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HORMESIS AND THE SALK POLIO VACCINE

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□ The production of the Salk vaccine polio virus by monkey kidney cells was generated using the synthetic tissue culture medium, Mixture 199. In this paper's retrospective assessment of this process, it was discovered that Mixture 199 was modified by the addition of ethanol to optimize animal cell survival based on experimentation that revealed a hormetic-like biphasic response relationship. This hormesis-based optimization procedure was then applied to all uses of Mixture 199 and modifications of it, including its application to the Salk polio vaccine during preliminary testing and in its subsequent major societal treatment programs.

Key Words: hormesis, biphasic, hormetic, polio, ethanol, Salk vaccine

INTRODUCTION

Jonas Salk is credited with the development of the first effective polio vaccine. After years of research, he developed the vaccine in 1952, with the results of subsequent pilot testing in children and adults published in 1953 (Salk, 1953). The vaccine was later successfully piloted again in children in the Pittsburgh, Pennsylvania area in 1954. Based on these findings, Salk's vaccine was then tested in a very large field trial directed by Thomas Francis at the University of Michigan. The "Francis Field Trial" was the largest medical experiment in history, a test that would include nearly two million children from 44 states. The study involved about 440,000 children receiving one or more vaccine injections, 210,000 children given a placebo while 1.2 million children received no vaccine, serving as an unexposed control group (Lambert and Markel, 2000). On April 12, 1955 the successful results of the field trial were announced (www.sph.umich.edu/about/polioannouncement.html). Soon afterwards the vaccine became licensed and vaccinations to prevent polio were introduced throughout the US.

In order to satisfy the goal of a mass vaccination program involving millions of children within a tight schedule it was necessary to develop the means to standardize and optimize the production of the virus from monkey kidney cells. The object of the present paper is to provide evidence that the tissue culture method used for the production of the Salk vaccine in preliminary testing and societal implementation was optimized

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by Parker *et al.* (1950) using what has proved to be the concept of hormesis. This finding was unexpectedly discovered during an assessment of the occurrence of hormesis in the wound healing process. An aspect of the history of wound healing involves how tissue culture has been used to screen agents for their capacity to enhance the healing process. During this paper's assessment the research of Raymond C. Parker was followed and it led to the unearthing of how he used the hormesis concept to optimize the capacity of the synthetic tissue culture medium, Mixture 199, to support cell proliferation and survival.

HORMESIS AND MIXTURE 199

Hormesis is a dose response phenomenon characterized by a low stimulation and a high dose inhibition (Calabrese, 2008a). The term hormesis was first used by Southam and Ehrlich (1943) to describe how extracts from the red cedar tree affected the metabolism of a number of fungal species. The actual concept of a biphasic dose-response preceded the term hormesis by nearly six decades, having been first reported by Schulz (1887, 1888) based on experiments assessing the effects of multiple disinfectants on the metabolism of yeasts (Calabrese, 2005a; Calabrese, 2009). Hormetic dose responses are now considered to be broadly generalized, being independent of biological model, endpoint measured and the class of chemical inducing agent (Calabrese and Blain, 2005, 2011; Calabrese, 2005b.; Calabrese 2008a; Calabrese 2008b; Calabrese, 2010). Hormetic dose responses also display specific quantitative features of the dose response, with the stimulatory response being modest, typically 30-60% greater than the controls, while the stimulatory range is usually in the 10-20 fold range, starting immediately below the estimated pharmacological or toxicological threshold (Calabrese and Baldwin, 1997, 2001; Calabrese and Blain, 2005; 2009).

Mixture 199 was developed as part of a cancer cell nutrition project under the direction of Parker (Morgan *et al.*, 1950; Morton *et al.*, 1950; Rhodes, 1956). Mixture 199 was a synthetic tissue culture medium without animal serum, offering the advantage of avoiding potential allergenic responses which would be very problematic in a widely distributed vaccine. Salk became aware of Mixture 199 and saw its potential use as an important component in the overall polio vaccine project, using it in the 1952 pilot polio study (Salk, 1953), and in a subsequent large scale test (Rhodes, 1956) and the major societal polio vaccine program.

It was within the context of maximizing the production of the virus that the hormesis concept entered the discussion. During attempts to optimize the functioning of Mixture 199, it was necessary to add a member of the Tween series of surface-active agents. Tweens serve both as a source of water-soluble fatty acids and as a way to dissolve and disperse fat-soluble compounds such as vitamins A and D and cholesterol throughout

the medium. However, before the vitamins and cholesterol could be added to Mixture 199 solution, it was necessary to make an alcoholic (ethanol) stock solution of these agents and to dilute this with Tween. This product yielded a clear solution when highly diluted in the aqueous medium. Follow-up experiments indicated that the survival of the cultured animal cells (i.e., chicken mesenchyme embryo cells), was reduced at certain concentrations of Tween and alcohol (Morton *et al.*, 1950; Morgan *et al.*, 1950). However, if the ethanol concentration was reduced to 0.001% the tissue culture survival returned to normal while the Tween could still be used at its optimal level. Since an adequate synthetic mixture for growing animal cells was believed to require other alcohol-soluble agents, Parker *et al.* (1950) decided it was important to establish the concentrations of ethanol that could be used within Mixture 199 with 0.002% Tween. The Parker study assessed the effects of ethanol upon the survival and outgrowth of fibroblast-like spindle cells from 11-day old chick embryos grown in the synthetic Mixture 199 with a broad range of ethanol concentrations using either roller tube or flask cultures (Parker *et al.*, 1950). In both cases there was a hormetic biphasic concentration-response relationship. The optimal response for these experiments (based on Table 2 and Figures 5-8 of Parker *et al.*, 1950) was in the 0.02-0.50% range, increasing cell survival and cell migration by about 30-50% (Figure 1).

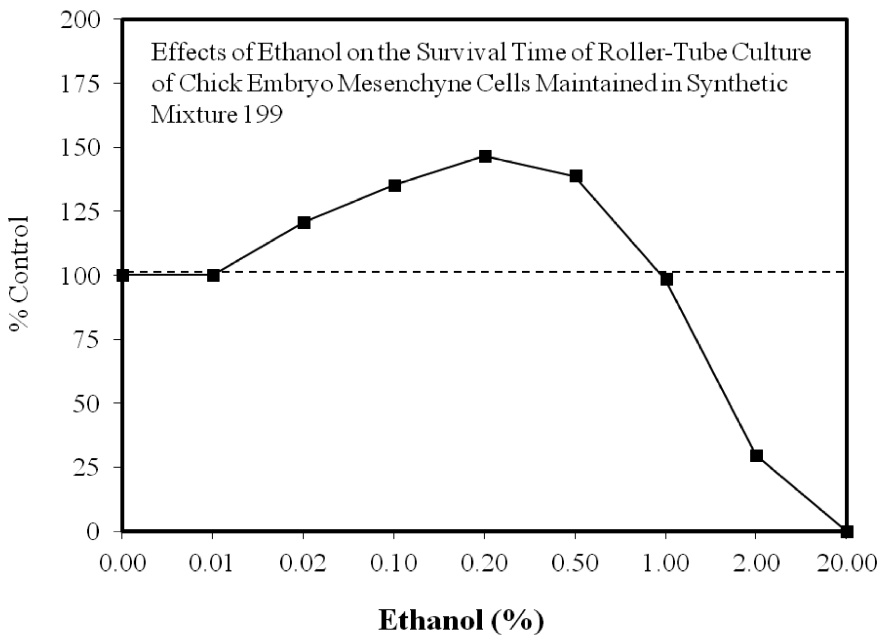


FIGURE 1. Effects of Ethanol on the Survival Time of Roller-Tube Culture of Chick Embryo Mesenchyme Cells Maintained in Synthetic Mixture 199 (Source: Parker *et al.*, 1950).

Parker *et al.* (1950) concluded that ethanol would not adversely affect cultured animal cells when kept < 1%. These findings were seen as important since they established the level of alcohol that could be used safely in tissue culture studies. The findings also provided the basis for changing the concentration of ethanol in Mixture 199 from 0.001% to 0.2%, based on the peak of the stimulatory (i.e. hormetic) response. Mixture 199 would become the culture medium used to grow the monkey kidney cells for the Salk Polio trials and vaccination program. Within a few years, Mixture 199 would become modified and be called H597. However, the concentration of ethanol was retained in the modified H597 (Healy *et al.*, 1971). Although optimizing of Mixture 199 to enhance cell survival was expected to play a key role in the tissue culture performance for the production of the polio virus, there is no evidence to indicate how increasing the ethanol concentration to 0.2% affected the survival of the monkey kidney cells and their capacity to produce polio virus. Decades after its initial role in the Salk Polio Vaccine project, it has been determined that the hormesis concept was practically implemented into the massive manufacturing process that resulted in the production of sufficient vaccine to immunize millions of children against the profoundly harmful effects of polio. What is not explicitly known is whether the increase in ethanol, which had a significant effect on the survival of chicken embryo cells, also had a similar enhancing effect on the monkey kidney cells employed for the polio virus production.

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