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On a colorimetric method of adjusting bacteriological culture media to any optimum hydrogen ion concentration.

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In most bacteriological laboratories of this country adjustment in the reaction of culture media by titration has largely replaced all other methods. The indicator most commonly employed is phenolphthalein, and the results are expressed in terms of the amount of normal alkali necessary to bring one liter of the medium to the desired reaction (Fuller Scale).

Recent studies have shown that the titrimetric method in its present form is inaccurate. The results of titrations done in this laboratory support the observations of Clark[1] that media titrated to the end point of phenolphthalein and corrected to definite degrees on the Fuller scale have different hydrogen ion concentrations.

An exact knowledge of the reaction of a medium can be gained only from a determination of its hydrogen ion concentration. It is our purpose to present a simple colorimetric method which makes possible the accurate and rapid determination of the hydrogen ion concentration of culture media and their adjustment to any optimum concentration of ionized hydrogen.

For our work we have made use of a set of standard solutions recommended by Levy, Rowntree, and Marriott[2] for determining the hydrogen ion concentration of the blood. These consist of standard phosphate mixtures containing phenolsulphonephthalein. The advantages of this indicator have been set forth by these workers.

The medium is tested first to ascertain what its ionization is before adjustment. This preliminary test can be carried out quickly: to 3 c.c. of fluid is added 0.3 c.c. of a 0.01 per cent.

[1] Clark, W. M., *Jour. Infect. Dis.*, 1915, XVII, 109.

[2] Levy, Rowntree, and Marriott, *Arch. Int. Med.*, 1915, XVI, 389.

solution of phenolsulphonephthalein, the fluid being read directly in the comparator.[1] In most instances the culture fluid has been roughly adjusted by the usual methods so that its reaction falls within the limits of the scale (pH^{+} = 6.4 to pH^{+} = 8.4).

If the medium has not received a preliminary adjustment of reaction, it may be too acid or too alkaline to be read directly. In that event a specimen of the medium is titrated as follows: to a 3 c.c. sample is added *n*/20 acid or alkali solution[2] depending upon the initial reaction of the medium, until a color is obtained which corresponds to the hydrogen ion concentration desired. The conversion of the amounts of *n*/20 solutions read on the pipette into *n*/1 solutions is made by referring to a curve plotted for an average medium in which the amounts of the *n*/20 solutions required are plotted as abscissæ and the corresponding amounts of *n*/1 solutions as ordinates. Final adjustment in reaction must be made with sterile acid or alkali in order to avoid the change in ionization caused by sterilization.

To illustrate the accuracy of the method the results of the titration of five media are given in the table.

TABLE.

No. of Exp.	Date.	Medium.	Titration by Fuller Scale.	Preliminary Test.	Standard Desired.	*n*/20 Alkali in c.c.	*n*/1 Alkali Added per 25 c.c.	Value of pH Obtained.
1	Aug. 6	Veal infusion	1.0	Below 6.4	7.5	0.46	0.225	7.45
2	Aug. 7	Plain broth	0.8	6.9	7.6	0.17	0.073	7.55
3	Aug. 12	Liebig's broth	0.8	6.9	7.6	0.195	0.087	7.55
					7.7	0.20	0.095	7.7
4	Aug. 16	Plain broth	0.8	7.15	7.6	0.13	0.06	7.6
					7.9	0.195	0.088	7.9
								8.15
5		Extract	1.0	6.9	7.5	0.08	0.04	7.55
					7.9	0.21	0.096	7.9

[1] In order to make direct comparisons possible even in the presence of the natural color of the fluid tested, we have constructed a simple device whereby the medium tested serves as a background for the standard test color to which it imparts its own characteristic quality of color.

[2] In order to keep the concentration of indicator during titration the same as its concentration in the standard comparison tubes (0.3 c.c. to 3 c.c. or 1 to 11) the solutions of *n*/20 acid and alkali are so made up that one eleventh of their volume is indicator solution. The solutions are kept in vessels protected against light, air, and moisture, and the apparatus so arranged that the solution can be delivered directly into a graduated one cubic centimeter pipette provided with a ground glass stopcock on the principle of a burette.

The colorimetric method will be found of great value in the adjustment of the hydrogen ion concentration of media for organisms which are sensitive to the reaction of their culture fluids. The method is comparable in a way to the fine adjustment of a microscope. The method of titratable acidity serving only to adjust media coarsely for the growth of the average organism.

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Sarcoma occurring in a guinea-pig.

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On May 10, 1915, a large male guinea-pig which had seemed to be in good health in the morning became suddenly ill in the afternoon and died within a short time. The animal had been injected some months previously with a culture of what was supposed to be diphtheria bacilli, but it had survived the injection. At autopsy a large freely movable mass was found in the mid-line of the neck on the ventral surface, which was adherent to the underlying tissues about mid-way between the lower jaw and the shoulder girdle. The tumor was apparently encapsulated and showed no attachment to the skin. It measured 3½ x 2½ x 2½ cm. in the various diameters, was yellowish in color and quite firm. The capsule was fibrous and cut with some difficulty, but the central portion was quite friable. The cut surface was yellow with many mottled patches which were dark red in color.

Surrounding the tumor and in the right axilla were a number of metastatic nodules, the largest of which measured 1½ x 1 x ½ cm., and the smallest being about the size of a grain of wheat. Section of the larger nodules showed a cut surface which was identical with that of the large tumor.

The thoracic and abdominal organs showed nothing unusual.

On microscopic examination the body of the tumor is seen to consist of round and ovoid cells which vary considerably in size. In places the cells are closely packed together but in others they are separated by a reticulum of connective tissue. There are