BUFFER EFFECTS OF TAP-WATER IN THE ESTIMATION OF CARBON DIOXIDE BY CHANGE IN HYDROGEN-ION CONCENTRATION.—

The hydrogen-ion concentration method of measuring respiration, as described by Haas (1), involves a consideration of the buffer effects of the reagents employed. In this method the respiring material is placed in water containing an indicator, and the time taken to change the medium from one known P_H value to another is noted. The P_H is determined by comparison with standard buffer solutions having the same concentration of indicator and contained in tubes of the same dimensions as the tube with the respiring material. After several closely agreeing determinations have been made, a solution of the reagent is substituted for the water and the effect noted. In this way the times taken to do the same amount of work are compared: that is, a measure of the reaction velocities is obtained. The normal rate of respiration is taken as the reciprocal of the time required to effect the standard change of P_H in water alone, and the rate under the influence of the reagent is expressed as per cent. of the normal.

Owing to the known injurious effect upon organisms of prolonged exposure to distilled water, tap-water is frequently used in these experiments. As a preliminary to a study of the effect of the hydrogen-ion concentration of the medium on the respiration of Wheat seedlings, the writer has made an investigation of the buffer effects of tap-water which brings out some points of interest.

Method. Water from the Edinburgh main supply was boiled in a 'Resistance' glass flask until free from carbon dioxide. The flask was closed with a clean stopper while still boiling, and allowed to cool to room temperature before being used. The temperature at which the experiments were performed was $16^{\circ}\text{C.} \pm 1$. This boiled water was titrated with a standard solution of carbon dioxide, prepared as follows: distilled water was charged with carbon dioxide under pressure in a 'Sparklet' siphon; 10 c.c. of this solution were mixed with 30 c.c. distilled water. This standard solution was approximately saturated (a saturated solution of CO_2 at 25°C. has a P_H of 4.8) and showed a P_H of 5 ± 0.05 with methyl red, and did not vary appreciably in four hours' exposure to the laboratory air in an open beaker. As each determination occupied about twelve minutes, the CO_2 solution may be assumed to be constant for that period. With a pipette of fine bore, graduated to 0.01 c.c., and provided with a rubber bulb, it was possible to drop accurately 0.05 c.c.

10 c.c. of the boiled tap-water were taken in a Jena glass test-tube ($4'' \times \frac{1}{2}''$) fitted with rubber tubing at the mouth to enable it to be clamped off easily. Three drops each of phenol red (1 in 10,000) and thymol blue (1 in 1,000) were added, and the resulting colour matched with a set of borate buffers made up according to Palitzsch's directions (2). These gave a range of P_H values from 9.24 to 6.77, and with the above mixture of indicators a well-graded and easily read series of colours was obtained. The boiled tap-water gave a very constant upper limit of P_H 9.24. One drop of the CO_2 solution was added, and the tube at once clamped off and quickly inverted. A small bubble of air included acted as stirrer. (Any error introduced in this way was a constant one.) This was repeated until the next (more acid) buffer standard in the series was matched, and the procedure continued until P_H 6.77 was reached

In order to discount as far as possible variations in the salt-content of the tap-water due to dilution by rain, &c., sets of experiments were made over a period of four weeks. There appeared to be no substantial variation from this cause.

Titration curve of boiled tap-water with carbonic acid of P_H 5 ± 0.05 . The curve represents the mean of nine experiments. Probable error of the mean, less than 2 per cent. of the mean, except for points corresponding to P_H 8.69 and 8.08, where it is 5 per cent. of the mean. Indicators: thymol blue (1 in 1,000) and phenol red (1 in 10,000), three drops of each to 10 c.c. water. Temperature $16^\circ C. \pm 1$.

The results may be expressed by the above curve, which is constructed from the mean of nine experiments. It will be seen that the buffer action is least

between P_H 8.98– P_H 7.88, and that the portions of the curve between P_H 9.24–8.98 and P_H 7.88–6.77 have the same slope. It follows that if the same P_H interval is used as standard in all measurements of respiration by this method the buffer action can be disregarded, provided that the same water is used for making up all the reagents: in which case only are all the experiments strictly comparable. If, however, it is intended to test the effect of varying hydrogen-ion concentrations on respiration, care must be taken to choose P_H intervals which represent equal amounts of work done (that is, CO_2 evolved). These can readily be determined from the titration curve. The particular curve presented was developed to fill a practical need, and cannot be regarded as directly applicable to tap-water in other towns, but the method described is offered as simple, practical, and reasonably accurate.

It is obvious that the same method can be used to determine the additional buffer action of reagents whose effect on respiration is being studied. In cases where there is marked buffer action it is necessary to adjust the standard interval to be used in determining the normal, so that the amount of CO_2 required to produce an appreciable change in the P_H of the reagent shall be the same as the normal amount. For example, if it requires 0.05 c.c. of the standard CO_2 solution to change the P^H of 10 c.c. of tap-water from 7.60 to 7.36, and 0.5 c.c. CO_2 solution to change the P_H of 10 c.c. of the reagent by the same amount, then the standard must be taken as the change produced in 10 c.c. tap-water by 0.5 c.c. CO_2 solution (in this particular case, P_H 8.51–7.24).

Precautions. All glass used must be of the best quality. Pyrex glass is the best for all indicator work, but where unobtainable Jena glass makes a reliable substitute. All the glass used in these experiments was carefully cleaned with nitric and chromic acids, then boiled in three changes of distilled water, and finally rinsed with alcohol. The rubber tubing used for closing the titration tube was boiled repeatedly and sealed on to the glass with fresh unused paraffin wax (m.p. 52°). The colours were matched by daylight (north light) against an unglazed white paper.

Conclusions. Tap-water shows a decided buffer action when titrated against a standard solution of carbonic acid.

That this relation can be expressed in the form of a titration curve from which the change in hydrogen-ion concentration produced by equal amounts of CO_2 (produced in respiration or otherwise), at any point on the curve, can be deduced.

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