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Stability of Antibacterial Preservatives in Parenteral Solutions III

Relationship Between Chemical Loss and Microbiological Activity in Multiple-Dose Vials

By L. LACHMAN, S. WEINSTEIN, T. URBANYI, E. EBERSOLD, and J. COOPER

The loss of preservative content due to degradation in solution and absorption by rubber stoppers was studied by chemical and microbiological analytical methods. The preservatives investigated were chlorobutanol and *p*-chloro-β-phenylethyl alcohol in conjunction with neoprene rubber stoppers. The contribution of rubber extractives and preservative degradation products towards enhanced antimicrobial activity is discussed.

THE LOSS of preservatives from solution due to degradation or absorption by rubber stoppers has been determined by chemical (1–4) and microbiological (4–6) methods. However, there are no reports in the literature quantitatively comparing the loss of preservative as determined chemically and microbiologically. This lack of information can be ascribed to two major factors: (*a*) the time consuming and cumbersome characteristics of microbiological procedures and (*b*) the fact that existing microbiological assay methods have not been of sufficient accuracy or precision to permit valid comparisons.

In a previous investigation (7) a turbidimetric microbiological assay method was described for chlorobutanol and *p*-chloro-β-phenylethyl alcohol. This would now permit a valid correlation between chemical and microbiological determinations of preservative content.

Because of the techniques employed in the manufacture of rubber stock to be used for molding rubber stoppers, it is not uncommon for the rubber to contain appreciable amounts of unreacted accelerators, activators, and antioxidants. It has been recognized that these unreacted materials, as well as certain reaction products (8–12), can be extracted by solutions coming in contact with the stopper. These materials have been shown to exert deleterious effects on active ingredients (13) and antibacterial agents (10) in injectable solutions.

In this study the influence of rubber closure extractives on the microbiological activity of the preservatives chlorobutanol and *p*-chloro-β-phenylethyl alcohol in vial solutions buffered to a pH 4 and stoppered with neoprene closures was determined. In addition, the contribution of preservative degradation products to antibacterial activity was ascertained. These two preservatives were chosen for study because chlorobutanol is representative of one which degrades in solution as well as being absorbed by the closure, whereas *p*-chloro-β-phenylethyl alcohol is representative of a preservative which essentially undergoes no degradation for the duration of study but which is lost from solution by absorption into the closure. Analyses for residual preservative content were performed both chemically and microbiologically and the results compared to determine whether rubber extractives or preservative degradation products influence the microbiological determinations.

EXPERIMENTAL

Materials.—0.275 *M* solution of citric acid–sodium phosphate buffer of pH 4.0; *p*-chloro-β-phenylethyl alcohol, Ciba, b.p. 80–83° at 1.07 mm; chlorobutanol, anhydrous U.S.P.; neoprene poly-

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mer closure No. 704, West Co.; U.S.P. type I, 10-ml. amber ampuls and vials, Kimble Glass Co.; and three-piece aluminum caps for vials Nos. 13–30, West Co.

Equipment.—Bausch & Lomb Spectronic 20 colorimeter; Beckman spectrophotometer model DU; Beckman pH meter model G; and Cary recording spectrophotometer model No. 11.

Preparation of Ampuls and Vials of Preservative Solution.—The rubber closures, vials, and ampuls used in this study were prepared in accordance with the procedures described in a previous publication of this series (14). Solutions of 0.3% *p*-chloro-*β*-phenylethyl alcohol and 0.5% chlorobutanol were prepared on a w/v basis with water for injection buffered to a pH of 4.0. The preservative solutions were then filtered through medium porosity sintered-glass filters. Each preservative solution was filled into 10-ml. amber ampuls and vials. The ampuls were closed by normal pull sealing techniques under an oxygen-gas flame. The vials of each preservative solution were stoppered with the neoprene closures. The stoppered vials were then sealed at a constant head pressure of 60 p.s.i. with a Westcapper. The preservative solutions in ampuls and vials were placed into a constant temperature oven regulated at 60° ± 1.5°. Half of the vials were stored in an upright position and half inverted. At prescribed time intervals, samples were withdrawn and tested chemically for residual preservative content, and microbiologically for residual antibacterial activity.

Analytical Methods.—*Chemical:* The assay methods employed for chlorobutanol and *p*-chloro-*β*-phenylethyl alcohol have been described in an earlier publication of this series (14).

Microbiological: A complete description of the method used for quantitatively determining the residual antibacterial activity of chlorobutanol and *p*-chloro-*β*-phenylethyl alcohol has been presented in the previous paper of this series (7).

RESULTS AND DISCUSSION

The loss of preservative content and its related antibacterial activity was determined by chemical and microbiological methods. Converting the antibacterial activity to preservative concentration permitted a comparison with the residual preservative content measured chemically. This then enabled a valid evaluation of the influence of closure extractives and preservative degradation on antibacterial activity.

TABLE I.—NEOPRENE CLOSURE COMPOSITION AND PHYSICAL PROPERTIES

Composition	Physical Properties
Neoprene polymer[a]	Specific gravity = 1.490
Sulfonated oil	Thickness[b] = 0.125 inch
Calcined clay	Weight[c] = 548 mg.
Barium sulfate	
Zinc oxide	
Iron oxide	
Stearic acid	
Mineral oil	
Imidazoline type accelerator	

[a] Rubber content is 42.4% of total composition. [b] Measured microscopically, and the value is an average of five measurements from different stoppers. [c] Value is an average of ten stoppers.

TABLE II.—PER CENT *p*-CHLORO-*β*-PHENYLETHYL ALCOHOL ABSORBED BY NEOPRENE CLOSURE AT 60°C.

Time, Days	Storage	Chemical Assay	Microbiological Assay
1	Vials upright	3.6	...
	Vials inverted	11.1	...
2	Vials upright	10.6	0
	Vials inverted	14.3	3.7
8	Vials upright	17.9	7.15
	Vials inverted	25.0	17.9
14	Vials upright	25.0	14.3
	Vials inverted	32.1	25.0
21	Vials upright	28.6	21.1
	Vials inverted	35.7	32.1
23	Vials upright	28.6	21.1
	Vials inverted	35.7	32.1
30	Vials upright	28.6	21.1
	Vials inverted	35.7	32.1

The neoprene closures were chosen for this study as a result of earlier data (14) showing that these preservatives exhibited the greatest loss from solution when in contact with these closures. The composition, per cent rubber content, and the physical properties of these closures are given in Table I.

Chemical and Microbiological Results

***p*-Chloro-*β*-phenylethyl Alcohol.**—The data summarized in Table II show the amount of *p*-chloro-*β*-phenylethyl alcohol absorbed by the rubber when the vials are stored at 60° in inverted and upright positions. When plotting these data rectilinearly, as shown in Fig. 1, it is evident from these graphs that at about 21 days the curves develop a plateau. This would indicate that the time required for the absorption to reach an equilibrium concentration is the same for the vials stored in an inverted or upright position. However, the equilibrium concentration of the preservative in the closure is higher for the vials stored in the inverted position as compared with those stored upright.

From the equilibrium portions of the curves it is apparent that the per cent preservative absorbed as determined microbiologically is less than that found chemically. This difference can be attributed to extractives being leached from the rubber stopper and exhibiting antimicrobial activity. When the preservative solutions are assayed microbiologically, the antibacterial activity found is a measure of the biological activity of the residual preservative plus that due to extractives. Therefore, when the microbiological assay data are converted from activity to concentration, it would suggest that less preservative was absorbed. Berry (10) has shown that the presence of tetramethylthiuram disulfide used as an accelerator in rubber compositions exerts a bactericidal effect. The present investigation indicates that the accelerator contained in the neoprene closures, namely 2-mercaptoimidazoline ethylene thiourea, also possesses antibacterial activity.

Since the total concentration of preservative in solution would be expected to be significantly greater than that in the vapor above the solution, there should occur less preservative absorption when the vials are stored upright. That this is the case is shown by comparing the difference between the equilibrium absorption concentrations for the vials stored upright and inverted. As measured

chemically, the difference is about 7% as compared to 11% when measured microbiologically. The 4% difference that exists between these two assay methods must be attributed to extractives being leached from the closure and exerting antibacterial activity. This is confirmed by comparing the equilibrium concentration of preservative absorbed for the vials stored in an inverted position and determined both chemically and microbiologically. The microbiological assay shows about 4% less preservative absorbed when compared with the chemical assay.

Chlorobutanol.—A comparison between chemical and microbiological assays measuring chlorobutanol loss in vial solutions stoppered with neoprene closures and stored at 60° in inverted and upright positions is presented in Table III. It is evident from the degradation data obtained for the ampul solutions that the microbiological assay does not indicate any loss of preservative until there is about a 6% reduction in concentration as measured chemically. It is postulated that this difference is due to the degradation products exerting antibacterial activity. These degradation products were identified by Nair and Lach (15) to be acetone, carbon monoxide, chloride ion, and alpha-hydroxybutyric acid.

It would appear from the data for the vial solutions that the difference between the residual concentration of preservative measured chemically and microbiologically can be mainly ascribed to the antimicrobial activity of the rubber extractives leached from the rubber and to a lesser degree to the antibacterial activity of the degradation products of the preservative.

TABLE III.—PER CENT RESIDUAL CHLOROBUTANOL AS MEASURED CHEMICALLY AND MICROBIOLOGICALLY AFTER STORAGE AT 60°C.

Time, Days	Storage	Chemical Assay	Microbiological Assay
2	Ampul	100	100
	Vials upright	85.6	86.4
	Vials inverted	83.6	88.4
8	Ampul	97.0	100
	Vials upright	69.4	88.4
	Vials inverted	67.4	84.3
16	Ampul	94.5	100
	Vials upright	67.4	78.5
	Vials inverted	63.2	74.5
23	Ampul	92.0	94.0
	Vials upright	65.3	76.5
	Vials inverted	63.2	72.5
30	Ampul	89.4	92.0
	Vials upright	59.2	68.5
	Vials inverted	53.0	68.5

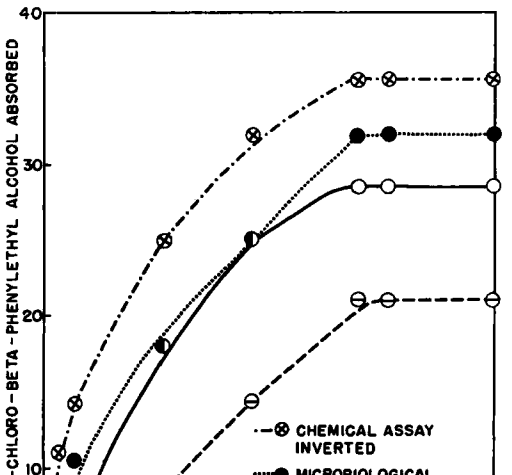

Fig. 1.—The per cent of *p*-chloro-β-phenylethyl alcohol absorbed by the neoprene rubber stoppers from vial solutions stored upright and inverted at 60° as determined chemically and microbiologically.

SUMMARY

This study was undertaken in an effort to determine whether chemical analysis for residual preservative in vial solutions stoppered with rubber closures is a true indication of residual preservative activity. The preservatives evaluated were chlorobutanol which degrades in solution and is absorbed by the rubber and *p*-chloro-β-phenylethyl alcohol which is apparently lost from solution entirely by absorption into the rubber stopper. The data may be summarized as follows:

1. The extractives leached from the neoprene stoppers were found to exhibit demonstrable antimicrobial activity causing the microbiological analysis for residual preservative to be higher than that determined chemically.

2. Although the degradation products of chlorobutanol showed some antibacterial activity, it was substantially less than the extractives leached from the rubber closure.

3. In order to properly determine the influence of rubber closures on preservatives in parenteral solutions, it is important to study the preservative systems both chemically and microbiologically.

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