Di Brom Thymol Sulhpone Phthalein as a Reagent for Determining the Hydrogen Ion Concentration of Living Cells.

By

W. R. G. Atkins, O.B.E., Sc.D., F.I.C.,

Head of the Department of General Physiology at the Plymouth Laboratory.

In work of a physiological or cytological nature it is desirable to ascertain whether the processes and appearance of the dead cell are truly representative of the condition in the living. This is especially true when dealing with the hydrogen ion concentration in a small organism which is of necessity accompanied by a considerable quantity of the medium in which it lives. Haas (1916, 4) accordingly ascertained the reaction of various coloured plant cells, such as those of petals with naturally occurring anthocyan indicators by noting the tints, and changes in tint, seen in living cells and comparing these with the colours given when the various anthocyans were added to buffer solutions of known concentration.

Harvey (1911, 1914), too, sought for indicators suitable for use in living tissues, and made use of neutral red, which had previously been of service to Bethe (1909) in similar work on permeability. Finding it impossible to stain living cells with any other dye which would act as an indicator for acid, Harvey (1915) studied penetration of acids in the tissues of a holothurian *Stichopus ananas*, certain cells of which have a dark red pigment which becomes orange in dilute acid, N/1000 to N/500 HCl, viz. at about pH3.

Extensive use has been made by Crozier (1912–1919) of the blue pigment found in a Bermudan Nudibranch, *Chromodoris Zebra*, which changes from blue to pink at pH5·6 in presence of sea salts as found naturally, at $^5/_8$ M total concentration. From the fact that the animal is normally blue it may be concluded that its reaction is less acid than pH5·6.

Certain sponges were also found by Crozier to show indicator changes from yellow to blue, from scarlet to brown-yellow and from colourless to green as acidity decreased in the respective varieties. Similar properties were exhibited by the pigments of a colonial hydrozoan and of various holothurians. He concludes that the tissues of marine animals are in

general more acid than the surrounding sea water, since the pigments appeared in the animals studied to denote reactions lying between pH6·0 and pH7·6.

Heidenhain (1907) and Ehrlich (1910) have reviewed the subject of the staining of the living cell, and the last edition of Lee (1921) mentions no addition to their lists of useful reagents for this purpose.

By comparing these lists with those given by Clark (1920) as indicators selected by Sörensen and other workers, the following are found to combine both properties :—

Substance.	Range pH.	Notes.
Methyl violet, 6B.	0·1–3·2	Slight penetrating power
Methyl orange	3·1–4·4	
Congo red	3–5	Rejected by Sörensen as indicator
Lackmus (lacmoid)	4·4–6·2	
Neutral red	6·8–8·0	Penetrates very rapidly. Has also a blue-red change in 2N to N acid
Cyanin	7–8	
Hæmatoxylin	0–1·0 and 6·0–11·0	
Alizarin	10·1–12·1	Very unreliable indicator

For work on living cells some substances in the list may be suitable as stains, but not as indicators, since they are outside the range to be studied. Neutral red is, however, available, and its changes in the Clark and Lubs standard buffer mixtures were studied. From pH6·6–7·0 it gives a good clear red, decreasing in intensity ; at pH7·2–7·4 it is reddish, at pH7·6 it is a dirty reddish, and at pH8·0 it is orange-red, beyond which it becomes more yellowish. Thus it may be seen that around the neutral point the changes are not such as to enable one to judge accurately the decimal points of the pH values in a tissue, though pH8 may be distinguished from the neighbourhood of pH7. That is to say, it is possible to judge whether the reaction of a cell or tissue is closely the same as that of sea water, pH8·2 approximately, or whether it has a reaction which is perceptibly different. That the latter is the case may be seen at once when organisms such as *Pleurobrachia pileus*, *Clytia Johnstoni*, medusa stage, and *Tiara pileata* are placed in dilute neutral red in sea water. Even when so dilute as to be imperceptible in a green glass jar the stain is taken up with remarkable rapidity, so that in five or ten minutes the bases of the tentacles, the canals and other structures are shown up with beautiful vividness in red. The tint appears to be in the neighbourhood of pH7, but beyond that it is not possible to judge. Lightly stained specimens remained actively motile in jars in the laboratory for three days, more deeply

stained specimens lived actively for a day, so the reaction indicated is that of the normal state.

In order to define the reaction more precisely the newer indicators selected by Clark and Lubs were tried. No trace of di-ethyl red was taken up even from a bright yellow solution, nor was cresol red able to penetrate when colouring the water deeply. *Pleurobrachia* and *Clytia* lived for two days in the former stain and one in the latter without showing any effects of toxic action.

Clytia was tried also in brom thymol blue, drops of a 0·02 per cent solution being added to sea water to produce a faint but quite perceptible blue tint. The indicator penetrated slowly and the manubrium, ocelli, and bases of the tentacles became a light green, judged to be pH6·6. The specimen was lost in course of transference to fresh sea water to observe the general staining. Experiments were continued on *Tiara*. When in light blue indicator for sixteen hours no trace of colour was taken up, as judged by examination in sea water without indicator. On increasing the indicator till a deep blue was produced and allowing a further sixteen hours to elapse the specimen was seen to be light yellowish green pH6·4, or possibly 6·2, in the circular canal of the mantle and in the tentacles, which were still actively motile. The umbrella was a good light blue, pH7·2 or over, but as the indicator changes only in intensity from this onwards to pH7·6, it was not possible to affirm that the reaction was not more alkaline than pH7·2. Experience with neutral red though shows that the reaction is nearer pH7 than pH8. It is possible that the mantle was pathologically permeable when blue, as medusæ may be observed pulsating even when the mantle has largely disintegrated.

Brom cresol purple was also tried, as a stain for *Vorticella*, but though non-toxic in the concentrations used it failed to penetrate. Neutral red, however, stained it deeply, the colour indicating pH7, or probably rather less. These deeply stained specimens were actively motile after sixteen hours, when observations were discontinued.

SUMMARY.

1. Brom thymol blue may be used in dilute solution for ascertaining the hydrogen ion concentration of certain marine organisms. It penetrates slowly, but the stained portions remain actively motile, so its toxic action does not appear to be great at the dilutions found serviceable.

2. The animals studied gave values from pH6·2 to about pH7·5, though possibly the more alkaline end of the range may be pathological. About pH0·2 should be subtracted from these figures for neutral salt error. The sea water used was initially at pH8·2, corrected.

REFERENCES.*

CLARK, W. M. 1920. The determination of hydrogen ions. Baltimore.

CROZIER, W. J. 1912–19. See Clark, also collected papers reprinted in Contributions from the Bermuda Biol. Sta., Vols. III and V.

EHRLICH, P., etc. 1910. Enzyklopädie d. mikroskop. Technik, **2**, 589. Berlin.

HARVEY, E. N. 1914. The relation between the rate of penetration of marine tissues by alkali and the change in functional activity induced by the alkali. Carnegie Inst. Wash., Publ. No. 183. Tortugas Lab. Papers, Vol. VI.

—— 1915. The permeability of cells for acids. *Loc. cit.* No. 212 and Vol. VIII.

HEIDENHAIN, M. 1907. Plasma und Zelle. Die Vitalfarbstoffe, s. 436. Jena.

LEE, A. B. 1921. The microtomist's vade-mecum. 8th Ed. London.

* Those given in Clark's bibliography are omitted from this list.