THE USE OF TRICINE BUFFER IN ANIMAL TISSUE CULTURES

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INTRODUCTION

The use of a nonvolatile buffer system is advantageous for pH control in animal cell cultures because it not only eliminates the necessity for a constant carbon dioxide environment but also facilitates the manipulations of cell populations during growth, maintenance, and experimentation without the concomitant fluctuations in pH of the medium.

In 1966, Good et al. (1) described the preparation of several amine buffers intended for use in biological systems. Among those mentioned, Tricine, HEPES[1] and TES[2] all had pK values in the physiological range for tissue culture. Tricine (*N*-Tris (hydroxymethyl) methylglycine) has a pK_a of 7.8 (37°) and a $\Delta pK_a/°C$ of -0.021 (1); is stable to autoclaving; can be made up at 1 molar concentration as the sodium salt; and is the most economical of the above mentioned buffers. It was, therefore, tested for its suitability in tissue culture media.

While these studies were in progress, HEPES and TES were reported to be effective buffers for HeLa and several other cell lines (2).

[1] HEPES: *N*-2 hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid.

[2] TES: *N*-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid.

MATERIAL AND METHODS

Tricine was obtained from the California Biochemical Corp. (Los Angeles) and Swim's S77 powder (without sodium bicarbonate) from Grand Island Biological Co. (Grand Island, N.Y.). Medium "213" was prepared as a modification of Swim's S77 medium (3). It was prepared by the NIH Media Unit by dissolving per liter of distilled water: 9.0 g of S77 powder in distilled water and then adding 0.5 g of sodium bicarbonate, 2.0 g of additional glucose, 0.05 mmoles of cystine, 20 mg of phenol red, 60 mg of penicillin G, 50 mmoles of Tricine adjusted to pH 7.6 (at 37°) with sodium hydroxide; fetal calf and bovine serum (previously heated at 57° for 45 min) were added to 5% final concentration each. The medium was then sterilized by passage through a millipore filter (0.22 μ). Prior to use, 290 mg/liter filtered glutamine was added. HTC cells (derived from the Morris rat hepatoma 7288C) (4) were cloned in medium 213 containing 0.3% agar in closed polypropylene tubes, as described by MacDonald and Bruce (5). Induction of the enzyme, tyrosine aminotransferase, by the steroid, dexamethasone phosphate, was studied in medium 213 modified by omitting nonessential amino acids and serum. Cells were grown to mid-log phase (about 5×10^5 cells/ml), harvested, washed, and cell extracts were prepared as described by Thompson et al. (4). Tyrosine aminotransferase activity was measured by using the assay of Diamondstone (6), with one unit of activity equal to 1 mμmole of *p*-hydroxybenzaldehyde formed per

minute at 37°. Protein concentration was measured by the method of Lowry et al. (7).

RESULTS AND DISCUSSION

Hepatoma tissue culture (HTC) cells grown in spinner cultures with Tricine-containing medium 213 (in an air atmosphere at 37°), and HTC cells grown under the conditions previously used, i.e. S77 spinner medium in a carbon dioxide atmosphere (4), both had a generation time of about 24 hr and came out of the logarithmic growth phase at a cell density of about 10^6 per ml. The pH of the medium containing Tricine remained at 7.6 (measured at 37°) at low cell densities and only dropped to 7.2 at a cell density of 10^6 per ml. The cell line has not changed its growth characteristics or morphology during 2 yr of maintenance in medium 213. The same medium can also be used for "monolayer" cultures of HTC cells in Falcon flasks.

HTC cells, cloned in an air atmosphere at 37° in medium 213 containing 0.3% agar, had a cloning efficiency of 30–60%. This value compares favorably with that previously reported for HTC cells at a density of 10 cells per ml (4).

HTC cells carry out a differentiated function characteristic of rat liver cells, namely the induction of tyrosine aminotransferase by dexamethasone phosphate (4). The induction of this transaminase by 10^{-5} M dexamethasone phosphate was also studied in HTC cells incubated in an air atmosphere in the modified medium 213 described above. The kinetics of enzyme induction and the maximal specific activity of the induced enzyme were similar to those previously reported for HTC cells (6). In a typical experiment, the basal level of tyrosine aminotransferase was 2.0 units/mg protein, and the induced level after incubation for 19 hr with the steroid was 43 units/mg. During this time period, the control (which lacked steroid) increased only to 2.8 units/mg.

The Tricine-containing medium was found to be particularly useful in experiments in which cells were manipulated outside the incubator. In media buffered by the CO_2-bicarbonate system, drastic changes in pH occur when the cell cultures are removed from the CO_2 incubator. No such pH changes have occurred with medium 213.

CONCLUSION

In this laboratory, Tricine has been introduced into the regular culture medium for HTC cells, and it has been used continuously over a 2-yr period. It appears to be an effective nonvolatile buffer which obviates the necessity for a carbon dioxide atmosphere during the culture and manipulation of these cells. It has been used in all media with no change in the growth characteristics of the cells or inducibility of tyrosine aminotransferase, and no evidence of toxicity has appeared. It is, therefore, recommended that this buffer be more widely used to control the pH of animal tissue cultures.

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REFERENCES

1. Good, N. E., G. D. Winget, W. Winter, T. N. Connelly, S. Isawa, and R. M. M. Singh. 1966. *Biochemistry.* **5**:467.
2. Williamson, J. D., and P. J. Cox. St. Mary's Hospital Medical School, London (preprint obtained from California Biochemical Corporation, Los Angeles, California).
3. Swim, H. E., and R. F. Barker. 1958. *J. Lab. Clin. Med.* **52**:309.
4. Thompson, E. B., G. M. Tomkins, and J. F. Curran. 1966. *Proc. Nat. Acad. Sci. U.S.A.* **56**:296.
5. MacDonald, K. B., and W. R. Bruce. 1968. *Exp. Cell Res.* **50**:471.
6. Diamondstone, T. I. 1966. *Analyt. Biochem.* **16**:395.
7. Lowry, O. M., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. *J. Biol. Chem.* **193**: 256.