

This Week's Citation Classic

Good N E, Winget G D, Winter W, Connolly T N, Izawa S & Singh R M M.
Hydrogen ion buffers for biological research. *Biochemistry—USA* 5:467-77, 1966.
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To the dismay of experimental biologists who did not want to spend time reinvestigating phenomena already worked over, 12 radically different hydrogen ion buffers were introduced. These were consciously designed to have desirable physical, chemical, and biological properties. They were also designed to have buffering (pKa's) in the biologically important range of 6.0 to 8.5. [The *SCJ*[®] indicates that this paper has been cited in over 1,025 publications since 1966.]

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"The idea of tailoring molecules for specific buffering purposes came from a game I used to play with one of my colleagues, Richard O'Brien, at the University of Western Ontario. He was interested in the effects of ionization on the activity of nerve poisons and related drugs. We developed between us a game of guessing pKa's on the basis of the oftentimes rather complex structures of the drugs he was studying. Meanwhile I had been investigating electron transport and ATP synthesis in chloroplast lamellar preparations, and I had discovered that various anions commonly used in buffers could uncouple electron transport from phosphorylation. How then to avoid the anion uncoupling and still have essential control of hydrogen ion concentration? Since the inner salt glycine had absolutely no uncoupling effect, it occurred to me that I might modify glycine in such a manner that its new pKa would bring it into an appropriate buffering range (glycine itself buffers at far too high a

pH for chloroplast research). It seemed obvious that replacing the amino group of glycine with tris(hydroxymethyl)aminomethane (Tris) would produce an amino acid with a pKa rather similar to that of Tris itself. Thus, Tricine was born.

"Subsequently, it also occurred to me that I could use sulfonic acids instead of carboxylic acids and thereby avoid much of the binding of polyvalent metal ions. Consequently, when I moved to Michigan State University, I ordered all of the simple primary and secondary amines commercially available and started making the appropriate N-substituted taurines and glycines, drawing on the intuition developed in the above-mentioned pKa guessing game. Hence the origin of most of the other buffers listed in the paper.

"Almost all experimental biologists and not a few analytical chemists were interested in the new buffers and consequently the paper received instant attention. The fact that the buffers often proved superior to anything before available made their wide use inevitable. It is a measure of their acceptance that the biological and chemical journals now allow the use of the trivial names of several—e.g., HEPES, Tricine—without definition and without reference.

"In a desultory way, I have continued to make and introduce more buffers along the same lines and I have occasionally enlisted the assistance of real chemists. A series of N-substituted 3-aminopropanesulfonic acids was prepared using propane sulfone and, more recently, W.J. Ferguson and I¹ have introduced a series of N-substituted 3-amino-2-hydroxypropanesulfonic acids, all excellent buffers.

"A summary of available biological buffers was compiled in 1972 by me and my colleague, S. Izawa."²

1. Ferguson W J, Braunschweiger K I, Braunschweiger W R, Smith J R, McCormick J J, Wasmann C C, Jarvis N P, Bell D H & Good N E. Hydrogen ion buffers for biological research. *Anal. Biochem.* 104:300-10, 1980.
2. Good N E & Izawa S. Hydrogen ion buffers. *Meth. Enzymology* 24:53-68, 1972.