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On the deterioration of crystalline strophanthin in aqueous solution.

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For clinical use, crystalline strophanthin is commonly dissolved in normal salt solution or water and marketed in glass ampules. Sterilization is accomplished by autoclaving after the ampules have been filled and sealed. In making biologic assays, by the cat method of Hatcher and Brody, of several lots of a commercial preparation of "ouabain" (g-strophanthin) wide variations in potency were found. On adding a drop of indicator, phenol red, to the contents of those ampules showing low potency, it was observed that they were decidedly alkaline in reaction, whereas freshly prepared, aqueous solutions of the drug are neutral or slightly acid. Experiments were undertaken to ascertain the cause of the deterioration in relation to the altered hydrogen ion concentration and to devise a method for preparing a stable solution for therapeutic purposes.

Doubly distilled water, pH 6.0, was autoclaved in various types of glass bottles and flasks, chosen at random from the laboratory supply. Immediately after autoclaving, the reaction of the water in the cheaper and softer varieties of container had become quite alkaline, the pH ranging from 6.3 to 9.0. In the hard glass flasks (Pyrex) no significant alteration in reaction occurred.

A similar experiment was done with sixteen types of glass ampules, obtained from a number of pharmaceutical firms, and used by them in marketing their products. The distilled water autoclaved in these ampules in every instance showed a change in pH, which now ranged, in different lots, from 6.2 to 9.0. In order to titrate back to neutrality (pH 7.0) the most alkaline solution in the series, 2.6 c.c. of N/50 HCl per 100 c.c. of water were required.

Next, a 2 per cent. solution of strophanthin was made in standard *M*/20 phosphate mixtures with pH 7.0, pH 8.6 and pH 5.0.

Biologically tested, the cat unit of these solutions was found to be 0.107 mg. per kg., their optical rotation $-0.97°$. After autoclaving for 20 minutes at 15 pounds pressure, no alteration in either optical activity or potency was observed in the acid or neutral solutions. The alkaline mixture, however, now had an optical rotation of $-0.93°$ and a cat unit value of 0.152. In short, when strophanthin is autoclaved in alkaline solution (pH 8.6) the molecule is partially decomposed, with resultant alteration in its ability to rotate polarized light and significant reduction in biologic activity.

For bedside use it is convenient to employ crystalline strophanthin in dilute concentration, usually 0.01 per cent. Such a solution autoclaved in a soft glass ampule, which, on heating, gives off enough alkali to alter the reaction of its contents to pH 9.0, becomes biologically practically inert, more than four times the calculated lethal dose having no appreciable effect on the cat's heart. The contents of a hard glass ampule, with no significant alteration in pH after sterilization, retain full potency.

To insure stability of reaction, it is advisable, therefore, to put up solutions of crystalline strophanthin in hard glass (Pyrex) ampules. In order to avoid even slight changes toward the alkaline side, the drug has been prepared for clinical use in $M/50$ standard phosphate solution, at pH 7.0. The buffer action of this mixture can compensate, with a wide margin of safety, for the amount of alkali yielded, on autoclaving, by the softest variety of glass ampule.