

# Stability of Antibacterial Preservatives in Parenteral Solutions IV

## Contribution of Rubber Closure Composition on Preservative Loss

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The loss of benzyl alcohol, phenylethyl alcohol, and methylparaben from solution in the presence of natural, neoprene, and butyl rubber stoppers was studied. The influence of closure extractives on the analysis for residual preservative was evaluated. To insure valid determinations of preservative loss, techniques for eliminating the interference of extractives in the analysis were investigated. Partition studies were performed on the preservatives to determine their relative preference for each rubber composition studied.

THE USE of rubber closures for parenteral solutions in multiple-dose vials has resulted in many compatibility problems (1-3). This can be attributed to the complexity of most rubber closure formulations which contain about 40-50% rubber, plus at least seven other components (4). These include vulcanizing agents, activators, accelerators, fillers, pigments, antioxidants, and softeners, all of which are reported to be essential if the stoppers are to possess the desirable physical and chemical properties.

In order to insure complete vulcanization of various type rubbers, excess quantities of accelerators, activators, and other additives are generally used. It has been shown that some of these unreacted agents and reaction products of the vulcanization process can be leached from the closure by parenteral solutions. These materials exert toxic and pyrogenic effects (5, 6) as well as deleterious effects on the stability of the injectable solution (7-10).

In previous investigations conducted at this laboratory (11, 12), rubber closures and rubber extractives were found to significantly influence preservative loss from solution and antimicrobial activity, respectively. In this study an attempt was made to determine (a) the effect of neoprene, natural, and butyl rubber closures on the loss of benzyl alcohol, methylparaben, and phenylethyl alcohol from vial solutions, (b) the manner in which rubber extractives interfere with the assay methods for residual preservative, and (c) the distribution tendencies of the above three preservatives between water buffered to a pH of 4 and each composition rubber stopper.

### EXPERIMENTAL

**Materials.**—Benzyl alcohol, reagent grade, Fisher Scientific Co., methylparaben, U.S.P.; phenylethyl alcohol, Eastman Organic Chemicals; heptaldehyde aniline reaction product, tetramethyl thiuram monosulfide, and zinc dimethyl dithiocarbamate, West Co.; 0.275 M citric acid-sodium phosphate buffer of pH 4.0; natural crepe, neoprene polymer, and butyl polymer rubber stoppers, West Co.; 10-ml. amber ampuls and vials, U. S. P. type I; and three-piece aluminum caps for vials, West Co., No. 13-30.

**Equipment.**—Beckman spectrophotometer model DU; Cary recording spectrophotometer model No. 11; and Beckman pH meter model G.

**Preparation of Ampuls and Vials of Preservative Solutions.**—The ampuls, vials, and stoppers were washed in a manner described in a previous study (11). Solutions of 1.0% benzyl alcohol, 0.2% methylparaben, and 0.5% phenylethyl alcohol were prepared on a w/v basis with water for injection buffered to a pH of 4.0. The solutions were filtered through a medium porosity sintered-glass filter. Each preservative solution was filled into 10-ml. ampuls and vials. The ampuls were closed by customary pull sealing procedures with an oxygen-gas flame. The vials of each preservative solution were stoppered with three different composition rubber closures and then sealed with three-piece aluminum caps at a constant sealing head pressure of 60 p.s.i. with a Westcapper. The preservative solutions in ampuls and vials were placed in constant temperature ovens regulated at 25, 40, 50, and 60° ± 1.5°. Half of the vials were stored upright and half inverted. At designated time intervals, samples were withdrawn and tested for residual preservative content, pH, and physical change.

**Apparent Distribution of Preservative Between Buffer Solution and Rubber.**—The procedure employed has been described in detail in a previous publication (11). This evaluation was done for the three stoppers: neoprene, natural, and butyl rubber, and the preservatives: benzyl alcohol, phenylethyl alcohol, and methylparaben.

### Analytical Methods

**Benzyl Alcohol.**—The concentration of preserva-

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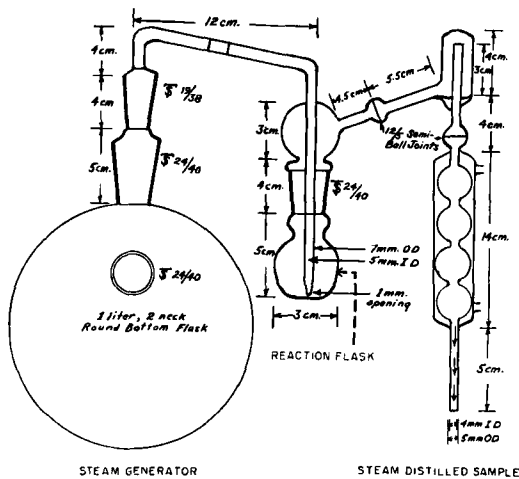


Fig. 1.—Microsteam-distillation apparatus.

tive was determined by pipetting 1 ml. of solution into a microsteam-distillation apparatus (Fig. 1) and the sample distilled to 25 ml. Absorbance was measured at 257  $m\mu$  where the  $A(1\%, 1 \text{ cm.}) = 18$ .

**Phenylethyl Alcohol.**—The concentration of preservative was determined by pipetting 3 ml. of solution into a microsteam-distillation apparatus and the sample steam distilled to 30 ml. The distillate was brought up to volume in a 50-ml. volumetric flask with distilled water. Absorbance was measured at 257  $m\mu$  where  $A(1\%, 1 \text{ cm.}) = 18$ .

**Methylparaben.**—A 3-ml. aliquot was pipetted into a 50-ml. volumetric flask and brought up to volume with methanol. Five milliliters of this solution was transferred to a 100-ml. volumetric flask and brought up to volume with methanol. Absorbance was measured at 256  $m\mu$  where  $A(1\%, 1 \text{ cm.}) = 1115$ .

TABLE I.—BUTYL RUBBER COMPOSITION AND PHYSICAL PROPERTIES

| Composition                                    | Physical Properties                   |
|--|---------------------------------------|
| Butyl polymer <sup>a</sup>                     | Specific Gravity = 1.609              |
| Barium sulfate                                 | Thickness <sup>b</sup> = 0.125 inches |
| Calcined clay                                  | Weight <sup>c</sup> = 597 mg.         |
| Carbon black                                   |                                       |
| Titanium dioxide                               |                                       |
| Zinc oxide                                     |                                       |
| Stearic acid                                   |                                       |
| Paraffin wax                                   |                                       |
| Sulfur   |                                       |
| Thiuram combined with aniline reaction product |                                       |

<sup>a</sup> Rubber content is 36.8% of total composition. <sup>b</sup> Measured microscopically, and the value is an average of five measurements from different stoppers. <sup>c</sup> Value is an average of ten stoppers.

## RESULTS AND DISCUSSION

The stability of several bacteriological preservatives in aqueous buffered solutions stored in multiple-dose vials was studied. The rubber stoppers used in this investigation are representative of three closure compositions frequently employed for multiple-dose vials. The composition, per cent rubber, and physical properties for the neoprene and natural rubber stoppers were presented in a previous paper (11). However, a different butyl stopper was used in place of the one used earlier because there was an obvious physical incompatibility with the preservative solutions used. The composition and physical properties for this closure are presented in Table I.

## Benzyl Alcohol

The influence of neoprene, natural, and butyl rubber stoppers on the stability of benzyl alcohol in aqueous solution buffered to a pH of 4.0 was investigated. The results obtained from ampul and vial solutions are summarized in Table II. It is evident from the data in this table that for the vials stoppered with butyl rubber closures, there appears to be a substantial increase in benzyl alcohol concentration with time instead of the expected loss or maintenance of concentration. This also seems to occur for the vial solutions stoppered with neoprene closures, but to a lesser degree. However, for the vials stoppered with the natural rubber closures, this effect was not observed.

As a result of this unexpected finding, investigations were initiated to determine whether extractives from the rubber stopper were responsible for the apparent increase in benzyl alcohol content. After reviewing the composition of the butyl rubber closure (Table I), it was presumed that the presence of unreacted accelerators or their reaction products would most likely be the causative agents for the difficulties being encountered. If these agents were leached from the closure by the benzyl alcohol solution they could interfere with the analysis for residual preservative content by exhibiting high absorptivity in the wavelength region of benzyl alcohol.

In order to verify this assumption, samples of the primary (heptaldehyde aniline reaction product) and secondary (tetramethyl thiuram monosulfide) accelerators and the reaction product of the accelerators (zinc dimethyl dithiocarbamate) were obtained from the rubber closure manufacturer and analyzed according to the procedure used for the preservative solution. For the heptaldehyde aniline reaction product, 1 ml. was steam distilled in a manner similar to that used for benzyl alcohol, while for the secondary accelerator and the reaction product of the accelerators, separate saturated solutions were prepared in pH 4 buffer and a 3-ml.

TABLE II.—PER CENT RESIDUAL BENZYL ALCOHOL AFTER STORAGE AT 60° C.

| Time, weeks | Ampul | Butyl Rubber |          | Neoprene Rubber |          | Natural Rubber |          |
|-------------|-------|--------------|----------|-----------------|----------|----------------|----------|
|             |       | Upright      | Inverted | Upright         | Inverted | Upright        | Inverted |
| 2           | 100   | 105          | 110      | 96              | 98       | 96             | 98       |
| 4           | 100   | 106          | 124      | 96              | 97       | 95             | 95       |
| 8           | 102   | 115          | 127      | 99              | 107      | 89             | 92       |
| 12          | 103   | 124          | 131      | 102             | 102      | 87             | 88       |

aliquot removed and steam distilled. The absorption curves in the ultraviolet region for the distilled samples are shown in Fig. 2. It is evident from these curves that a volatile component of the heptaldehyde aniline reaction product exhibits strong absorption in the region of 257  $m\mu$ , the wavelength where measurements are made for benzyl alcohol. The other two components, namely tetramethyl thiuram monosulfide and zinc dimethyl dithiocarbamate, show very low absorption at 280  $m\mu$  and no absorption at 257  $m\mu$ .

The influence of the closure extractives on the absorption characteristics of benzyl alcohol stored as a buffered solution in vials stoppered with the butyl rubber closure is illustrated in Fig. 3. By studying the curve for the zero time sample, it is apparent that the absorbance values for the minimum at 253  $m\mu$  and the maximum at 263  $m\mu$  are equal.

However, the curve for the sample stored for 12 weeks at 60° shows a large difference between the absorbance at 253  $m\mu$  and 263  $m\mu$ . This difference can be ascribed to the influence of the absorption characteristic of the volatile component of the primary accelerator which is leached into the benzyl alcohol solution from the rubber stopper. The effect at 253  $m\mu$  and 263  $m\mu$  causes a concurrent effect at 257  $m\mu$ , the absorption maximum used for benzyl alcohol analysis.

In order to correct the absorption readings for

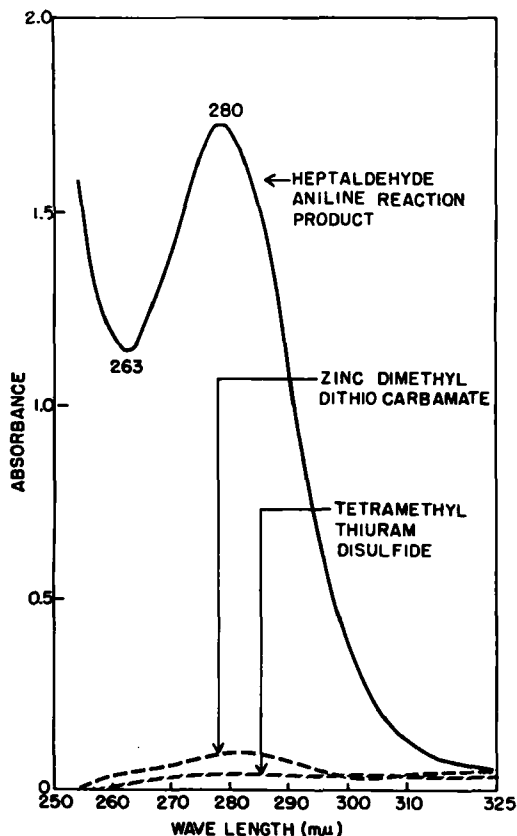


Fig. 2.—The ultraviolet absorption spectra of the accelerators and a reaction product found in the butyl rubber stoppers.

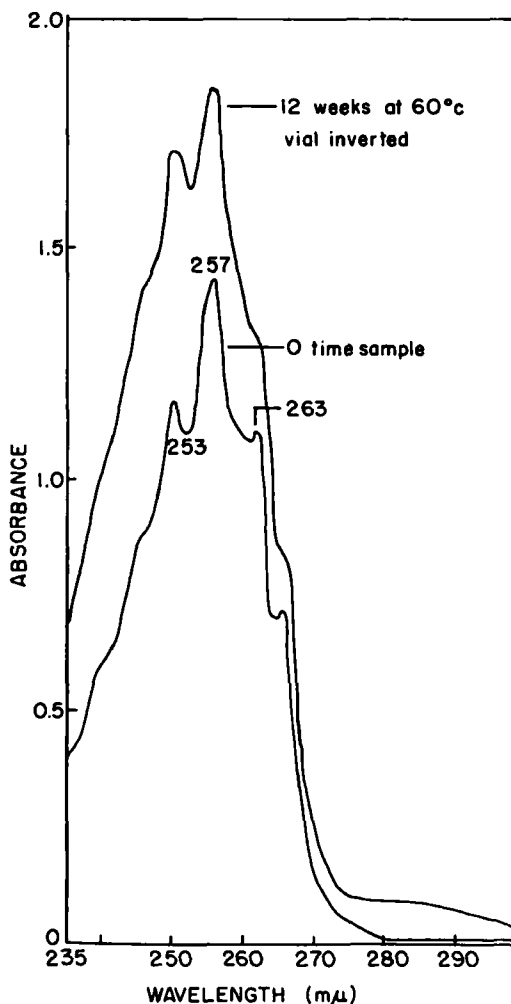


Fig. 3.—Plots showing the influence of butyl rubber closure extractives on the absorption characteristics of benzyl alcohol.

benzyl alcohol to deduct the amount due to extractives, two procedures were evaluated.

**Method I.**—The following equation was developed to correct for the absorbance due to extractive and thus give the true absorbance at 257  $m\mu$  for benzyl alcohol:  $A = B - (C - D)$  where  $A$  = corrected absorbance at 257  $m\mu$ ;  $B$  = observed absorbance at 257  $m\mu$ ;  $C$  = observed absorbance at 253  $m\mu$ ;  $D$  = observed absorbance at 263  $m\mu$ .

Using this equation, the uncorrected data in Table II for the vials stoppered with butyl rubber stoppers were recalculated and are presented in Table III. It is evident from this table that the corrected values are still higher than the original concentration in most instances. However, if the data are now further corrected for benzaldehyde development measured at 280  $m\mu$ , the results presented in the last two columns of Table III are obtained. These data now show that no absorption of benzyl alcohol by the butyl rubber stopper results when the vials are stored in an inverted or upright position.

**Method II.**—An alternate method used to correct

TABLE III.—CORRECTED PER CENT RESIDUAL BENZYL ALCOHOL IN VIALS STOPPERED WITH BUTYL RUBBER AFTER STORAGE AT 60° C.

| Time, weeks | Uncorrected |          | Corrected           |                      |                                      |                                       |
|-------------|-------------|----------|---------------------|----------------------|--------------------------------------|---------------------------------------|
|             | Upright     | Inverted | Extractives Upright | Extractives Inverted | Extractives and Benzaldehyde Upright | Extractives and Benzaldehyde Inverted |
| 2           | 105         | 110      | 100                 | 101                  | 100                                  | 100                                   |
| 4           | 106         | 124      | 101                 | 105                  | 99                                   | 101                                   |
| 8           | 115         | 127      | 105                 | 107                  | 101                                  | 100                                   |
| 12          | 124         | 131      | 103                 | 108                  | 100                                  | 102                                   |

for the absorbance at 257 mμ due to extractives was a modification of the Morton-Stubb technique (13). A model is presented in Fig. 4 representing pure benzyl alcohol and benzyl alcohol containing extractives which influence the characteristics of the absorption curve. Points A and B in Fig. 4 are representative of the equal absorbance at the minimum of 253 mμ and maximum of 263 mμ shown in Fig. 3 for pure benzyl alcohol. These wavelengths in the model are represented by λ<sub>2</sub> and λ<sub>3</sub>. The wavelength of 257 mμ used in the analysis for benzyl alcohol is represented in the model by λ<sub>1</sub>. Using the curves in Fig. 4 as simplified models of the benzyl alcohol absorption curves shown in Fig. 3, an equation was derived which corrects for the absorbance due to extractives.

CE = increase in absorbance at 257 mμ due to extractives; CD =  $\frac{DG}{FG} \times HF$  by trigonometry;

where HF represents the difference between the observed absorbance at λ<sub>2</sub> and λ<sub>3</sub>. DG = λ<sub>3</sub> - λ<sub>1</sub> and FG = λ<sub>3</sub> - λ<sub>2</sub>.

If DE is denoted by X, then the corrected value for the absorbance at λ<sub>1</sub> is Aλ<sub>1</sub> corrected = Aλ<sub>1</sub> observed - CD - X and at λ<sub>2</sub> is Aλ<sub>2</sub> corrected = Aλ<sub>3</sub> observed - X. Therefore, the ratio of these must be equal to the known ratio for the pure substance designated as K

$$K = \frac{A_{\lambda_1} \text{ observed} - CD - X}{A_{\lambda_3} \text{ observed} - X} = 1.31$$

for pure benzyl alcohol.

Since DG = 263 mμ - 257 mμ = 6 mμ and FG = 263 mμ - 253 mμ = 10 mμ then  $\frac{DG}{FG} = 0.6$

$$K = \frac{A_{257}^{1\%} - (A_{263}^{1\%} - A_{263}^{1\%}) 0.6 - X}{A_{263}^{1\%} - X}$$

Solving for X

$$A_{257}^{1\%} - (A_{263}^{1\%} - A_{263}^{1\%}) 0.6 - X = (A_{263}^{1\%} - X) 1.31 - X + 1.31 X = [(A_{263}^{1\%}) 1.31] - [A_{257}^{1\%} - (A_{263}^{1\%} - A_{263}^{1\%}) 0.6]$$

$$X = \frac{[(A_{263}^{1\%}) 1.31] - [A_{257}^{1\%} - (A_{263}^{1\%} - A_{263}^{1\%}) 0.6]}{0.31}$$

$$CD = (A_{257}^{1\%} - A_{263}^{1\%}) 0.6$$

$$A_{257}^{1\%} \text{ corrected} = A_{257}^{1\%} \text{ observed} - (X + CD)$$

The data for the vials stoppered with the butyl rubber closures were corrected according to this equation and the corrected data were found to be in agreement with the data obtained from the equation in Method I. This indicates that either Method I or II satisfactorily corrects for the absorbance due to extractives. However, since Method I

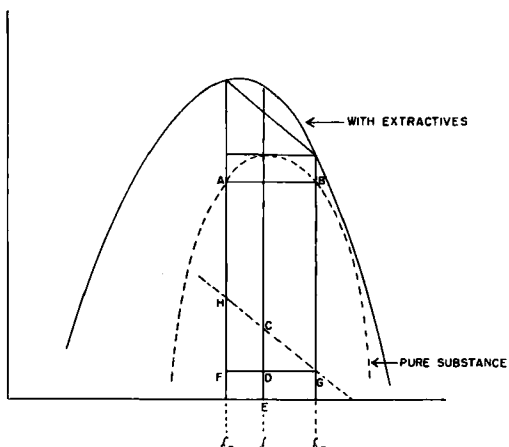


Fig. 4.—Model curves developed from the absorption plots shown in Fig. 3 for use in developing the modified Morton-Stubb's correction for benzyl alcohol assay.

is simpler than Method II, it was used to correct the data for the vials stoppered with the neoprene closures.

The results in Table IV show the corrected and uncorrected values for the per cent preservative

TABLE IV.—CORRECTED AND UNCORRECTED PER CENT RESIDUAL BENZYL ALCOHOL IN VIALS STOPPERED WITH NEOPRENE RUBBER AFTER STORAGE AT 60° C.

| Time, weeks | Uncorrected |          | Corrected |          |
|-------------|-------------|----------|-----------|----------|
|             | Upright     | Inverted | Upright   | Inverted |
| 2           | 96          | 98       | 94        | 95       |
| 4           | 96          | 97       | 92        | 92       |
| 8           | 99          | 107      | 90        | 92       |
| 12          | 102         | 102      | 91        | 90       |

absorbed by the neoprene rubber stoppers. These stoppers behave differently from the butyl rubber, in that they absorb preservative from solution as well as leach out extractives. This is particularly apparent from the data for the vials stored in an inverted position. The decrease in apparent benzyl alcohol concentration after 12 weeks storage indicates that the benzyl alcohol is continuing to be absorbed by the closure while the amount of extractives being leached from the closure has apparently reached an equilibrium concentration somewhere between 8 and 12 weeks.

In Table V is presented the corrected per cent residual benzyl alcohol for the ampul and vial solutions stoppered with the three closures. It can be seen from this table that the natural rubber

TABLE V.—THE CORRECT PER CENT RESIDUAL BENZYL ALCOHOL AFTER STORAGE AT 60° C.

| Time, weeks | Ampul | Butyl Rubber |          | Neoprene Rubber |          | Natural Rubber |          |
|-------------|-------|--------------|----------|-----------------|----------|----------------|----------|
|             |       | Upright      | Inverted | Upright         | Inverted | Upright        | Inverted |
| 2           | 100   | 100          | 100      | 94              | 95       | 96             | 98       |
| 4           | 100   | 99           | 101      | 92              | 92       | 95             | 95       |
| 8           | 101   | 101          | 100      | 90              | 92       | 89             | 92       |
| 12          | 100   | 100          | 102      | 91              | 90       | 87             | 88       |

absorbs slightly more benzyl alcohol than the neoprene rubber. However, it is interesting to note that for both stoppers the amount of preservative absorbed is the same for the vials stored in an upright and inverted position.

From the data presented above, it is apparent that extractives leached from closures may have a significant effect on the assay for residual preservatives. Consequently, when assay results for preservative content appear to be irregular or show an increase with storage, consideration should not only be given to the assay method but also to the possibility that extractives are being leached from the rubber stoppers and interfering with the assay. If the assay method is a spectrophotometric one, it is necessary to run the complete absorption curves in order to adequately demonstrate the interfering characteristics of the extractives.

#### Methylparaben

The stability of a 0.2% solution of methylparaben (pH 4.0) in vials stoppered with natural, neoprene, and butyl rubber closures and stored at 60° was investigated. According to the data presented in Table VI, methylparaben appears to exhibit good stability in the presence of butyl rubber and fair stability with natural and neoprene rubber, with the latter causing the greater deleterious effect. As in the case of benzyl alcohol, the amount of methylparaben lost from solution was the same in vials stored upright or inverted.

It is interesting to note that although residual methylparaben was assayed spectrophotometrically at 256  $m\mu$  which is extremely close to the 257  $m\mu$  used for benzyl alcohol, no interference was observed due to extractives. This may be explained as follows: (a) since methylparaben is a solid, it does not exert any solubilizing effect on the unreacted accelerator in the rubber, (b) the steam distilling of the benzyl alcohol solution before assaying could concentrate the volatile component responsible for interfering with the assay for benzyl alcohol, and (c) the high  $A_{1\text{cm}}^{1\%}$  of 1115 for methylparaben compared to 18 for benzyl alcohol could diminish any effect due to the absorptivity of the extractives.

#### Phenylethyl Alcohol

The influence of neoprene, natural, and butyl rubber stoppers on the preservative content of a 0.5% aqueous buffered solution of phenylethyl alcohol (pH

4.0) stored in vials at 60° was studied. It became evident early in the study that the solutions in the vials as well as in the ampuls developed a very fine precipitate which remained fairly well distributed throughout the solution. This precipitate was found to interfere with the spectrophotometric assay for residual preservative since it appeared to distill over from the aliquot of phenylethyl alcohol solution taken for assay, causing high assays for phenylethyl alcohol. In fact, after 12 weeks storage at 60°, the ampuls assayed 112% phenylethyl alcohol. Because of the interfering properties of the precipitate on the assay results, it was felt that the data were not adequate for presentation in this report.

It is believed that the precipitate is due to oxidation products of phenylethyl alcohol, namely, phenylacetaldehyde and phenylacetic acid. Further information will be presented on this preservative at a later date when the studies presently underway to elucidate the responsible factors for the precipitate development are completed.

**Apparent Distribution of Preservative Between Rubber and Solution.**—In an earlier report (11) data were presented on the distribution of *p*-chloro- $\beta$ -phenylethyl alcohol and phenylethyl alcohol between rubber and water buffered to a pH of 4.0. These tests were performed with neoprene and natural rubber stoppers.

Similar studies were performed in this investigation for the preservatives chlorobutanol, benzyl alcohol, and methylparaben. The apparent partition coefficients for these three preservatives, as well as for the two studied in the earlier investigation, are summarized in Table VII. These data indicate that of the five preservatives, benzyl alcohol exhibits the least tendency to distribute into either the natural or neoprene rubber of the five preservatives tested. For each preservative the neoprene stoppers absorb to a greater degree than the natural rubber.

From the distribution data in this table, the natural rubber stoppers show the following order of absorption of preservative: benzyl alcohol < methylparaben < phenylethyl alcohol < *p*-chloro- $\beta$ -phenylethyl alcohol < chlorobutanol. The results for the neoprene stoppers are as follows: benzyl alcohol < phenylethyl alcohol < methylparaben < chlorobutanol < *p*-chloro- $\beta$ -phenylethyl alcohol. It is interesting to note that the order of absorption is not the same for the two composition closures. The preservative being most absorbed by the natural

TABLE VI.—PER CENT RESIDUAL METHYLPARABEN AFTER STORAGE AT 60° C.

| Time, weeks | Ampul | Butyl Rubber |          | Neoprene Rubber |          | Natural Rubber |          |
|-------------|-------|--------------|----------|-----------------|----------|----------------|----------|
|             |       | Upright      | Inverted | Upright         | Inverted | Upright        | Inverted |
| 2           | 100   | 100          | 100      | 100             | 100      | 100            | 100      |
| 4           | 100   | 100          | 100      | 100             | 94.5     | 100            | 100      |
| 8           | 100   | 100          | 100      | 94.5            | 94.5     | 100            | 94.5     |
| 12          | 100   | 100          | 100      | 89              | 89       | 94.5           | 94.5     |

TABLE VII.—APPARENT DISTRIBUTION OF PRESERVATIVE BETWEEN RUBBER AND BUFFER SOLUTIONS<sup>a</sup> AFTER FOUR WEEKS STORAGE
$$K_T = \frac{C_R}{C_B}$$

| Preservative                                   | Closure  | Temperature, °C. |       |
|--|----------|------------------|-------|
|  |          | 25               | 40    |
| Phenylethyl alcohol                            | Natural  | 1.72             | 1.39  |
|  | Neoprene | 4.23             | 4.13  |
| <i>p</i> -Chloro- $\beta$ -phenylethyl alcohol | Natural  | 6.05             | 5.70  |
|  | Neoprene | 16.40            | 21.80 |
| Chlorobutanol                                  | Natural  | 9.85             | 6.83  |
|  | Neoprene | 14.50            | 14.50 |
| Benzyl alcohol                                 | Natural  | 0.63             | 0.63  |
|  | Neoprene | 1.66             | 1.93  |
| Methylparaben                                  | Natural  | 1.36             | 1.43  |
|  | Neoprene | 7.27             | 8.40  |

<sup>a</sup> Solutions buffered to a pH of 4.0.

rubber is chlorobutanol while for the neoprene rubber it is *p*-chloro- $\beta$ -phenylethyl alcohol.

It is common practice for rubber closure manufacturers to use an excess of accelerators and additives in rubber closure formulations. As a result, there exists at the completion of the manufacturing process certain unreacted components in the rubber stock. The quantity of unreacted accelerators present in synthetic rubbers would be expected to be greater than in natural rubber. This is due to the fact that the synthetic rubber, such as butyl and neoprene, is generally more inert than natural rubber, thus making the vulcanization process more difficult. In order to minimize this difficulty, higher dosages of accelerator are used (14).

The results obtained in this investigation, as well as those reported by other investigators, show that the unreacted components of the closure could be extracted by the solution in the vials. These extractives could be extremely dangerous in that they can (a) affect the toxicity of the injectable solution, (b) interact with components in the solution and cause physical and chemical instability, (c) influence bactericidal activity, and (d) as illustrated in this investigation, seriously interfere with the analysis of the solution. Consequently, it is essential that rubber stopper manufacturers use more precise formulations and adequate quality standards in the manufacture of rubber closures.

### SUMMARY

The interaction between several composition rubber stoppers and three preservatives was evaluated. Aqueous buffered solutions of the preservatives benzyl alcohol, methylparaben, and

phenylethyl alcohol were filled into multiple-dose vials and stoppered with natural, neoprene, and butyl rubber closures. These vials were then stored at several temperatures and tested at designated time intervals for the effect of closure on preservative content. The data from this study may be summarized as follows:

1. Two methods were developed to correct for the interference of rubber closure extractives on the analytical procedure used for benzyl alcohol.

2. The butyl rubber closures, although leaching extractives into the preservative solutions, exhibited the least absorbing tendencies toward benzyl alcohol and methylparaben.

3. The amount of benzyl alcohol and methylparaben lost from vial solutions was not significantly influenced by storage of the vials in an upright or inverted position.

4. Distribution studies have shown that both the natural and neoprene rubber stoppers absorbed preservative from solution with the latter closure exhibiting greater absorbing tendencies.

5. From the distribution data in this and a previous study, the natural rubber stoppers exhibit the following order of preservative absorption: benzyl alcohol < methylparaben < phenylethyl alcohol < *p*-chloro- $\beta$ -phenylethyl alcohol < chlorobutanol. For the neoprene stoppers the following order exists: benzyl alcohol < phenylethyl alcohol < methylparaben < chlorobutanol < *p*-chloro- $\beta$ -phenylethyl alcohol.

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