SCIENCE PAPERS AND DISCUSSIONS

LOSSES OF BACTERIOSTATS FROM INJECTIONS IN RUBBER-CLOSED CONTAINERS

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The losses of bacteriostats from rubber-closed multidose containers of injections are confirmed, and the mechanism of these losses by absorption, diffusion and volatilisation is discussed. The present B.P. methods of pretreating rubber closures are inadequate, and methods for their more satisfactory equilibration with various bacteriostatic solutions are given. From measurements of the partition ratios of the B.P. bacteriostats and of benzyl alcohol between rubber and water, phenol and benzyl alcohol appear to be the most stable and promising and phenylmercuric nitrate the least stable. The value of using closures equilibrated for phenol and benzyl alcohol is demonstrated and methods for preventing the diffusion losses are discussed.

THAT rubber-closed containers of injections containing phenolic bacteriostats lose substantial quantities of these on storage has long been known. As early as 1923 Masucci and Moffat[1] reported the loss of 50 to 70 per cent of cresol and 20 to 40 per cent of phenol from rubber-capped vials of solutions which had been stored for eighteen months at room temperature, and in the late 1930's similar observations were made in these laboratories; we also showed considerable differences in the uptake of phenol, cresol and chlorocresol by different types of rubber closures when they were heated with successive quantities of the respective bacteriostatic solutions, but unfortunately all of these records were lost in 1941. We still have the records, however, of work by a colleague who in the period 1945 to 1947 examined vials of insulin returned from tropical countries and which showed losses of from 60 to 70 per cent of cresol after storage for 12 to 18 months in these areas.

More recently, Berry[2] has adequately summarised the lack of information on this topic in his contribution to the symposium on Containers and Closures at the British Pharmaceutical Conference in 1953. Since then Weiner[3] has discussed the absorption of thiomersalate by rubber, and there have been contributions by Wing[4–6] on the absorption of phenol and chlorocresol by rubber and the effects of varying the chemical composition of the rubber mix on this absorption.

Since 1952 we have made many tests with various types of rubber closures used for injectable products. Our results with phenol and chlorocresol are in broad agreement with other workers in this field, but our data also include observations with cresol, phenylmercuric nitrate and benzyl alcohol, and this does not appear to have been covered elsewhere.

Most of the tests were made with rubber diaphragms or plunger components used in medical cartridges, and they involved storage periods up to three years.

EXPERIMENTAL

The B.P. Treatment of Rubber Closures

The British Pharmacopoeia, 1953, states on page 850 that rubber caps are "either boiled under a reflux condenser for thirty minutes, or stored for not less than forty-eight hours, in a solution containing the same bacteriostatic in the same concentration, or preferably in twice the concentration used in preparing the injection". The uncertainty of the method is implied, for, apart from the fact that it permits two strengths of solution for treatment which will obviously give different results, it states on the same page "rubber so treated is liable to continue to absorb bacteriostatic from the injection".

TABLE I

THE ABSORPTION OF PHENOL, CRESOL AND CHLOROCRESOL BY RUBBERS

	Concentration per cent of bacteriostat remaining in solution in contact with different rubber components					
	Phenol with		Cresol with		Chlorocresol with	
Time of contact	Red rubber	White rubber	Red rubber	White rubber	Red rubber	White rubber
Hot treatment						
Initial	1·00	1·00			0·20	0·20
5 min.	0·96	0·97				
15 min.	0·91	0·92				
30 min.	0·87	0·90			0·12	0·14
1 hr.	0·83	0·88			0·10	0·12
2 hr.	0·80				0·09	0·10
4 hr.	0·77	0·80				
6 hr.	0·77	0·78			0·08	0·09
Cold treatment						
Initial	1·00	1·00	0·32	0·32	0·20	0·20
1 day	0·90	0·95	0·26	0·31	0·09	0·14
2 days	0·88	0·93	0·21	0·29	0·06	0·12
6 days					0·05	0·08
7 days	0·84	0·89				
9 days			0·19	0·27		
14 days	0·82	0·86				
21 days	0·80	0·84				

That the treatment is inadequate can easily be shown by experiment. For the present work it was demonstrated with phenol, cresol and chlorocresol by treating three types of rubber components for different periods. In each there was one component weighing approximately 0·33 g. to each 1 ml. of bacteriostatic solution. For the hot treatments, separate sealed containers were used, the containers being heated together in flowing steam and one container being removed at each appointed time for analysis of the residual solution. For the cold treatments, one large container was used, the ratio of component to residual solution being kept constant by removing the appropriate number of components at each sampling. The phenols were estimated by the normal bromination method, and the results of these tests are given in Table I.

Effect of Repeated Treatments

In further examination of the hot treatment, red rubber and white rubber components were subjected to a number of successive treatments each of 30 minutes at 100° and each time with a fresh solution. The first few heatings were on the same day and thereafter they were at varying time intervals up to several weeks. When the treatments were on the same day the components were rinsed and dried each time and immediately put back into fresh solution, but where the interval between heatings was of a day or more they were kept at room temperature during this period. Again one component was used to each 1 ml. of solution, and the residual bacteriostat was estimated by bromination. From this estimate, the uptake of the phenol by the rubber was calculated, and the results are given in Table II.

TABLE II

UPTAKE OF PHENOLS BY RUBBER AT SUCCESSIVE HEAT TREATMENTS

	Uptake of bacteriostat by rubber (mg./g.) from:—					
	Phenol, 1 per cent		Cresol, 0·3 per cent		Chlorocresol, 0·2 per cent	
	Red component	White component	Red component	White component	Red component	White component
Successive heatings for 30 min. at 100° on same day:—						
1st	3·0	2·1	2·4	1·5	3·3	2·7
2nd	1·2	1·2	0·9	0·6	2·1	1·8
3rd	0·3	0·6	0·6	0·6	1·2	0·9
4th	Nil	0·3	0·6	0·6	0·3	0·3
Subsequent heatings for 30 min. at 100° on:—						
day 2	0·9	0·9			2·1	1·8
day 4			1·2	0·6	1·8	1·5
day 11					1·5	1·5
day 18			0·6	0·6	1·8	0·9
day 22	2·1	1·5				

The near approach to apparent equilibration after four treatments on the same day followed by further substantial uptakes of bacteriostat when the components are retreated after standing for some time can have at least two explanations: (i) the bacteriostat is absorbed on the surface layers only of the rubber, and so gives an impression of quick saturation during the initial treatments, but subsequently it diffuses into the deeper layers, thus freeing the surface layers to absorb more (a point already noted by Sykes[7]); and (ii) the bacteriostat is lost to the atmosphere by the normal process of volatilisation.

To investigate this, red and white rubber components were treated with a 1 per cent solution of phenol by the B.P. hot method, after which they were rinsed and dried. The phenol was then extracted from these components, some immediately and some after exposure to the atmosphere for six weeks, by heating *in water* in a sealed container for 30 minutes at 100°. Untreated rubber controls were also subjected to the same extraction process. The following figures were obtained:—

	Red rubber	*White rubber*
Phenol absorbed (mg./g. of rubber) during initial treatment	3·0	2·1
Phenol extracted (mg./g. of rubber) by immediate water treatment	2·4	1·5
Phenol extracted (mg./g. of rubber) after six weeks storage	2·0	1·3
Control: phenol extracted from untreated rubber	0·0	0·0

These show clearly that even after six weeks exposure most of the absorbed phenol can still be extracted and, therefore, that the main reason for the continued absorption of the phenol is due to its diffusion and consequent further dissolution in the rubber.

Partitioning of Bacteriostats between Rubber and Water

The acknowledged solubilities of phenols and other bacteriostatic substances in rubber suggests that in a rubber-water system, as in all systems containing two immiscible solvents, a normal partitioning of the bacteriostat takes place between the rubber and water and that in each example a partition ratio might be calculated. Because one of the solvents is a solid the time taken to reach equilibrium may be considerable, depending on the rate of diffusion of the substance throughout the rubber.

TABLE III

THE PARTITIONING OF BACTERIOSTATS BETWEEN RUBBER AND WATER

Bacteriostat per cent	Approximate distribution per cent between rubber and water	
Benzyl alcohol 1	15	85
Phenol 0·5	25	75
Cresol 0·3	33	67
Chlorocresol 0·2	85	15
Chlorbutol 0·5	80–90	10–20
Phenylmercuric nitrate 0·002 ..	>95	<5

Wing[4,5] has already drawn attention to this in relation to phenol and chlorocresol and has shown that with chlorocresol the equilibration time may be as long as 58 days at 2° or 23 days at 37°. In general he used about 2 g. of rubber to each 10 ml. of solution and showed that the partition coefficient varies with different types of rubber, but that in each the value for chlorocresol is much greater than that for phenol. Thus, there is always a much higher proportional absorption of chlorocresol than of phenol into any type of rubber.

On similar lines, we have made a large number of tests with several different rubbers and using varied proportions of rubber and bacteriostatic solution. The bacteriostats examined were benzyl alcohol and those recommended in the B.P. 1953, and a ratio of 1 g. of rubber to 3 ml. of solution was employed, one month being allowed at room temperature for substantial equilibrium to be reached. From such tests the partition ratios as given in Table III were obtained. These values were all about the same for the several rubbers examined, although there was one exception, an oil-resistant rubber, which absorbed some three to four times as much phenol as did other rubbers. Our findings with phenol and chlorocresol were very similar to those of Wing[5], in that (*a*) he found uptakes of

chlorocresol between 73 and 80 per cent for latex rubbers and up to 91 per cent for other rubbers against our average value of 85 per cent, and (*b*) he obtained partition ratios for chlorocresol "which are about 20 times greater than for phenol", whilst our value was about 17.

Tests with Equilibrated Rubbers

From the foregoing observations it is clearly possible to equilibrate any rubber closure with any bacteriostat in solution. But in view of the large differences in the partitioning of bacteriostats and the time required, it is necessary to consider each type of rubber component separately. It is for these reasons that the treatments at present prescribed in the B.P. are inadequate.

From the evidence available it requires about one month at room temperature to allow of adequate diffusion of a bacteriostat into rubber, and using this as a basis equilibration treatments were worked out for red and white components for phenol, chlorocresol and benzyl alcohol. Different concentrations of bacteriostatic solution and different volumes of solution in relation to the amounts of rubber were examined, and the following treatments were found satisfactory:—

To equilibrate with phenol, 0·5 per cent	a 1 per cent solution using 1 ml. for each component;
To equilibrate with chlorocresol, 0·2 per cent	a 1 per cent suspension, using 1 ml. for each component;
To equilibrate with benzyl alcohol, 1 per cent	a 2 per cent solution using 1 ml. for each two components.

The efficacy of these equilibrations was demonstrated by filling cartridge tubes with the appropriate bacteriostatic solution, closing them with either (*a*) components treated as above, (*b*) components treated by the B.P. process, using twice the bacteriostatic concentration, or (*c*) untreated components, and then storing them at room temperature. At intervals up to one month the solutions were assayed for their phenolic or benzyl alcohol* content. The results (Table IV) show practically no change in concentration of the bacteriostats in contact with rubbers subjected to the equilibration treatment, but significant losses in those untreated or treated by the B.P. process. Thus it is possible in practice to ensure that a bacteriostat will remain in an injection at the required concentration for at least a few weeks, and such conditions obtain with injections prepared extemporaneously or in normal hospital practice.

But from the pharmaceutical manufacturers point of view this is not the complete answer, for a bacteriostatic stability for one, two or even more years is required.

* The benzyl alcohol estimations were made spectrophotometrically in the Physical Assay Division of the Standards Department, Boots Pure Drug Co. Ltd.

In this connection, attention has already been drawn to the fact that bacteriostats are not only absorbed by rubber, but they also diffuse through the rubber and are ultimately lost by volatilisation. In a multi-dose container, therefore, there is a continuous slow diffusion of the bacteriostat from the injection through the rubber to the atmosphere.

TABLE IV

THE EFFICACY OF "EQUILIBRATED" RUBBER COMPONENTS IN CARTRIDGES CONTAINING BACTERIOSTATIC SOLUTIONS

Period of storage	Concentration of bacteriostat remaining in solution		
	Untreated components	Components treated by B.P. cold method	"Equilibrated" components
Phenol, 0·5 per cent, in 1 ml. cartridges			
0 days	0·51	0·51	0·51
2/3 days	0·46	0·50	0·52
1 week	0·45	0·48	0·51
2 weeks	0·43	0·46	0·51
1 month	0·42	0·45	0·51
Chlorocresol, 0·2 per cent, in 1 ml. cartridges			
0 days	0·21	0·21	0·21
2/3 days	0·11	0·20	0·21
1 week	0·08	0·16	0·22
2 weeks	0·07	0·12	0·21
1 month	0·06	0·10	0·19
Benzyl alcohol, 1 per cent, in 2 ml. cartridges			
0 days	1·00	1·00	1·00
1 week	0·97	0·98	1·02
2 weeks	0·93	0·96	1·00
1 month	0·90	0·95	1·01

The rate of this diffusion and loss will depending on the volatility and concentration of the bacteriostat, the size, thickness and type of the rubber closure, the amount of solution in relation to the amount of rubber and the storage conditions, and the effects of some of these factors can be seen in the results of some experiments quoted in Table V. In these tests, different sizes of containers were filled with a 0·3 per cent solution

TABLE V

LOSSES OF CRESOL FROM DIFFERENT RUBBER-CLOSED CONTAINERS

Period of storage	Concentration per cent of cresol remaining in			
	2·2 ml. cartridge with 1 red diaphragm and 1 white plunger (0·66 g. rubber)	5 ml. vial with brown latex plug, metal rimmed (0·4 g. rubber)	10 ml. vial with white waxy plug, metal rimmed (1·8 g. rubber)	5 ml. vial with obsolete red rubber cap (0·7 g. rubber)
Nil	0·30	0·30	0·30	0·30
1 week	0·23	0·26	0·27	0·25
1 month	0·21	0·24	0·27	0·23
3 months	0·17	0·22	0·26	0·15
6 months	0·15	0·18	0·25	0·10
1 year	0·11	0·12	0·23	0·04

of cresol and the cresol contents estimated after different periods of storage. None of the closures used had been subjected to any pretreatment, and so it may be assumed that the losses recorded during the first month or so are due to absorption effects whilst those occurring later give a measure of the diffusion and volatilisation. The high rate of loss with the obsolete red rubber cap can be readily attributed to its thin

construction and comparatively large surface area, whereas the low rate of loss from the 10 ml. vial is due not only to the larger volume of solution in the vial, but also to the different quality of the rubber containing a high proportion of wax and to the protective metal seal over part of the closure. The cartridge pack, in spite of the small volume of solution it contains and its rubber seal at both ends, assumes an intermediate position on account of the small surface area of the rubber plug and its thickness.

Curtailment of Diffusion Losses

An obvious method of preventing diffusion losses is to seal the rubber surface with some less penetrable seal, and several attempts have been made to do this. There are, however, other factors to be considered such as the resistance of the seal to needle puncture, the possibility of needle blockage by the sealing material, the shedding of particles into the medicament, and the possible reaction of the seal with the medicament on long-term storage, and it is for these reasons that several of the more obvious solutions are rejected.

TABLE VI

EFFECT OF A PARAFFIN WAX SEAL IN PREVENTING LOSS OF BACTERIOSTATS FROM RUBBER-CLOSED CONTAINERS

Period of storage	Concentration of bacteriostat remaining in 1 ml. cartridges with					
	Phenol, 0·5 per cent, and			Chlorocresol, 0·2 per cent, and		
	Untreated components	Equilibrated components	Equilibrated components + paraffin wax seal	Untreated components	Equilibrated components	Equilibrated components + paraffin wax seal
Nil	0·51	0·51	0·50	0·21	0·21	0·20
1 month	0·42	0·51	0·51	0·06	0·19	0·20
2 months	0·41	0·49	0·51	0·05	0·17	0·18
3 months	0·40	0·46	0·50	0·05	0·14	0·18
6 months	0·35	0·41	0·48	0·04	0·11	0·15
9 months	0·32	0·36	0·45	0·03	0·09	0·15
1 year	0·26	0·31	0·41	0·02	0·07	0·15
1½ years	0·20	0·23	0·39	0·02	0·06	0·14
2 years	0·16	0·17	0·32	0·02	0·05	0·13
3 years	0·09			0·01		

Viscose, paint, paraffin wax, sputtered metal and various metal overseals and resin-coated foils were all tried in turn. Of these, the metal overseals effected some reduction in losses, but the results were variable and seemed to depend on the rimming procedure. The most successful results were obtained with a paraffin wax coating, but this, as pointed out by Berry[2], is liable to cause needle blockage. With this material the rate of initial loss of the bacteriostat was markedly reduced, but not eliminated, and typical results with phenol and chlorocresol are quoted in Table VI. It is evident that the paraffin seal, as with other seals, has only a delaying effect and that after a sufficient passage of time the overall losses will be the same.

Benzyl Alcohol

Benzyl alcohol is now used as a bacteriostat in several preparations administered parenterally at concentrations ranging between 0·9 and 2

per cent. Earlier in this communication the favourable partition ratio of benzyl alcohol between rubber and water was noted (Table III), and consequently the slow uptake by rubber from an aqueous solution (Table IV), and for these reasons a series of long term storage tests was made with cartridge packs containing a 1 per cent solution of benzyl alcohol. The cartridges were stored for 2 years at normal room temperature and the residual benzyl alcohol concentrations determined at varying intervals with the results recorded in Table VII. Even at the end of the test period a substantial amount of the benzyl alcohol remained and in this respect it compares favourably with phenol, the best of the pharmacopoeial bacteriostats.

TABLE VII

COMPARATIVE RATES OF LOSS OF BENZYL ALCOHOL AND PHENOL FROM RUBBER-CLOSED CONTAINERS

Period of storage	Concentration per cent of bacteriostat remaining			
	Benzyl alcohol, 1 per cent, in 2 ml. cartridges with		Phenol, 0·5 per cent, in 2 ml. cartridges with	
	Untreated components	Equilibrated components	Untreated components	Equilibrated components
Nil	1·00	1·00	0·51	0·51
1 month	0·90	1·01	0·42	0·51
2 months	0·87	0·97	0·41	0·49
3 months	0·80	0·90	0·40	0·46
6 months	0·69	0·83	0·35	0·41
9 months	0·66	0·78	0·32	0·36
1 year	0·61	0·72	0·26	0·31
1½ years	0·48	0·57	0·20	0·23
2 years	0·39	0·48	0·16	0·17

Phenylmercuric Nitrate

Reference is made in Table III to the large absorption of phenylmercuric nitrate by rubber, a point which was commented upon by one of us a few years ago[8]. The estimation of phenylmercuric nitrate in low concentration is a complex analytical procedure, but for the present purposes comparative observations only were required and these were obtained by a simple microbiological technique. It consisted briefly in preparing serial twofold dilutions of the test solutions in a diluted nutrient broth and finding the level in each case at which a test organism (*Pseudomonas pyocyanea*) would just grow. Using this technique, the uptake of phenylmercuric nitrate from a 0·002 per cent solution was assessed for both red and white rubber components. The numbers of components and the amounts of solution were varied to give ratios of 1 component to each 1, 2, 3, 5 and 10 ml. of solution, and measurements were made at intervals of 1, 3 and 7 days at room temperature.

With the red components, at least $\frac{7}{8}$ths of the available phenylmercuric nitrate was absorbed and lost within 3 days even with only 1 component to each 10 ml. of solution, and with the white components similar losses were found after 7 days. On this basis, phenylmercuric nitrate is not a satisfactory bacteriostat in rubber-closed containers, especially in medical cartridges, because the amount absorbed by the rubber is much too great.

Discussion

No complete solution to the problem of the losses of bacteriostats from multidose containers of injections is as yet in sight. There is no rubber or other suitable elastomer closure which will completely prevent such losses. However, it is possible to reduce them substantially by selecting rubbers with a relatively low absorbency and to design the closures so that they have a maximum thickness and present a minimum surface area to the bacteriostatic solution.

The initial absorption of a bacteriostat from an injection can be dealt with by a proper equilibration treatment, details of which can be worked out for any particular closure. The subsequent diffusion and volatilisation losses are more difficult to prevent, but they can be partially overcome by some form of sealing. Further work with nonvolatile or less volatile preservatives may also be of advantage.

With regard to the bacteriostat itself, choice can be made from those with a more favourable partitioning between rubber and water and with small subsequent diffusion losses. On this basis, phenol and benzyl alcohol both come high on the list and phenylmercuric nitrate is contra-indicated; in fact it would seem that none of the phenylmercuric salts is suitable as a bacteriostat for any injection in a rubber-closed container.

It might be possible to extend the shelf life of an injection by including where possible a higher concentration of the chosen bacteriostat than that normally recommended. But this is not always feasible for various reasons; also the advantage may be temporary since the higher the concentration of a bacteriostat in a solution the greater is its diffusion loss.

References

1. Masucci and Moffatt, *J. Amer. pharm. Ass.*, 1923, **12**, 117.
2. Berry, *J. Pharm. Pharmacol.*, 1953, **5**, 969.
3. Wiener, *ibid.*, 1955, **7**, 118.
4. Wing, *ibid.*, 1955, **7**, 648.
5. Wing, *ibid.*, 1956, **8**, 734.
6. Wing, *ibid.*, 1956, **8**, 738.
7. Sykes, *ibid.*, 1955, **7**, 658.
8. Sykes, *ibid.*, 1953, **5**, 1018.

DISCUSSION

The paper was presented by MR. A. ROYCE.

MR. C. L. J. COLES (Greenford). Was latex or a synthetic mix used?

MR. G. R. WILKINSON (London). Was the composition of the rubber given by the makers? Phenylmercuric nitrate and rubber might react to form metallic mercury under certain conditions. Had lacquered plugs been tested?

MISS A. E. ROBINSON (London) suggested that ultra-violet absorption techniques would be more satisfactory than the bromination method for estimating phenol.

MR. E. ADAMS (Plymouth). Did solutes affect the loss of bacteriostats; had flexible collodion been tried to reduce diffusion?

MR. R. L. STEPHENS (Portsmouth). Had the use of a rubber mix containing phenol been considered?

MR. HOBBS (Bristol). Had any other method of pre-treatment been tried?

MR. A. G. FISHBURN (Rochdale). Some rubber stoppers, after as many as twenty separate boilings with water, still yielded an extractive which gave a high titration figure with permanganate.

DR. L. M. ATHERDEN (Sunderland). Had sealed ampoules stored under the same conditions for the same length of time been used as controls for decomposition of the bacteriostats?

MR. T. D. WHITTET (London) confirmed that phenylmercuric nitrate reacted chemically with rubber; it seemed to be a surface reaction.

MR. J. R. ELLIOTT (London). How was the 1 per cent suspension of chlorocresol made?

DR. H. DAVIS (London). Were "expiry date" and "shelf life" synonymous?

MR. D. F. SMITH (Bournemouth). Did the rate of loss change after the cap had been punctured?

MR. F. STOWELL (Liverpool). Had the inclusion of a barrier, like nylon, within the rubber cap been considered?

DR. L. SAUNDERS (London) suggested that the partial polymerisation of phenol might reduce its volatility and rate of diffusion.

MR. A. ROYCE replied. Plugs of latex and red rubber mixes were used. The formulae for some of the mixes were known, but no special approach had been made to the makers. There was a chemical reaction between rubber and phenylmercuric nitrate. No experiments were made with lacquered plugs. Some were made with plugs coated with flexible collodion, but no difference had been observed. The bromination method for estimating phenol was used, as the equipment for physical methods was not available in his own laboratory. An assessment of the effect of solutes had not been made. Rubbers containing 0·5 per cent and 0·75 per cent phenol were used, but phenol was still lost by diffusion. No other pre-treatment had been tried. They had not used ampoules as suggested. The chlorocresol was not specially suspended. When a mixture of 1 per cent chlorocresol in water is in contact with rubber the undissolved chlorocresol goes rapidly into solution as the rubber absorbs the chlorocresol from the solution. All tests were made on intact closures. The barriers used had been put on top and underneath the plug.

MR. G. SYKES added that if a bacteriostat was going to be lost from a multidose container of an injection, the product should have an expiry date. He had not considered that there was any difference between an "expiry date" and "shelf life".