

REFERENCES

- (1) Linde, S., *Svensk Farm. Tidskr.* **35**, 875(1955).
- (2) Windheuser, J., and Cooper, J., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 542(1956).
- (3) Wolff, J. E., U. S. pat. 2,757,124 (1956).
- (4) Klump, T. G., Wolff, J. E., and Kaplan, L. J., U. S. pat. 3,019,169 (1962).
- (5) Cooper, J., and Windheuser, J., U. S. pat. 2,887,438 (1959).
- (6) Blubaugh, F. C., Zapapas, J. R., and Sparks, M. C., *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 857(1958).
- (7) James, K. M., Jeffrey, J. G., and MacAuley, W. C., *Can. Pharm. J., Sci. Sect.*, **1958**, 456.
- (8) Swintosky, J. V., U. S. pat. 2,971,889 (1961).
- (9) Millar, J. F., and Lindner, G., U. S. pat. 3,018,221 (1962).
- (10) Lachman, L., Speiser, P. P., and Sylwestrowicz, H. D., *J. Pharm. Sci.*, **52**, 379(1963).
- (11) Bennett, C. A., and Franklin, N. I., "Statistical Analysis in Chemistry and Chemical Industry," John Wiley & Sons, Inc., New York, N. Y., 1951, p. 197.

Lined and Unlined Rubber Stoppers for Multiple-Dose Vial Solutions II

Effect of Teflon Lining on Preservative Sorption and Leaching of Extractives

By LEON LACHMAN, WAYNE A. PAULI, PRAVIN B. SHETH, and MIRIAM PAGLIERY

This report demonstrates the protective action of Teflon linings on the sorption and leaching characteristics of polyurethan and natural rubber stoppers. This lining was effective in essentially eliminating extractives from appearing in water, 50 per cent polyethylene glycol 300, 50 per cent *N,N*-dimethylacetamide, 10 per cent ethanol, and 2 per cent benzyl alcohol when these solvents were in contact with the closures for 6 hr. at 115°. Sorption of the preservative, *p*-chloro- β -phenylethyl alcohol, from aqueous solution was effectively retarded by the Teflon lining on the stoppers. Reduced protection against sorption and leaching was found when Teflon lined closures, which previously underwent multiple puncture with a 20-gauge hypodermic needle, were used.

WITHIN recent years, the influence of rubber closures on the contents of multiple-dose vial solutions has received considerable attention (1, 2). It has been shown that both leaching of rubber extractives from rubber closures into solution and sorption of materials from solution by rubber stoppers can be detrimental to multiple-dose injectable preparations. Since many of the materials extracted from closures are reactive chemicals, they could cause stability (3-5) or toxicity and pyrogenicity (6, 7) problems with the vial contents, as well as interfere with assay methods (2), making it difficult or impossible to quantitatively identify active ingredients. The loss of antibacterial preservatives from multiple-dose vials resulting from sorption into rubber closures and/or reaction with rubber extractives is recognized as a serious problem. Since these agents are added to multiple-dose injectable preparations to insure bacteriostasis

for the life of the product, any significant loss of the antimicrobial agent from solution can seriously undermine sterility maintenance of the product. Various attempts have been made to retard sorption of materials from vial solutions and to reduce the amount of extractives leached from closures into solutions. Certain rubber stopper manufacturers have attempted to eliminate these incompatibilities between vial solutions and closures by application of a lacquer lining to the inner surface of their closures. This lining appears to be essentially noneffective in retarding both sorption and leaching effects (8). A recent report from these laboratories (9) contains an evaluation of the protective action of an epoxy lining on rubber stoppers of varying composition. Although this lining was found to afford partial protection against leaching, no protective action against sorption was observed.

This study was initiated to determine the extent of protection afforded by Teflon linings, described by Hopkins (10), on polyurethan and natural rubber stoppers against sorption and leaching. The sorption characteristics of the lined and unlined elastomer closures were tested

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with an aqueous buffered solution of the preservative, *p*-chloro- β -phenylethyl alcohol, stored in multiple-dose vials. The leaching tendencies of water, 10% ethanol, 50% polyethylene glycol 300, 50% *N,N*-dimethylacetamide, and 2% benzyl alcohol on the closures were evaluated. Identification of the extractives leached from the stoppers was attempted.

EXPERIMENTAL

Materials.—*p*-Chloro- β -phenylethyl alcohol (Ciba), b.p. 80–83°, at 1.07 mm.; thiazole-type accelerator, thiuram-type accelerator, dithiocarbamate reaction product, urethan monomer glycol-amine curing agent, and an organo-metallic catalyst were supplied by the rubber manufacturer.

Polyethylene glycol 300 (Union Carbide); *N,N*-dimethylacetamide (E. I. du Pont); benzyl alcohol, reagent grade (Fisher Scientific Co.), double distilled at Ciba; 0.275 *M* citric acid–sodium phosphate buffer of pH 4.0; polyurethan and natural rubber stoppers Teflon lined and unlined, 13 mm. (West Co.); U.S.P. type 1, 10-ml. clear ampuls and vials (Kimble Glass Co.); three-piece aluminum caps for vials (West Co.), No. 13-30 were also utilized.

Equipment.—Beckman model DB recording spectrophotometer, Beckman model G pH meter, and microdistillation apparatus, as previously described in an earlier report (2), were employed.

Preparation of Ampuls, Vials, and Stoppers.—These units were washed and dried in accordance with the procedures described in a previous publication from this laboratory (1).

Sorption of Preservative from Solution.—To evaluate the effectiveness of Teflon lining against preservative sorption, lined and unlined closures of the same basic rubber formulations were used. The preservative solution used in the evaluation, 0.3% *p*-chloro- β -phenylethyl alcohol was prepared on a weight to volume basis with water for injection buffered to a pH of 4.0. Before being filled into 10-ml. clear glass ampuls and vials, the preservative solution was filtered through a medium porosity sintered-glass filter. Ampul closure was accomplished by the customary pull sealing technique with an oxygen-gas flame. The vials containing the preservative solution were then divided into four equal parts and stoppered with Teflon lined and unlined polyurethan and natural rubber stoppers. The stoppered vials were sealed with three-piece aluminum caps using a Fermpress hand crimper. The ampuls and vials containing the preservative solution were then placed into constant-temperature cabinets regulated at 25, 50, and 60 \pm 1.5°. Half of the vials were stored upright and half inverted; and at designated time intervals, samples were withdrawn and evaluated for residual preservative content.

To investigate the effect of multiple puncture of lined closures on preservative sorption, Teflon lined natural rubber stoppers were punctured 5 times with a 20-gauge hypodermic needle. Ten-milliliter clear vials containing the 0.3% buffered preservative solution were stoppered using these closures and then capped as described previously. Half of the vials were stored upright and half inverted at the same conditions employed for the other vials.

Sealed 10-ml. clear ampuls of the preservative solution served as controls at the various storage conditions.

Rubber Closure Extractives.—To determine the effectiveness of the Teflon lining on the two different closures against leaching of unreacted materials and reaction products into the vial solution, several solvents commonly found in parenteral formulations were employed. These solvents, including water, 10% ethanol, 50% polyethylene glycol 300, 50% *N,N*-dimethylacetamide, and 2% benzyl alcohol, were filled into 10-ml. clear glass vials and stoppered with the unlined and Teflon lined polyurethan and natural rubber closures. Multipunctured Teflon lined natural rubber stoppers were also employed to evaluate the possible loss of protection resulting from the multiple perforations in the Teflon film. The stoppered vials were then sealed with three-piece aluminum caps again using the hand crimper described previously. After autoclaving the vials in an inverted position at 115° (10 p.s.i.) for 6 hr., the solutions in the vials were scanned spectrophotometrically to obtain ultraviolet absorption curves of the rubber extractives.

Identification of Extractives.—For the various organic additives and their possible reaction products of the two rubber compositions used in this study, saturated solutions were prepared in the five solvent systems and scanned spectrophotometrically to obtain their characteristic ultraviolet absorption spectra. This made possible a qualitative identification of the materials extracted from the polyurethan and natural rubber closures.

Analytical Method.—The assay method employed for *p*-chloro- β -phenylethyl alcohol has been described in an earlier publication from these laboratories (1).

RESULTS AND DISCUSSION

The protective action of a Teflon lining on polyurethan and natural rubber stoppers against sorption of the preservative, *p*-chloro- β -phenylethyl alcohol, from solution and leaching of extractives from the stoppers by several solvents was investigated. The composition and per cent rubber content of these stoppers are presented in Table I.

Sorption Studies.—The sorption tendencies of Teflon lined, multipunctured Teflon lined, and unlined rubber stoppers were evaluated with vial solutions of *p*-chloro- β -phenylethyl alcohol buffered to a pH of 4.0. The protective action of the Teflon lin-

TABLE I.—CLOSURE COMPOSITION

Natural Rubber	Polyurethan Rubber
Natural crepe ^a	Urethan monomer ^b
Calcined clay	Amine-glycol curing agent
Barium sulfate	Organo-metallic catalyst
Zinc oxide	Titanium dioxide
Iron oxide	
Stearic acid	
Paraffin wax	
Thiazole-type accelerator	
Thiuram-type accelerator	

^a Rubber content: 56% of total composition. ^b Rubber content: 95% of total composition.

TABLE II.—EFFECTIVENESS OF TEFLON LINING ON NATURAL RUBBER STOPPERS AGAINST SORPTION OF *p*-CHLORO- β -PHENYLETHYL ALCOHOL FROM VIAL SOLUTIONS REPRESENTED AS PER CENT RESIDUAL PRESERVATIVE

Time, Wk.	Temp., 25°							Temp., 50°						Temp., 60°								
	Upright			Inverted				A	Upright			Inverted			A	Upright			Inverted			A
L ^a	PU	P	L	PU	P	A	L		PU	P	L	PU	P	A		L	PU	P	L	PU	P	
0	100
2	99	98	96	99	95	90	100	99	91	90	99	90	79	98	100	90	84	99	89	78	98	98
4	100	98	95	100	95	88	99	100	91	83	98	84	77	99	100	88	78	100	84	75	99	99
8	99	98	88	100	94	81	101	99	84	74	99	81	70	101	99	77	63	100	81	63	92	92
12	102	95	91	102	91	82	102	102	80	72	102	80	71	102	102	74	64	108	74	63	102	102

^a L = Teflon lined; PU = multipunctured Teflon lined; P = plain; A = ampul.

TABLE III.—EFFECTIVENESS OF TEFLON LINING ON POLYURETHAN RUBBER STOPPERS AGAINST SORPTION OF *p*-CHLORO- β -PHENYLETHYL ALCOHOL FROM VIAL SOLUTIONS REPRESENTED AS PER CENT RESIDUAL PRESERVATIVE

Time, Wk.	Temp., 25°					A	Temp., 50°					A	Temp., 60°									
	Upright		Inverted				L	Upright		Inverted			L	Upright		Inverted			L			
L ^a	P	L	PU	P	L	P		L	PU	P	L	P		L	PU	P	L	PU		P	L	PU
0	100
2	99	85	98	51	97	97	99	74	97	37	98	99	69	98	38	98	98	38	98	98	98	98
4	96	63	97	42	97	97	96	51	96	29	97	96	45	97	30	97	96	45	97	30	97	97
8	97	61	96	38	95	97	97	39	97	26	97	101	24	96	23	97	101	24	96	23	97	97
12	100	78	99	36	99	100	100	34	99	23	99	97	20	101	19	101	97	20	101	19	101	101

^a L = Teflon lined; P = plain; A = ampul.

TABLE IV.—ABSORBANCE VALUES OF EXTRACTIVES LEACHED BY SEVERAL SOLVENTS IN 10-ml. VIALS FROM TEFLON LINED AND UNLINED RUBBER STOPPERS AFTER AUTOCLAVING THE VIALS IN AN INVERTED POSITION AT 115° FOR 6 hr.

Solvent	Wave-length, m μ	Natural Absorbance			Wave-length, m μ	Polyurethan Absorbance	
		P ^a	PU	L		P	L
Water	310	0.74	0.02	0.01	276	0.07	0.01
	230	0.83	0.07	0.02			
Ethanol, 10%	308	0.86	0.03	0.01	274	0.17	0.01
	228	0.91	0.11	0.02			
Benzyl alcohol, 2%	312	0.77	0.03	0.01	280	0.14	0.02
Polyethylene glycol 300, 50%	324	0.66	0.13	0.02	270	0.85	0.12
	248	0.71	0.40	0.27	250	0.88	0.21
<i>N,N</i> -Dimethylacetamide, 50%	326	1.95	...	0.01	278	1.55	0.01

^a P = plain; PU = multipunctured Teflon lined; L = Teflon lined.

ing on natural rubber closures against sorption of the preservative from solution can readily be seen from the data in Table II. At all temperature conditions throughout the 12-week period, no decrease in preservative content is noted for any of the vials with lined closures. The observed loss of *p*-chloro- β -phenylethyl alcohol from vials with plain closures appears to level off at around the 8-week period. This plateau could indicate the presence of an equilibrium condition. It is interesting to note that only for the unlined closures do vial solutions stored in an inverted position show greater loss of preservative content than those stored upright.

To evaluate the effectiveness of the Teflon lining after the stopper was punctured several times by a hypodermic needle, Teflon lined natural rubber closures were punctured 5 times with a 20-gauge hypodermic needle and employed as closures for vials containing the preservative solution. These vials were stored at the same conditions and for the same time period as the other group. Results show that even when perforated several times by a hypodermic needle, the Teflon lining still affords some protection against sorption when compared with loss of preservative for the unlined closures.

The data in Table III summarize the sorption tendencies of lined and unlined polyurethan closures for *p*-chloro- β -phenylethyl alcohol at the various temperature conditions. Excellent protection was afforded by the Teflon lining against sorption at all storage conditions throughout the 12-week evaluation. Again, only for the unlined closures do the vial solutions stored in an inverted position show a greater loss of preservative than those stored upright.

Since there was no diminution of preservative concentration in the ampul solutions, the loss in the vial solutions stoppered with natural and polyurethan closures must be attributed to closures.

Although no physical changes in the natural rubber closures were noted at the various elevated temperature conditions employed, both the lined and unlined polyurethan closures deformed markedly at these same temperatures. In fact, the unlined closures were so badly deformed that the loss data of preservative in the vials could be due to loss around the closure and out to the atmosphere in addition to that sorbed by the closure.

Leaching of Extractives.—Several solvents commonly used in injectable formulations were employed to evaluate the leaching of extractives from

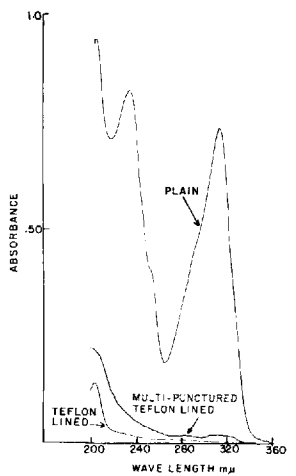


Fig. 1. — Ultraviolet absorption curve of leached extractives. Rubber, natural; solvent, water; storage condition, 6 hr. at 115°, vial inverted.

Teflon lined and unlined stoppers when used on multiple-dose vials. Table IV lists the ultraviolet maxima and corresponding absorbances representing extractives from the polyurethan and natural rubber closures appearing in the various solvents. It can readily be seen that for each of the rubber compositions employed and in each of the solvent systems, the Teflon lining afforded considerable protection against leaching of extractables from the closures as evidenced by the extremely low absorbance values obtained when lined closures were used. Considerable protection against leaching was also provided using the multipunctured Teflon lined closures. Representative ultraviolet absorption spectra for the extractives found in the vial solutions containing water and stoppered with lined, multipunctured lined, and unlined natural rubber stoppers are given in Fig. 1.

It is interesting to note from the data in Table IV that of the five solvents investigated with the lined closures, only 50% polyethylene glycol 300 appeared to be successful in leaching out a significant amount of extractives from both polyurethan and natural rubber. To explore whether decomposition of the polyethylene glycol 300, when exposed to the extreme conditions of the evaluation, gave degradation products which also showed ultraviolet absorption, samples of this solvent were sealed in clear glass ampuls and heated for the same period at the same temperature as the vials. The ultraviolet absorption spectra of the 50% polyethylene glycol 300 solution heated in ampuls and an untreated control are given in Fig. 2. The appearance of an absorption peak at approximately 250 $m\mu$ for the heated glycol solution indicates that the decomposition of the glycol most likely contributes to the relatively high absorbance values found with this solvent from vials using Teflon lined closures.

Identification of Extractives.—Materials of the rubber compositions studied, which would tend to give absorption in the ultraviolet region, were obtained from the rubber stopper manufacturer and ultraviolet absorption spectra taken in the solvents studied. This information was employed to aid in the identification of the extractives leached from the polyurethan and natural rubber stoppers. The absorption data for these various ingredients are

summarized in Table V. With this information, positive identification of each of the various extractables was made possible.

For the natural rubber stoppers, the material responsible for the absorption at 308–326 $m\mu$ and 228–230 $m\mu$ is believed to be the thiazole-type accelerator. The solvent decomposition products and/or the dithiocarbamate reaction product could account for the absorption at 248 $m\mu$ in 50% polyethylene glycol 300. In the case of the polyurethan closures, the absorption at 270–280 $m\mu$ can be ascribed primarily to the organo-metallic catalyst employed with the possibility of minor contribution from the urethan monomer. The observed peak found for 50% polyethylene glycol 300 of approximately 250 $m\mu$ can be attributed to solvent decomposition and not to extractives from the rubber. Even though slight ultraviolet absorption was observed for the glycol-amine curing agent, the absorption spectra of this agent in the various solvent systems employed were rather diffuse with no definable maxima.

SUMMARY

Teflon lined and unlined polyurethan and natural rubber closures were evaluated for their sorption and leaching characteristics. Multiple-dose vials containing an aqueous buffered solution of the preservative, *p*-chloro- β -phenylethyl alcohol, were stoppered with the lined and unlined rubber closures for the sorption studies. Several solvents commonly used in injectable formulations were employed to evaluate the leaching of extractives from the lined and unlined closures when used on multiple-dose vials. The solvents used were water, 10% ethanol, 2% benzyl alcohol, 50% *N,N*-dimethylacetamide, and 50% polyethylene glycol 300. The following results were obtained.

1. The Teflon lining was found to afford excellent protection against sorption of the preservative into either the polyurethan or natural rubber stoppers.
2. The lining was found to be effective in preventing the leaching of extractives from the rubber closures by the various solvents employed.
3. The extractives leached by the different sol-

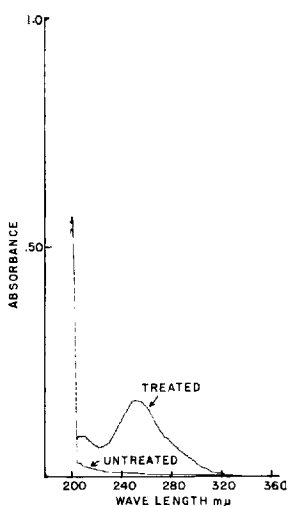


Fig. 2. — Ultraviolet absorption spectra of a 50% polyethylene glycol 300 solution after autoclaving in sealed ampuls for 6 hr. at 115°, 10 p.s.i., and an untreated control.

TABLE V.—ULTRAVIOLET ABSORPTION MAXIMA OF RUBBER CLOSURE INGREDIENTS

Rubber Stopper	Wavelength, m μ				
	Water	10% Ethanol	2% Benzyl Alcohol	50% N,N-Dimethylacetamide	50% Polyethylene Glycol 300
Natural					
Thiazole-type accelerator	312	312	312	322	322
	227	230	228
Thiuram-type accelerator	274	274	278	276	278
Dithiocarbamate reaction product	274	276	...	274	274
	...	250	...	257	253
Polyurethan					
Urethan monomer	288	287
Organo-metallic catalyst	272	274	272	274	274

vents from both the unlined polyurethan and natural rubber closures were qualitatively identified.

4. Teflon lined natural rubber stoppers, which were perforated several times by a hypodermic needle, still afforded some protection against sorption and considerable protection against leaching.

REFERENCES

- (1) Lachman, L., Weinstein, S., Hopkins, G., Slack, S., Eisman, P. C., and Cooper, J., *J. Pharm. Sci.*, **51**, 224(1962).
- (2) Lachman, L., Urbányi, T., and Weinstein, S., *ibid.*, **52**, 244(1963).
- (3) Royce, A., and Sykes, G., *J. Pharm. Pharmacol.*, **9**, 814(1957).
- (4) Weiner, S., *ibid.*, **7**, 188(1955).
- (5) Wing, W. T., *ibid.*, **5**, 969(1953).
- (6) Berry, H., *ibid.*, **5**, 1008(1953).
- (7) Christiansen, E., *Medd. Norsk Farm. Selskap.*, **13**, 121, 135(1951).
- (8) Garnet, J. R., *Australian J. Pharm.*, **September 29, 1962**, 908.
- (9) Lachman, L., Sheth, P. B., and Urbányi, T., *J. Pharm. Sci.*, **53**, 211(1964).
- (10) Hopkins, G. H., *ibid.*, **54**, 138(1965).

Notes

Phosphorus-Nitrogen Compounds V. Some Guanidine and 2-Aminopyrimidine Derivatives

By LINDLEY A. CATES and NOEL M. FERGUSON

Five amidino(thio)phosphoramidates and six 2-pyrimidyl(thio)phosphoramidates were prepared for screening in four cancer test systems. Three of these derivatives were also tested against *Plasmodium berghei*. None of the compounds exhibited significant antineoplastic or antimalarial activity.

THE SYNTHESIS of phosphoramidopyrimidines was undertaken to further ascertain the effect of phosphorus bonding on the cytotoxic properties of heterocyclic amines. The preparation of similar pyridine-containing compounds was described in an earlier report in this series (1).

The rationale for antineoplastic activity by these derivatives originates from an hypothesis concerning the mechanism of action of cyclophosphamide. According to this theory, cyclophosphamide exists as an inactive transport form *in vivo* until the P-N

bond is cleaved by the enzymatic action of phosphamidases or phosphatases to yield the alkylating ethylenimmonium ion (2). Thus, replacement of the bis(2-chloroethyl)amino group by an aminopyrimidine moiety might give rise to an antimetabolic activity of a more specific nature than would be realized by administration of the parent compound. More recent evidence (3), however, suggests that the conversion of cyclophosphamide *in vivo* into a cytostatically effective activation product is the result of a metabolic process dependent on the presence of oxygen and not the result of simple hydrolysis. At this time it can be stated that no direct proof of the postulated biochemical transformations of cyclophosphamide and related congeners is available.

Since guanidine and some of its derivatives are known to possess antiprotozoal properties (4) three of the amidino(thio)phosphoramidate intermedi-

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