weakened organism. If the salt be left out of the Rideal-Walker broth the results will be considerably lower than those obtained with the Hygienic Laboratory broth, in spite of the fact that the hydrogen ion concentration and titratable acidity of the Rideal-Walker broth are not changed.

While the above is sufficient to show the fallacy of attempting, by phenolphthalein titration, to adjust the reaction of culture media of different composition to the same point; there are many other points in our present methods of preparing culture media which make it practically impossible always to prepare even the same medium with a uniform hydrogen ion concentration. In a culture medium titrated with phenolphthalein there is no sharp end point. No two people will judge the "faint but distinct pink color" in exactly the same way. It is true that one person can, with practice, so train his eye as to obtain generally consistent results. There is no question, however, but that different workers, no matter how skillful and painstaking with their titration, cannot produce media of uniform reaction. This point is well illustrated in table 3.

After solution of the ingredients, a batch of Hygienic Laboratory medium was divided into three equal portions and

TABLE 3

Variations in coefficients obtained in different observers

ADJUSTED BY	FINAL TITRATION BY			HYGIENIC LABORATORY PHENOL COEFFICIENT OF DISINFECTANT A
	A	B	C	
A.....	1.48	1.91	1.26	$\frac{\frac{1100}{80}+\frac{1600}{100}}{2}$ = 14.75 average of 4 tests
B.....	1.21	1.56	1.08	$\frac{\frac{1100}{80}+\frac{1500}{100}}{2}$ = 14.37 average of 4 tests
C.....	1.75	2.17	1.41	$\frac{\frac{1300}{80}+\frac{1700}{100}}{2}$ = 16.62 average of 4 tests

each of three bacteriologists asked to adjust the acidity of one portion. After the media were completed each man was asked to determine the final reaction of the three lots without in any case knowing the identity of any of the samples. These results are shown above, together with the Hygienic Laboratory coefficients of coal tar disinfectant A resulting from the use of these media. The hydrogen ion concentration of the media was as follows.

Medium adjusted by bacteriologist A = 5.9
Medium adjusted by bacteriologist B = 5.6
Medium adjusted by bacteriologist C = 4.7

In the above determinations, as well as in all such tests reported in this paper, the medium used for growing the test culture and for subcultures was the same. The test organism was transferred daily for seven days and no longer, upon the particular medium indicated before being employed for a test. Great care was taken to have the test cultures in all cases exactly twenty-four hours old. We consider this an important point and are convinced that a few hours difference in the age of the test cultures has a very marked influence upon the uniformity of results. In order to eliminate every possible chance of variation, one jar of Liebig's extract of meat and one bottle

of Witte's peptone were set aside for use exclusively with the experimental media, so that all media described in this paper were made from the same jar of extract and the same bottle of peptone, unless otherwise indicated. In no case have conclusions been based upon the results of less than four tests, and usually six or more duplicate tests were made on different days and with different lots of media.

TABLE 4

Variation in coefficients with different methods of preparing media

Hygienic Laboratory technique

DISINFECTANT	DILUTION	MEDIUM: STANDARD R.-W. BROTH						MEDIUM: R.-W. BROTH, REDUCED TO + 1.5 WITHOUT NUETRALIZING					
		Minutes of exposure											
		2½	5	7½	10	12½	15	2½	5	7½	10	12½	15
A	1: 900							−	−	−	−	−	−
	1: 1000							+	−	+	−	−	−
	1: 1200							+	+	+	−	−	−
	1: 1300							+	+	+	+	+	+
	1: 1400	−	−	−	−	−	−	+	+	+	+	+	+
	1: 1600	+	−	−	−	−	−	+	+	+	+	+	+
	1: 1800	+	+	−	−	−	−						
	1: 2000	+	+	−	−	−	−						
	1: 2200	+	+	+	−	−	−						
	1: 2400	+	+	+	+	+	+						
Phenol	1: 80	−	−	−	−	−	−	−	−	−	−	−	−
	1: 90	+	−	−	−	−	−	+	+	−	−	−	−
	1: 100	+	+	−	−	−	−	+	+	+	+	−	−
	1: 110	+	+	−	+	−	−	+	+	+	+	+	+
	1: 120	+	+	+	+	−	−	+	+	+	+	+	+
	1: 130	+	+	+	+	+	+						
Coefficient		17.91						11.62					
P_H		7.8						8.4					

The Hygienic Laboratory method states simply that the medium should be adjusted to a reaction of + 1.5. A simple solution of the ingredients for the Hygienic Laboratory broth has an initial reaction of about + 0.9. Should this solution be neutralized and then brought up to + 1.5, or should sufficient acid be added to bring the reaction to the desired point without

neutralization? The Rideal-Walker broth has an initial acidity of + 3.5. This method specifies that the medium shall be neutralized and then 15 cc. of HCl per liter shall be added. It would seem therefore that there could be no excuse for simply adding sufficient alkali to bring the reaction down to the desired point.

TABLE 5

Variation in coefficients with different methods of preparing media
Hygienic Laboratory technique

DISINFECTANT	DILUTION	MEDIA: H.-L. BROTH, NEUTRALIZED AND THEN RAISED TO + 1.5 WITH HCl						MEDIA: H.-L. BROTH, RAISED TO + 1.5 WITH HCl WITHOUT NEUTRALIZATION					
		Minutes of exposure											
		2½	5	7½	10	12½	15	2½	5	7½	10	12½	15
A	1:1000	−	−	−	−	−	−	−	−	−	−	−	−
	1:1100	−	−	−	−	−	−	+	−	−	−	−	−
	1:1200	−	−	−	−	−	−	+	+	−	−	−	−
	1:1300	+	−	−	−	−	−	+	+	+	−	−	−
	1:1400	+	+	+	−	−	−	+	+	+	+	−	−
	1:1500	+	+	+	+	−	−	+	+	+	+	+	−
	1:1600	+	+	+	+	−	−	+	+	+	+	+	+
	1:1700	+	+	+	+	+	−	+	+	+	+	+	+
	1:1800	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	1: 80	−	−	−	−	−	−	−	−	−	−	−	−
	1: 90	−	−	−	−	−	−	+	−	−	−	−	−
	1: 100	+	+	−	−	−	−	+	+	+	−	−	−
	1: 110	+	+	+	+	−	−	+	+	+	+	+	+
	1: 120	+	+	+	+	+	+	+	+	+	+	+	+
Phenol coefficient		14.94						13.75					
P_H value		5.2						5.7					

The effects of such differences in the preparation of culture media are illustrated by tables 4 and 5. In obtaining the results shown in these tables a single batch of Hygienic Laboratory broth and one of Rideal-Walker broth were prepared. Before adjusting the acidity, each lot was divided into two portions, one portion being neutralized with NaOH and then without filtration brought up to a reaction of + 1.5 with HCl, while to the other portion sufficient acid or alkali, as the case might be, was added to bring the reaction to the desired point. The differ-

ences in hydrogen ion concentration caused by such a variation in the method of preparing the media are also shown in the table. While this difference is not great, it is undoubtedly the cause of at least part of the discrepancies shown in the coefficients. It is, however, probable that a greater effect is produced by the changes in composition caused by the different methods of preparation.

When any medium is neutralized with alkali a considerable precipitate forms which only partially redissolves upon the addition of acid. This precipitate which separates out on the addition of acid or alkali consists of protein material and a considerable amount of phosphate, and its removal has a decidedly deleterious influence upon the subsequent growth of organisms. The hydrogen ion concentration of the medium is materially raised, due probably to the removal of "buffers." The increased hydrogen ion concentration, together with the reduced nutritive value, and the absence of phosphate, result in a very poor growth of the typhoid organism. On the finished culture being used for tests the results are not only much too high but the charts obtained are very irregular and unsatisfactory. This point is well illustrated by table 6. As would be expected, this phenomenon is more marked with the Hygienic Laboratory broth than with the Rideal-Walker medium.

It has probably been the experience of every worker that the amount of alkali indicated by titration is never sufficient to bring about complete neutralization of the media, it being necessary to add a considerable excess over the amount indicated. There will be a very decided difference in the coefficient obtained, depending upon how close to the neutral point the reaction has been carried before the addition of acid.

As previously mentioned, the Rideal-Walker method specifies that, after the medium has been neutralized, 15 cc. of N/1 HCl per liter shall be added. This should give the final product a reaction of + 1.5, but the acidity is always considerably less than the standard. Wide variations in hydrogen ion concentration with resulting variations in coefficients are obtained, depending upon whether or not an attempt is made to correct the medium to exactly 1.5.

In order to ascertain, if possible, just what influence variations in hydrogen ion concentration have upon culture media, a series of experiments was performed with media of varying P_H values. In these tests the proportion of the meat extract and peptone was as specified in the Hygienic Laboratory method, and in most cases the P_H values were adjusted by simply adding sufficient

TABLE 6

Variation in coefficients with different methods of preparing media
Hygienic Laboratory technique

DISINFECTANT	DILUTION	MEDIUM: H.-L. BROTH, NEUTRALIZED AND BROUGHT UP TO + 1.5						MEDIUM: H.-L. BROTH, NEUTRALIZED, FILTERED AND THEN BROUGHT UP TO + 1.5					
		Minutes of exposure											
		2½	5	7½	10	12½	15	2½	5	7½	10	12½	15
A	1:1200	−	−	−	−	−	−						
	1:1400	+	+	−	−	−	−						
	1:1600	+	+	+	+	−	−	−	−	−	−	−	−
	1:1800	+	+	+	+	+	+	+	−	−	−	+	−
	1:2000	+	+	+	+	+	+	+	+	−	−	−	−
	1:2200							+	+	−	−	+	−
	1:2400							−	+	+	+	−	−
	1:2600							+	+	+	+	+	+
Phenol	1: 80	−	−	−	−	−	−						
	1: 90	+	−	−	−	−	−						
	1: 100	+	+	+	−	−	−	−	−	−	−	−	−
	1: 110	+	+	+	+	+	−	+	−	−	−	−	−
	1: 120	+	+	+	+	+	+	+	+	−	+	−	−
	1: 130							+	+	−	−	+	−
	1: 140							+	+	+	+	+	+
Coefficient		14.77						17.72					
P_H		5.26						4.92					

NaOH or HCl, to bring the reaction to the desired point. Considerable work, however, was done in which the P_H value was adjusted by the use of phosphoric, lactic and citric acids, and sodium acid phosphate. Little difference could be detected in media prepared with different acids, provided the hydrogen ion concentration was uniform. In fact, all our work pointed to the conclusion that ordinary variations in the character and amount

of titratable acid present are of little importance, except in so far as they influence the P_H value.

The method of preparing the medium was as follows. The peptone, meat extract, and salt were boiled 15 minutes in the proper amount of distilled water. Then sufficient acid or alkali, as the case might be, was added to bring the P_H value to the desired point, the medium then being sterilized under 10 pounds pressure for twenty minutes. Different lots of medium were prepared in which the P_H values increased regularly by one-half unit from 8.5 to 4.5, the entire series being repeated three times. The relative amount of precipitate in each lot of medium was observed. A precipitate always formed upon the addition of more than a trace of alkali, and usually upon the addition of acid. In some instances the precipitate was not observable at once, but was present after the medium had been sterilized and allowed to cool. In all cases the amount of precipitate recorded is the amount present in the finished product.

It was found that the solubility of the peptone and meat extract was largely dependent upon the hydrogen ion concentration. This is graphically shown in curve 2, Chart II. When the P_H value fell between 6 and 7 no precipitate of any kind was present in the finished medium. A slight increase or decrease in acidity on either side of this point was however sufficient to cause a marked precipitation of the media. While the acidity of ordinary culture media probably never runs beyond $P_H = 4.5$, it is worthy of mention here that, when the P_H value is over 4, the peptone and meat extract again becomes perfectly soluble, the maximum precipitation occurring at approximately $P_H = 4.75$. It is also worthy of note that a chemical examination of these precipitates showed that that formed on the acid side of the chart consists almost entirely of protein, while that found on the alkaline side contains relatively large quantities of phosphates.[1]

[1] The writer feels that he should acknowledge his indebtedness to Dr. I. J. Kligler of the American Museum of Natural History, who, while discussing the results of some similar investigations of his own with an American peptone, first called the writer's attention to the fact that the solubility of peptone depended largely upon the hydrogen ion concentration of the solvent.

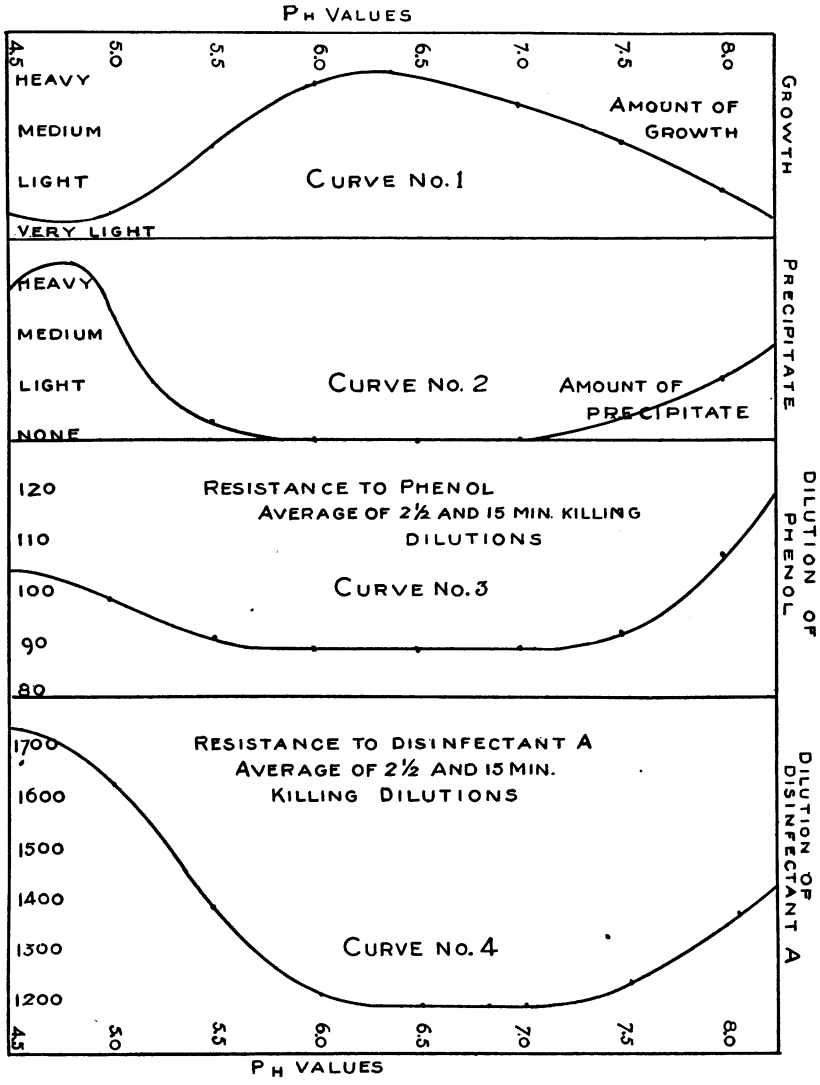

CHART 2

Examinations of a large number of different batches of media showed that the P_H value of standard Hygienic Laboratory broth varies from 4.75 to 5.75, while that of standard Rideal-Walker broth varies from 7.5 to 8.5. It will be seen therefore that the

composition of these media which is dependent largely upon a variable hydrogen ion concentration must consequently itself be variable. Such variations in chemical composition are undoubtedly one cause of the differences in the coefficients obtained with different lots of the same culture medium, although the indefinite hydrogen ion concentration is, of itself, without any reference to its influence upon solubility, an important point.

The development of the test organism in these media also forms an important index of their suitability for purposes of standardization. Five tubes of each broth were inoculated with *Bact. typhosum* Hopkins and were transferred daily for ten generations. These cultures were incubated at 37°C. and after twenty-four hours the amount of growth in each was recorded. The relationship between the amount of growth and the hydrogen ion concentration of the media is shown in curve 1, Chart II. The close agreement of this curve with that showing the relative amount of precipitate is quite remarkable and, as might be expected, the most luxuriant growth occurs in the same zone as that in which the peptone and meat extract are completely soluble.

Finally, these media were employed in determinations of the phenol coefficient of a coal tar disinfectant. At least five tests were run with each kind of medium on different days, using the same medium for both subcultures and for preliminary cultivation of the test organisms. The resistance of *Bact. typhosum* Hopkins in its relation to the various P_H values of the media is shown in curves 3 and 4, Chart II.

The curves plotted in each case were obtained by taking the mean of the two and one-half and 15 minute killing dilutions, as shown by the average result of not less than five check tests. It will be seen that the general outline of these curves corresponds with the two previous ones, the most important point being that between $P_H = 6$ and $P_H = 7$ the resistance of the organism is strong and constant, as indicated by the flatness of the curve. As the acidity is increased above $P_H = 6$ the resistance to phenol decreases slightly, while the resistance to the coal tar disinfectant decreases very rapidly, resulting in a

large increase in coefficient. On the other hand, when the acidity is reduced below $P_H = 7$ the resistance to phenol increases more rapidly than the resistance to a coal tar disinfectant, the result being a lowered coefficient. We have no explanation to offer for this phenomenon unless it be the fact that with increased acidity a large amount of protein material, but no phosphate, is removed from the media, while with a decreased acidity a large amount of essential phosphate and a comparatively small amount of protein is removed.

From these results it would seem, however, that we are at least justified in assuming that in order to obtain uniform results the hydrogen ion concentration of a culture medium should be between $P_H = 6$ and $P_H = 7$. This is further borne out by a study of the uniformity of coefficients obtained in duplicate tests with media of the same hydrogen ion concentration. Our results show that between $P_H = 6$ and $P_H = 7$ our phenol coefficients varied less than 10 per cent; between $P_H = 4.5$ and $P_H = 5.5$ our phenol coefficients varied from 30 to 40 per cent; while between $P_H = 7.5$ and $P_H = 8.5$ our coefficients varied from 20 to 25 per cent. In this connection it is of importance to note that a simple solution of the ingredients with no adjustment of the acidity whatever gives a P_H value most favorable for uniform results, namely, 6.3 to 6.7. It would seem, therefore, that there is no legitimate excuse for spoiling a good thing by juggling the reaction.

The Hygienic Laboratory broth has been often criticised on the ground that it contains insufficient nutriment for the normal growth of the typhoid organism, resulting in weakened and unreliable cultures. That this is not true is well demonstrated by our results, for when the hydrogen ion concentration of the culture medium is approximately $P_H = 6.5$ and the formula is as specified in the Hygienic Laboratory method *Bact. typhosum* grows luxuriantly and the cultures have been found to possess a remarkable degree of uniformity in their resistance to the action of disinfectants. The light, variable cultures of *Bact. typhosum* obtained with the regular Hygienic Laboratory broth are due not to a lack of a sufficient amount of nutriment, but to

an altered composition and high acidity brought about by a misguided attempt to adjust the reaction. However, it was thought wise to determine the effect of varying the proportion of different ingredients in culture media. A series of experiments was therefore performed as indicated in the following tables. In this work no attempt was made to adjust the acidity, the ingredients being simply dissolved, filtered, and sterilized; otherwise the details throughout the procedure were exactly the same as those previously employed.

TABLE 7

Showing effect upon culture media of varying amounts of Witte's peptone. All media in this table contained Liebig's Meat Extract, 3 grams, and NaCl, *5 grams, to each 1000 cc. of distilled water, in addition to the amount of peptone indicated*

AMOUNT OF WITTE'S PEPTONE PER LITER	PH VALUE	TITRATABLE ACIDITY TO PHENOLPHTHALEIN	RELATIVE AMOUNT OF GROWTH SHOWN IN PRELIMINARY CULTURES	RESISTANCE TO DISINFECTANT AVERAGE OF 2½ AND 15 MINUTE KILLING DILUTIONS		HYGIENIC LABORATORY COEFFICIENT OF DISINFECTANT A*
				Phenol	Disinfectant A	
grams.						
5	6.6	+0.75	Medium	1:95	1:1500	15.77
10	6.5	+0.93	Heavy	1:90	1:1200	13.33
15	6.4	+1.12	Heavy	1:89	1:1200	13.48
20	6.4	+1.35	Heavy	1:88	1:1190	13.52
25	6.3	+1.51	Heavy	1:89	1:1190	13.37
30	6.3	+1.74	Heavy	1:91	1:1210	13.29

* Average results of five check tests.

Table 7 shows the influence of adding varying amounts of Witte's peptone to culture media, the proportions of the other ingredients remaining constant. One medium was prepared containing no peptone. In this medium *Bact. typhosum* grew very poorly indeed, so that it was not considered worth while to continue the experiment. With 5 grams of the peptone a good growth of the test organism was obtained. The resistance of the organism was, however, markedly weaker than when grown in any other medium in this series. The use of amounts of Witte's peptone varying from 10 to 20 grams per liter made no apparent difference in the growth of the organism nor in

its relative resistance to the action of disinfectants. It would seem, therefore, that there is no good reason for using a larger quantity of this ingredient than is now called for by the Hygienic Laboratory method.

That some meat extract is essential is shown by the results obtained with the first medium in table 8. Where no extract was used it was impossible to obtain more than an exceedingly light growth of the test organism, and when such a culture was used for testing a disinfectant the coefficient was not only

TABLE 8

Showing effect upon culture media of varying amounts of Liebig's extract of meat. All media in this table contained Witte's peptone, 10 grams and NaCl, *5 grams, to each 1000 cc. of distilled water in addition to the amount of meat extract indicated*

AMOUNT OF LIEBIG'S EXTRACT OF MEAT PER LITER	PH VALUE	TITRATABLE ACIDITY TO PHENOLPHTHALEIN	RELATIVE AMOUNT OF GROWTH SHOWN IN PRELIMINARY CULTURES	RESISTANCE TO DISINFECTANT AVERAGE OF 2½ AND 15 MINUTE KILLING DILUTIONS		HYGIENIC LABORATORY COEFFICIENT OF DISINFECTANT A*
				Phenol	Disinfectant A	
grams						
None	6.9	+0.29	Very light	1:99	1:1710	17.27†
3	6.5	+0.81	Heavy	1:89	1:1190	13.37
5	6.4	+1.14	Heavy	1:91	1:1280	14.06
10	6.2	+1.81	Heavy	1:93	1:1380	14.85
15	6.2	+2.62	Heavy	1:93	1:1490	16.02
20	6.1	+3.44	Medium	1:95	1:1510	16.52
25	6.1	+4.23	Medium	1:97	1:1650	17.01

* Average results of five check tests.

† Very irregular.

very high but the charts obtained were very irregular and unsatisfactory in every particular. With 3 grams of meat extract the growth of the test organism was fully as good as that obtained with any medium tested. Its resistance to the action of disinfectants was strong and uniform, so that there was no difficulty in getting satisfactory checks with duplicate tests. As the amount of meat extract was increased there was, apparently, a steady decrease in the resistance of the test culture, and as this was more pronounced in the case of the coal tar disinfect-

ants than with phenol there was a corresponding increase in the phenol coefficient. This weakened resistance, however, is more apparent than real and is due largely to the fact that media containing large percentages of meat extract are sufficiently antiseptic to inhibit the growth of an organism which has been weakened but not killed by exposure to disinfectants. This is shown by a series of experiments in which the second medium in this table was used for subcultures with test cultures grown in each of the media shown in the table. In these experiments there was also an increase in coefficient with an increased percentage of meat extract. This, however, was only about one-

TABLE 9

Showing effect upon culture media of varying amounts of NaCl. *All media in this table contained Witte's peptone, 10 grams and Liebig's Extract of Meat, 3 grams, to each 1000 cc. of distilled water in addition to the amount of* NaCl *indicated*

AMOUNT OF NaCl PER LITER	P_H VALUE	TITRATABLE ACIDITY TO PHENOL-PHTHALEIN	RELATIVE AMOUNT OF GROWTH SHOWN IN PRELIMINARY CULTURES	RESISTANCE TO DISINFECTANT AVERAGE OF 2½ AND 15 MINUTE KILLING DILUTIONS		HYGIENIC LABORATORY COEFFICIENT OF DISINFECTANT A*
				Phenol	Disinfectant A	
grams						
None	6.6	0.93	Medium	1:94	1:1410	15.00
5	6.5	0.93	Heavy	1:90	1:1191	13.22
10	6.5	0.94	Medium	1:94	1:1480	15.74
15	6.4	0.93	Medium	1:96	1:1590	16.56
20	6.3	0.94	Light	1:98	1:1670	17.02

* Average result of five check tests.

half that obtained when the higher amounts of meat extract were introduced in the subculture media as well as in that used for preliminary cultivation. Moreover, this inhibitive action seems to vary materially with different lots of Liebig's extract of meat, so that it would seem wise to reduce this variable factor to a minimum by employing for a standard method as small an amount of meat extract as is consistent with the needs of the organism. From our results it seems clear that 3 grams per liter is ample.

It will be noted that in table 8 there is a very marked increase in titratable acidity when the larger amounts of meat extract

are used and at the same time there is a slight increase of the hydrogen ion concentration. It may possibly be contended that the inhibitive influence of the meat extract is due to this increased acidity. Such, however, is not the case as was shown by the fact that when the titratable acidity of these media was in all cases reduced to + 1.0 the resulting coefficients, while somewhat lower as would be expected with the decreased P_H value, still showed the same steady increase with increased percentages of meat extract.

That some salt is desirable in culture media is shown by the results in table 9, the best growths and the most resistant cultures being obtained with the medium containing 0.5 per cent. As the percentage of salt was increased the growth became less luxuriant with a corresponding increase in the phenol coefficient. As in the case of meat extract, the increased coefficient is partly due to the inhibitive action of the larger amount of salt upon the exposed organism rather than to an actual lowering of its resistance. In this connection it is of interest to note that, as Liebig's Meat Extract contains over 30 per cent of inorganic salts and Witte's peptone 3 to 10 per cent, a medium made up of 3 grams of the former, 10 grams of the latter, and 5 grams of NaCl would have an inorganic salt concentration closely approximating that of physiological salt solution. This is significant in view of the fact that this medium, of all those tested, has been the most favorable for the growth of the *Bact. typhosum* and has in every respect given the most uniform and satisfactory results.

A medium such as the above, in which no attempt is made to adjust the acidity, always has, in our experience, a P_H value of 6.3 to 6.6 which has been shown to be within the zone of hydrogen ion concentration most conducive to uniform and reliable results. The percentages of peptone, meat extract, and salt are those which have been found most favorable for a normal unrestricted growth of the test organism, as well as least liable to introduce disturbing variations. The phenol coefficient obtained with this medium is considerably lower than that given by either of the old methods, and hence may be considered un-

desirable by some manufacturers of disinfectants. However, if all manufacturers would guarantee their products upon the same basis, it is hard to see where anyone would suffer an injustice.

The most important need in a standard method for control of disinfectants is, of course, a reliable procedure for the accurate determination of the relative germicidal strength of disinfectants of the same class, and it makes no particular difference whether the results be high or low, so long as the results are uniform and the actual relative differences between different products are clearly shown. This seems to be easily accomplished if a culture medium such as the above be employed. Up to the present time more than 150 different determinations of phenol coefficients upon several disinfectants have been made with this medium, including the use of 50 different batches of media and 12 lots each of Witte's peptone and Liebig's extract of meat. The results of these tests have been very satisfactory throughout; the experimental error shown by duplicate tests has never exceeded 8 per cent and has almost always been much less than 5 per cent. It is hoped that, provided these results are confirmed, such a culture medium may be adopted as an official standard.

The writer is of the opinion that it is of little importance whether one employs the Hygienic Laboratory or the Rideal-Walker technique, provided a proper culture medium be used; personally, we are slightly inclined to favor the Hygienic Laboratory procedure and feel that the results obtained with it are slightly more uniform than with the Rideal-Walker. It is also quite possible that some entirely new technique may be developed which will prove superior to either of the present procedures. Whether old or new technique finally prevail, a reliable culture medium is the first essential.

Witte's peptone, owing to present European conditions, is very hard to obtain, and the small quantities available are almost prohibitive in price. As it is uncertain when a new supply will be available upon the American market, it would be of the utmost value if a product of American manufacture could

be found which might safely be substituted for Witte's. In order that data on this subject might be available for the use of the Committee on Standardization of Disinfectants of the American Public Health Association a study of various American peptones was undertaken, in conjunction with Prof. Earle B. Phelps, Chairman of the above committee.

The different manufacturers were asked to submit samples representing as many different batches of their product as possible. In all, fifteen samples from four manufacturers were received, all of which were examined in comparison with Witte's peptone. For this work media were prepared from the different peptones, using the formula previously described as having given the most uniform results with Witte's; namely, 10 grams of peptone, 3 grams of Liebig's extract, and 5 grams of salt to each liter of distilled water, no attempt being made to adjust the acidity. The details of the experiments were in each case the same as those employed in the previous work. The influence of the different peptones upon the resistance of *Bact. typhosum* to phenol and to a coal tar disinfectant is shown graphically in Chart III. The solid portions represent those dilutions which kill in two and one-half minutes, while the shaded portions represent those dilutions which kill at fifteen minutes. The results of four separate tests with each lot of peptone are shown. The hydrogen ion concentrations, as well as the titratable acidities to phenolphthalein, are indicated on the chart. For comparative purposes similar results obtained with different lots of Witte's peptone are also included.

It will be noted that the hydrogen ion concentrations of the medium prepared with Armour's peptone varied from $P_H = 5.9$ to $P_H = 6.3$, thus practically falling within the zone where our previous work would lead one to expect uniform and reliable results. With these media the preliminary growth of the test organism was luxuriant. The resistance of the test organism, both to the action of phenol and to disinfectant B is remarkably uniform and in close agreement with the results obtained with Witte's peptone. In so far as can be determined from the limited number of samples examined, different lots of Armour's

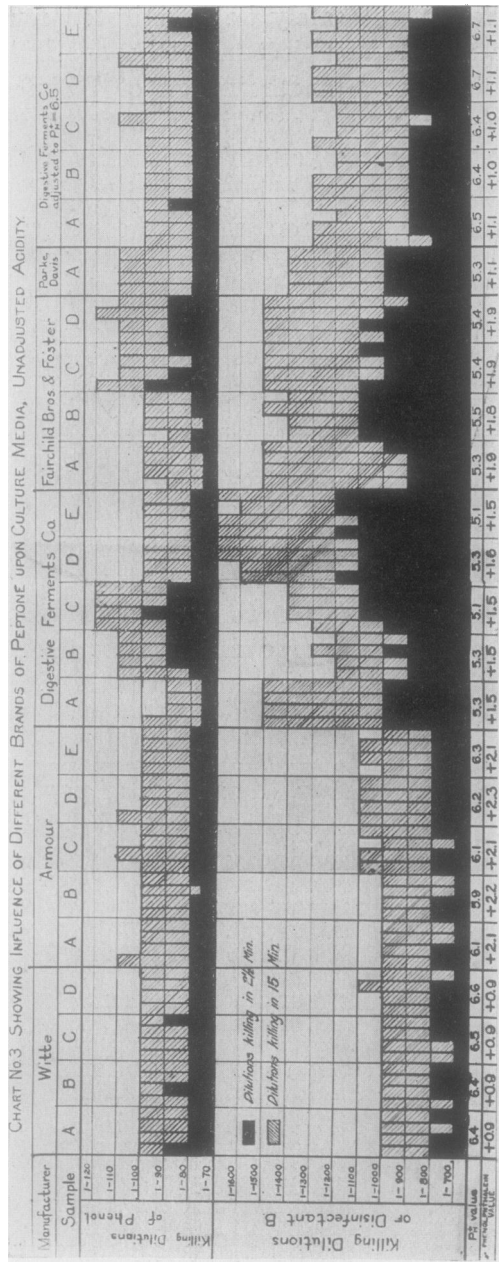
Chart No 3 Showing Influence of Different Brands of Peptone upon Culture Media, Unadjusted Acidity
Manufacturer
Witte
Armour
Digestive Ferments Co
Fairchild Bros. & Foster
Parke, Davis
Digestive Ferments Co adjusted to Pt=6.5
Sample
A B C D A B C D E A B C D E A B C D A A B C D E
Killing Dilutions of Phenol
1-120
1-110
1-100
1-90
1-80
1-70
Killing Dilutions or Disinfectant B
1-1600
1-1500
1-1400
1-1300
1-1200
1-1100
1-1000
1-900
1-800
1-700
Dishes killing in 2½ Min.
Dishes killing in 15 Min.
Pt value
Acidity Difference value
6.4 6.5 6.5 6.6 6.1 5.9 6.1 6.2 6.3 5.3 5.3 5.1 5.3 5.1 5.3 5.5 5.4 5.4 5.3 6.5 6.4 6.4 6.7 6.7
+0.9 +0.9 +0.9 +0.9 +2.1 +2.2 +2.1 +2.3 +2.1 +1.5 +1.5 +1.5 +1.6 +1.5 +1.9 +1.8 +1.9 +1.9 +1.1 +1.1 +1.0 +1.0 +1.1 +1.1

peptone seemed to possess a sufficient degree of uniformity, so that in this medium the product may possibly prove a satisfactory substitute for Witte's peptone. It must be remembered, however, that only five samples were examined, and it is quite possible that other lots may not prove so satisfactory.

Media prepared from the 5 samples of peptone furnished by the Digestive Ferments Company had a hydrogen ion concentration of from 5.1 to 5.3; media prepared from the 4 peptones furnished by Fairchild Brothers & Foster had a hydrogen ion concentration of from 5.3 to 5.5; while that from the one sample furnished by Parke, Davis & Company[2] had a hydrogen ion concentration of 5.3. It will be seen, therefore, that the acidities of these media lie outside of the zone in which uniform results were obtained with Witte's peptone, and as might be expected from the high acidity the results obtained with these products were much less satisfactory than those obtained with Armour's. The resistance of the test culture to the action of phenol was irregular and as a rule quite low. The resistance to the action of disinfectant B was also irregular and at the same time was very much less than when either Armour's or Witte's peptones were used. It is of interest to note that while all of these products were practically completely soluble in cold water, there was almost invariably a heavy precipitate in the finished sterilized medium, and that the growth of *Bact. typhosum* obtained was in all cases much less luxuriant than that given by either Armour's or Witte's peptones. This checks up very well with our previous results, which indicated that a medium having a hydrogen ion concentration in the neighborhood of 5 was variable in composition and in every way unsatisfactory for a normal development of the test organisms.

It was thought desirable to determine the effect of reducing the hydrogen ion concentration of these media to a point approximating that given by Witte's peptone. For this purpose the titration curves of batches of media prepared from each of the

[2] Only one sample of peptone was furnished by this company and it is understood that the preparation of this product is in an experimental stage and that it is not being marketed.

Digestive Ferments Company's peptones were determined. From these curves the amount of alkali necessary to bring the P_H value to 6.5 was calculated. Various lots of media were then prepared in which the ingredients were mixed and the amount of alkali indicated by the titration curve added, after which the mixture was boiled for fifteen minutes, filtered, tubed, and sterilized. As in the previous experiments, these media were then employed in the determination of the phenol coefficient of disinfectant B. These results are also shown in Chart III together with the final hydrogen ion concentrations, which will be seen to approximate closely the desired point.

The test organism grew luxuriantly, the growth obtained being fully equal to that given with the unadjusted medium prepared from Witte's or Armour's peptone. There was no evidence of any precipitate in the finished media, and as will be seen from the chart the resistance of the test organism was very uniform in all cases, so that the actual variations in coefficient are negligible. It will be noted, however, that the resistance to disinfectant B is still somewhat weaker than was shown with either Witte's or Armour's peptone and as a result the phenol coefficient is somewhat higher. Thus while the adjustment to a P_H value of approximately 6.5 of media prepared from the Digestive Ferments Company's peptone enables one to obtain uniform coefficients, these coefficients are not strictly comparable with those obtained with Witte's and Armour's. It seems quite clear, therefore, that there are differences in peptone other than acidity which are capable of causing variations in the resistance of *Bact. typhosum* to the action of disinfectants. While we have not taken the time to repeat these experiments on Fairchild Brothers & Foster's, or Parke, Davis & Company's peptones, we see no reason why a similar test should not give similar results.

From these results it becomes apparent that with the possible exception of one peptone, the American products cannot be safely substituted for Witte's peptone under the above conditions. It should be borne in mind, however, that these results were obtained upon culture media in which no attempt was

made to adjust the acidity. When the acidity is adjusted by first neutralizing and then bringing up to an acidity of + 1.5 to phenolphthalein entirely different results are to be expected. The results obtained with media adjusted in this way are shown in Chart IV.

A comparison of this chart with Chart III will bring out a remarkable difference in the behavior of the various peptones under these conditions. No better illustration could be desired of the futility of attempting to adjust the acidity of culture media to a definite point by phenolphthalein titration. Here we have sixteen different lots of culture media, all with a phenolphthalein acidity of + 1.5, in which the hydrogen ion concentration varies from $P_H = 4.6$ to $P_H = 7.0$. In the case of Witte's and Parke, Davis & Company's peptones, with which the initial titratable acidities were less than + 1.5, there is a marked increase in the hydrogen ion concentration. The media from Fairchild Brothers & Foster's and Armour's peptones, having an unadjusted acidity to phenolphthalein greater than + 1.5, show when adjusted to this point reduced P_H values. The Digestive Ferments Company's peptone, with which the initial phenolphthalein acidity was practically + 1.5, is not particularly affected except that there is a slightly greater variation in the P_H values of the adjusted media, which again illustrates the fallacy of our present method of adjusting the reactions of culture media.

Chart IV shows the same general relationship between the resistance of the organism to the action of disinfectants and the P_H value of the medium as was found with the unadjusted media given in Chart III. With the exception of greater variability in the phenol results, the resistance of the test organism when grown in media prepared with Digestive Ferments Company's peptone is practically identical with that shown by Witte's peptone. Fairchild Brothers & Foster's and Armour's peptones, owing to their low hydrogen ion concentration, cause a marked increase in the resistance of the test culture.

It will be noted that with adjusted culture media made from the Digestive Ferments Company's peptone the cultures are

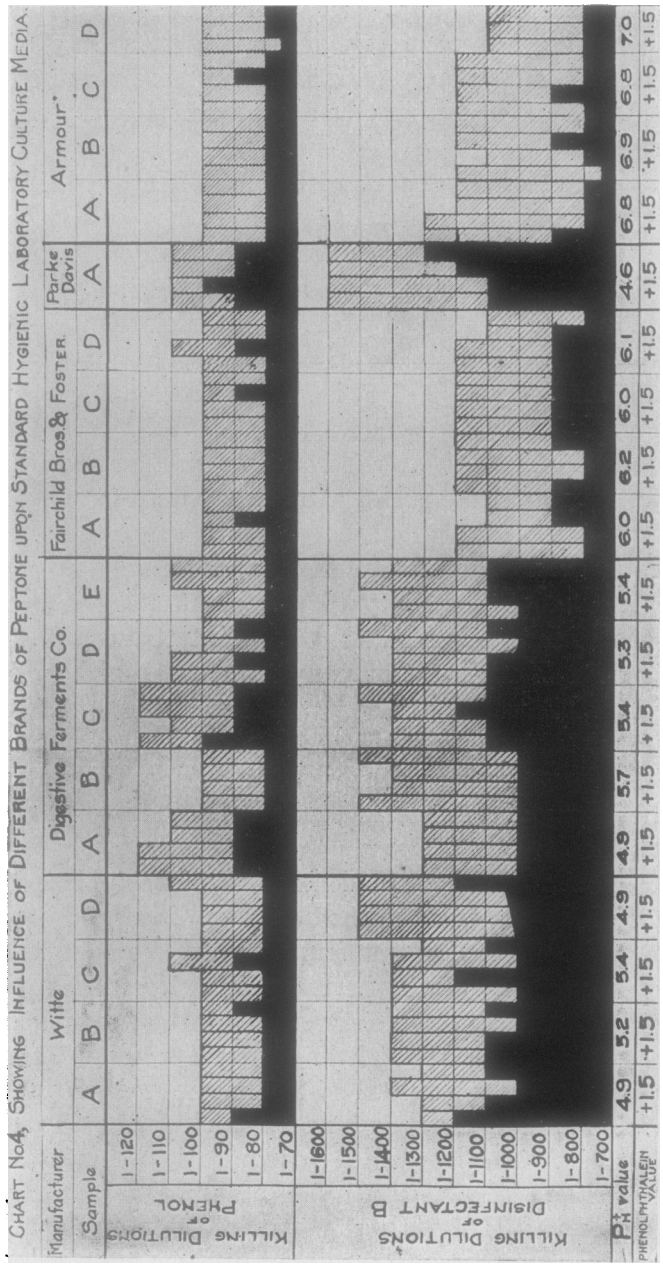
CHART No 4, SHOWING INFLUENCE OF DIFFERENT BRANDS OF PEPTONE UPON STANDARD HYGIENIC LABORATORY CULTURE MEDIA.
Manufacturer
Witte
Digestive Ferments Co.
Fairchild Bros. & Foster.
Parke Davis
Armour.
Sample
A B C D A B C D E A B C D A A B C D
KILLING DILUTIONS OF PHENOL
1-120
1-110
1-100
1-90
1-80
1-70
KILLING DILUTIONS OF DISINFECTANT B
1-1600
1-1500
1-1400
1-1300
1-1200
1-1100
1-1000
1-900
1-800
1-700
PH value 4.9 5.2 5.4 4.9 4.9 5.7 5.4 5.3 5.4 6.0 6.2 6.0 6.1 4.6 6.8 6.9 6.8 7.0
PHENOLPHTHALEIN VALUE +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5 +1.5

slightly more resistant and at the same time more uniform than with the unadjusted media. This certainly cannot be explained on the basis of hydrogen ion concentrations and the difference is too great to be laid to accident. This phase of the problem is worthy of careful study and involves painstaking investigations

TABLE 10

Showing phenol coefficients of disinfectant B obtained with different peptones

PEPTONE		AMOUNT OF GROWTH SHOWN IN PRELIMINARY CULTIVATION		PHENOL COEFFICIENT OF DISINFECTANT B AVERAGE RESULT OF FOUR TESTS	
		Medium adjusted	Medium unadjusted	Medium adjusted	Medium unadjusted
Witte	A	Light	Heavy	13.28	9.72
	B	Light	Heavy	13.45	9.72
	C	Light	Heavy	12.97	9.58
	D	Light	Heavy	13.28	10.00
Digestive Ferments Company	A	Medium	Light	11.21	14.92
	B	Light	Light	13.61	10.88
	C	Light	Light	12.75	11.52
	D	Light	Light	13.44	15.41
	E	Light	Light	13.78	15.83
Fairchild Brothers & Foster	A	Heavy	Medium	10.95	13.79
	B	Heavy	Medium	11.38	14.06
	C	Heavy	Medium	11.56	12.59
	D	Heavy	Medium	10.94	12.34
Armour	A	Heavy	Heavy	11.52	9.58
	B	Heavy	Heavy	11.04	9.79
	C	Heavy	Heavy	11.09	10.13
	D	Heavy	Heavy	10.58	10.41
	E	Heavy	Heavy		10.22
Parke, Davis & Company	A	Medium	Medium	13.21	12.70

into the chemistry of peptones. It does not, however, come within the scope of the present paper.

The average phenol coefficient obtained with each of the above peptones is shown above in table 10.

From a study of these results it seems clear that, with the possible exception of Armour's, none of the American peptones

could be safely substituted for Witte's in unadjusted culture media. The results obtained with Armour's peptone are fairly uniform and are in sufficiently close agreement with Witte's to warrant a more extended investigation of this product and to justify the hope that it may prove a satisfactory substitute for the imported peptone for use in unadjusted media, which we trust may soon be adopted as a standard for this work.

It will undoubtedly be some time, however, before such a change can be made in the official procedure. It would be of value, therefore, if an American product could be found which would give results comparable to and at least no worse than those given by Witte's peptone in the present Hygienic Laboratory media. A study of our results will show that the Digestive Ferments Company's peptone is the only one which could possibly fulfill these requirements and even with this the results are much less uniform than with Witte's. In justice to this product it should be stated, however, that the results given for Witte's peptone are from lots of media selected from a long series of tests and represent the average results which may be expected with this product, rather than the actual limits of variability. It should also be pointed out that these variations do not represent actual differences between the different samples of the same product. We have found in different batches of media made from the same bottle of Digestive Ferments Company's peptone variations fully as great as are shown here with different samples. The trouble does not appear to be due so much to actual variations in the peptone itself, as to the fact that with a high hydrogen ion concentration it is practically impossible to control the procedure so as always to produce solutions of uniform characteristics, even from identical materials.

SUMMARY

The essential points brought out by this work may be summed up as follows.

1. Variations in culture media are the cause of the majority of the discrepancies obtained in the bacteriological examination of disinfectants.

2. There is no indication that these variations in culture media are in any way due to lack of uniformity in Witte's peptone. Liebig's Extract of Meat, however, should be regarded with suspicion.

3. The greater part of the difficulty lies in our present methods of adjusting the acidity.

4. The hydrogen ion concentration of a culture medium has important influences upon its composition and upon its suitability for the growth of *Bact. typhosum.*

5. There is a marked relationship between the hydrogen ion concentration of the culture medium and the resistance of the test organism to the action of disinfectants.

6. The most satisfactory and uniform results have been obtained with a culture medium in which the P_H value falls between 6 and 7. This condition is easily obtained with a medium containing 10 grams of Witte's peptone, 3 grams of Liebig's meat extract, and 5 grams of salt, boiled fifteen minutes, filtered, tubed, and sterilized, with no attempt to adjust the acidity.

7. It has been found that 3 grams of meat extract are ample and that an increased amount is liable to cause disturbing variations.

8. It has also been found that 10 grams of Witte's peptone is sufficient for all the needs of the test culture.

9. The best results have been obtained with a medium containing 5 grams of salt per liter, an increase of salt content causing an increased coefficient due to the inhibitive action of the salt upon the exposed organism.

10. Of the different brands of American peptone only one has been found which could possibly be safely substituted for Witte's in the unadjusted medium. Insufficient samples, however, have been tested to enable one to make this statement with certainty.

11. The Digestive Ferments Company's peptone gives results most closely approximating those of Witte's when used in standard Hygienic Laboratory media.

In conclusion it should be stated that this work was made

possible by the financial support and interest of the West Disinfecting Company through their chemist, Dr. W. Dreyfus. The writer's appreciation is also due to Dr. H. D. Pease of the Lederle Laboratories for his interest in the work, and to Prof. E. B. Phelps of the Hygienic Laboratory, Washington, D. C., who very kindly had many determinations of hydrogen ion concentration made in his laboratory.

Since this article was written the Digestive Ferments Company and Fairchild Brothers & Foster have submitted new samples of peptone based upon the results of our experimental work. In these peptones they have attempted to standardize the hydrogen ion concentration so that the finished media with unadjusted acidity will have a desirable P_H value. We are at present examining these products, and while we have not as yet done a sufficient amount of work to warrant drawing definite conclusions, the present indications are that the new products will prove much more satisfactory than the old ones.