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This Week's Citation Classic™

Hanks J H & Wallace R E. Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exp. Biol. Med.* **71**:196-200, 1949. [Leonard Wood Memorial, Dept. Bacteriology and Immunology, Harvard Medical Sch., Boston, MA]

During attempts to secure the growth of *Mycobacterium leprae* in cells cultivated from susceptible leprosy patients, an autoclavable balanced salt solution (BSS) was prepared. In long-term cultures, supersaturation with Ca xPi inhibited the growth of host cells and caused precipitates in the explants and embedding chicken plasma. These problems were circumvented by restricting Ca levels to 5 mg percent and Pi to 2.2 mg percent and by maintaining 10:1 ratios of mammalian serum to chick embryo extract. The BSS became known because it was adopted by John Enders *et al*.[1] in their propagation of the polio virus in cell cultures. [The *SCI*® indicates that this paper has been cited in over 1,045 publications since 1955.]

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"This story illustrates the art of producing a quoted paper by being an unremitting failure in one's primary purpose. The steps were: (a) stubborn investigation of a problem that has not yet been solved after 110 years of effort by scores of investigators, (b) making sense by making an autoclavable balanced salt solution (BSS), and (c) helping John Enders *et al.* to develop cell cultures for the propagation of polio virus.

"These accidents happened because in 1939 I had been induced by the Leonard Wood Memorial (American Leprosy Foundation) to investigate the microbiology of leprosy at the Philippine Culion Leprosarium (then the largest in the world), a remote 8 X 25 mile island outpost at the edge of the China Sea, just 12° north of the equator. I was given free rein to search for the central problems in bacteriology or immunology and to investigate the problem I thought most important. It became evident that without cultivating the causative agent, one cannot gain the knowledge required to cope with a stubborn infectious disease.

"After about one year I had shot my bolt of brilliant ideas for inducing M. *leprae* to grow in bacteriologic media. The glowing report of Timofejewsky[2] suckered me into the belief that, if only I could learn to maintain human cells from susceptible patients in tissue culture, a useful (though more costly) tool could be made available. The trials and errors doubled sweat production in that sticky environment. The recommended filter sterilization of high-bicarbonate BSS opened a can of worms. Dissolved CO_2 (and changes in pH) were modified by pressures, temperature, and CO_2 concentrations. I terminated this foolishness[3] by adjusting the ratio of phosphage buffers to pH 6.8 to limit the hydrolysis of glucose, and by including phenol red color indicator so that pH could be seen across the room at any time and by autoclaving. Stimulation of cell respiration was ensured by one-tenth the usual bicarbonate or by the bicarbonate in serum. If interested in tissue cultivation, hundreds of biochemists, microbiologists, or country boys might have done the same.

"The third step arose from a long-standing friendship with Enders at Harvard University. He, Tom Weller, and/or Frank Robbins began coming to my lab to discuss uncertainties or problems in their efforts to obtain standard replication of the polio virus in an optimal cell culture system.[1] Their investigation resulted in replacing $30 monkeys with $.12 cell cultures (and a Nobel prize). One of their rare mistakes was to recommend that Hanks BSS was the way to go.

"The paper including BSS probably owes its popularity to the innate wisdom and laziness of humanity. It is useful to autoclave all possible reagents employed in cell cultivation. If contamination occurs, one has a specific list of components which need not interfere with sleeping at night."

1. **Enders J F, Weller T H & Robbins F C.** Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human tissues. *Science* **109:**85-7, 1949. (Cited 215 times since 1955.)
2. **Timofejewsky A D.** Explantationsversuche von leprösem Gewebe. *Arch. Exp. Zellforsch.* **9:**182-202. 1930.
3. **Hanks J H.** Calcification of cell cultures in the presence of embryo juice and mammalian sera. *Proc. Soc. Exp. Biol. Med.* 71:328-34. 1949.