

**Wolf K.** Physiological salines for fresh-water teleosts.

*Progr. Fish-Cult.* 25:135-40, 1963.

[Eastern Fish Disease Laboratory, US Bureau of Sport Fisheries and Wildlife, Kearneysville, WV]

The work is a spin-off of pioneer developments in fish tissue culture. Values for inorganic constituents of brown trout sera were used to prepare a truly physiological saline for freshwater teleosts. That saline proved to be essentially the same as salines for mammals. [The *SCI*® indicates that this paper has been cited in over 210 publications.]

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This paper was a product of earlier work in lower-vertebrate cell and tissue culture,<sup>1</sup> which gave rise to the first permanent fish cell line<sup>2</sup> and to the isolation of the first fish virus.<sup>3</sup>

Notification of the frequent citation of a 23-year-old report in a relatively obscure US Government serial publication came as a surprise, and, as a result, I reread a now brittle reprint. The scope of the report was somewhat greater than I had remembered; rereading brought to mind earlier days of cell culture. I recalled listening to Wilton Earle of the National Cancer Institute describe how he had developed the mammalian-type balanced salt solution (BSS) that bears his name. Earle designed his BSS to provide physiological levels of glucose and essential inorganic ions in a buffered isotonic solution based on human serum values.

In order to formulate a BSS for teleosts, we at the Eastern Fish Disease Laboratory of the US Bureau of Sport Fisheries and Wildlife needed values for blood of a normal healthy fish, and A.M. Phillips of our bureau's Fish

Nutrition Laboratory obliged us. Phillips chose the brown trout (*Salmo trutta*) as representative of propagated salmonids and carried out the analyses. The values for the trout were found to be very similar to those of humans. When a BSS or physiological saline was formulated on values for constituents of trout serum, the component ingredients and their quantities proved to be much like those of Earle's BSS and Hanks's BSS—two widely used physiological salines.

Because he had derived the trout data, I asked Phillips if the new saline could bear his name; he demurred but accepted the name *Cortland* for the New York town for which the laboratory was named.

The Cortland "salt solution" proved to be wholly appropriate for freshwater teleost cells, but practical considerations limited our particular uses. True, when supplemented with serum and egg ultrafiltrate, the Cortland formulation supported growth of the RTG-2 line of rainbow trout cells. However, perfectly suitable "mammalian type" media and BSS were available commercially and at reasonable prices. It behooved us, therefore, to buy BSS and media for trout-cell propagation and to concentrate on virological applications. Empirical evidence had shown us that cell culture products designed for use with mammalian materials were wholly appropriate for freshwater teleost tissues and cells.<sup>4</sup> In fact, BSS originally intended for mammals is also appropriate for birds, reptiles, amphibia, and teleosts. The cited paper was drafted to dispel popular misconceptions that teleosts are somehow "different" and to document the components of a physiological saline specifically tailored for teleosts.

One of the more vivid recollections of developing the new saline was determination of its freezing point. Never lavishly funded, we could not afford an osmometer. Instead, we were obliged to use a differential thermometer. Reuniting a separated mercury column and setting the instrument for maximum accuracy proved to be a challenge.

1. Wolf K, Quimby M C, Pyle E A & Dexter R P. Preparation of monolayer cell cultures from tissues of some lower vertebrates. *Science* 132:1890-1, 1960.
2. Wolf K & Quimby M C. Established eurythermic line of fish cells in vitro. *Science* 135:1065-6, 1962. (Cited 160 times.)
3. Wolf K, Salessko S F, Dunbar C E & Pyle E A. Virus nature of infectious pancreatic necrosis in trout. *Proc. Soc. Exp. Biol. Med.* 104:105-8, 1960. (Cited 80 times.)
4. Wolf K & Abne W. Fish cell culture. (Maramorosch K, ed.) *Advances in cell culture*. New York: Academic Press, 1982. p. 305-28.