THE STABILITY OF ANTIBACTERIALS IN POLYETHYLENE GLYCOL MIXTURES

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The antibacterial activity of penicillin and bacitracin is rapidly destroyed in polyethylene glycol bases. Aminoacridine, neomycin, oxytetracycline and streptomycin are affected less. Attempts to improve the stability of incorporated penicillin by modifications to the base, such as reformulation, adjustment of pH to neutrality and heat treatment, were unsuccessful. Commercially available polyethylene glycols, particularly the lower molecular weight polymers, showed oxidising activity thought to be due to peroxides. Catalase and horse blood destroyed the oxidising activity of polyethylene glycol bases and prevented the destruction of added penicillin during a test period of 10 days. The addition of reducing agents, although apparently removing the oxidising activity when tested chemically, did not improve the stability of penicillin mixed with the treated bases. Since catalase did not completely protect aminoacridine, neomycin and oxytetracycline from inactivation by polyethylene glycol bases, it was concluded that other inactivating factors were operative.

Consideration of the physical properties of polyethylene glycols, especially their water miscibility and excellent solvent qualities for many organic medicaments, suggests their usefulness as ointment bases for topical therapy. There is, however, conflicting evidence regarding their compatibility with antibiotics, in particular, penicillin.

Aburaya and Shirahiga (1952) reported that a polyethylene glycol base was the best of four tested and the penicillin level was still effective after storage for 35 days at room temperature. Meleney (1946) reported losses of 50 per cent with penicillin in these bases after storage for 6–12 weeks at 5°. On the other hand, Ferlauto and Clymer (1947) and Sherwood and Mattocks (1951) record rapid inactivation in polyethylene glycol mixtures. Simone and Popino (1955), in their investigations of neomycin, observed that penicillin was unstable in polyethylene glycol mixtures. Bacitracin has been noted by a number of authors to be unstable in polyethylene glycol 400 or mixtures (Bond, Himelick, and MacDonald, 1947; Plaxco and Husa, 1956; Simone and Popino, 1955).

As most of this work was carried out on polyethylene glycols manufactured in the United States, an investigation of the compatibility of presently available material from British sources was thought necessary. The following report describes the compatibility of these ointment bases with a range of antibacterials and a more detailed study of the mechanism responsible for the destruction of added penicillin.

Materials and Methods

The method of assay was a modified agar plate diffusion technique. The following medium with a very low content of sulphonamide antagonists was chosen. 10 g. Difco Certified Casamino acids; 10 g. Oxoid

Lab Lemco; 3 g. sodium chloride; 12 g. of appropriate agar and water to 1,000 ml. The final pH was 7·2–7·4 and the medium was sterilised by autoclaving at 10 lb./sq. in. for 20 min.

The choice of agar was dictated by the following considerations. Oxoid New Zealand Agar had been found to give the greatest diffusion with quaternary ammonium compounds and this agar was used for all assays except the large molecular weight antibiotics, bacitracin, neomycin and polymyxin B, where, following a recommendation by Bechtle and Scherr (1958), Ionagar No. 2 (Oxoid) was used.

The molten agar was poured into plates to a depth of at least 3 mm. and when solidified the surface was flooded with a suspension of the test organism standardized to approximately 100×10^6 organisms per ml.

TABLE I

CONCENTRATION OF ANTIBACTERIAL SUBSTANCES IN OINTMENT BASES AND ASSAY ORGANISMS

Antibacterial substance	Concentration in base	Assay organism
Aminoacridine	10 mg./g.	*Staph. pyogenes* Oxford H strain
Bacitracin	200 i.u./g.	" " " "
Benzalkonium chloride	10 mg./g.	" " " "
Chloramphenicol	2·5 mg./g.	" " " "
Chlorhexidine diacetate	2·5 mg./g.	" " " "
Chlortetracycline	5 mg./g.	" " " "
Neomycin sulphate	100 i.u./g.	" " " "
Oxytetracycline	1000 µg./g.	" " " "
Penicillin G	100 i.u./g.	" " " "
Penicillin V	100 i.u./g.	" " " "
Phenoxetol	200 mg./g.	*Escherichia coli*
Polymyxin B	100 µg./g.	" "
Streptomycin (sulphate)	1000 µg./g.	*Staph. pyogenes* Oxford H strain
Sulphathiazole	20 mg./g.	*Escherichia coli*

After drying the plates, holes of 6·25 mm. diameter were cut through the medium and the bottom of the wells sealed with a drop of molten medium. The polyethylene glycols used in the experimental formulations were obtained from Union Carbide Co. Ltd. and from Shell Chemical Co. Ltd. A 20 g. sample of each experimental base was used and the antibacterial was added either directly to the base at 45–48° or from a concentrated aqueous solution to the base at 45–48°. The final water concentration never exceeded 1–2 per cent. Two drops of the molten base, incorporating the antibacterial, were added to each test well, and standards were similarly added. Four test samples and four samples of each of five levels used to produce a standard curve, were assayed on every occasion. Test and standard samples were randomly distributed and the plates incubated at 37° for 18 hr. The resulting zones of inhibition were measured with callipers. To obviate temperature effects the standard solutions were held at 45–48° for the same period as the experimental bases.

Antibacterial mixtures were assayed immediately after mixing and after storing for 24 hr. at 28°, a temperature which it was thought could be encountered under some storage conditions and during topical therapy. Standard solutions were stored in the same conditions and results were calculated as percentage potency remaining and are comparative with

aqueous solutions maintained in the same environment. Concentrations of antibacterials and assay organisms used are in Table I.

RESULTS

Results of initial testing using base A (83 per cent polyethylene glycol 400; 17 per cent polyethylene glycol 4000) and the repeat tests where there was inactivation of an antibacterial substance, are found in Table II.

TABLE II

POTENCY REMAINING IN OINTMENT BASE A

Antibacterial substance	Potency remaining per cent	
	Immediate	24 hr. at 28°
Aminoacridine	12 (51)	14 (20)
Bacitracin	19	0
Benzalkonium chloride	100	100
Chloramphenicol	100	100
Chlorhexidine diacetate	100	100
Chlortetracycline	100	78
Neomycin	12 (38)	0 (37*)
Oxytetracycline	100 (42)	10 (45)
Penicillin G	0 (0)	0 (0)
Penicillin V	70	0
Phenoxetol	100	100
Polymyxin B	100	100
Streptomycin sulphate	47 (80)	47 (27)
Sulphathiazole	100	100

* 24 per cent after 3 days at 28°. ( ) repeated test.

With the confirmation that polyethylene glycols had a rapid inactivating effect on penicillin, attempts were made to improve the stability of penicillin by modifications to the ointment formulation. Tests on the individual constituents had shown that the higher molecular weight polyethylene glycols and glycerol were not so incompatible with penicillin.

TABLE III

EFFECT OF OINTMENT BASE FORMULATION ON PENICILLIN STABILITY

Ointment base	Ingredients per cent							Penicillin potency per cent	
	Polyethylene glycol			Carbitol	Hexylene glycol	Glycerol	Water	Immediate	24 hr. at 28°
	400	1500	4000						
A	83	—	17	—	—	—	—	0	0
B	—	20	20	60	—	—	—	0	0
C	—	—	25	—	75	—	—	100	0
D	—	—	45	—	—	35	20	100	0

The results are recorded in Table III, and show that penicillin stability is increased in formulations in which polyethylene glycols 400 and 1500 are omitted.

In view of the limited increase in stability achieved with this approach, analytical investigations of polyethylene glycol mixtures were undertaken, which showed that heavy metals were absent. It was found, however, that the mixtures had oxidising activity when tested with an acid potassium iodide solution. This was thought to be due to peroxides as detailed tests for other common oxidising agents proved negative. Chemical

methods of improving penicillin stability, such as adjustment of the pH to 7·3 with alkali, and incorporation of antoxidants were tried, but the lack of success achieved can be seen in Table IV.

TABLE IV

EFFECT OF CHEMICAL AND PHYSICAL TREATMENTS OF OINTMENT BASE ON PENICILLIN STABILITY

Treatment of ointment base A	Penicillin potency per cent	
	Immediate mixing	24 hr. at 28°
Control base	0	0
Base autoclaved at 20 lb./sq. in. for 30 min.	77	0
Base adjusted to pH 7·3	67	0
Base adjusted to pH 7·3 and autoclaved	77	0
Base with Na metabisulphite 0·02 per cent	100	0
Base with ascorbic acid 0·02 per cent	100	0
Base with ferrous sulphate 0·02 per cent	100	0
Base at pH 7·3 ferrous sulphate 0·02 per cent	72	0
Base with Na dithionite 0·02 per cent	48	0
Base with Na dithionite 0·1 per cent	11	0
Base with Na dithionite 0·2 per cent	23	0
Base with Na thioglycollate 0·01 per cent	28	0
Base with Na thioglycollate 0·22 per cent	80	0
Base with isopropanol 5–10 per cent	0	0

TABLE V

THE EFFECT OF CATALASE AND OTHER BIOLOGICAL MATERIALS ON PENICILLIN STABILITY IN OINTMENT BASE A

Additions to ointment base A (100 g.)	Penicillin per cent		
	Immediate	24 hr. at 28°	3 days at 28°
Base alone	38 (0)	0 (0)	— (—)
Base with 5 mg. per cent catalase	100	0	—
Base with 10 mg. per cent catalase	100	59	—
Base with 50 mg. per cent catalase	100 (100)	100 (64)	100 (52)
Base with 100 mg. per cent catalase	100	100	100*
Base with 10 per cent horse blood	100 (100)	100 (75)	100 (—)*
Base with 10 per cent horse serum	100 (70)	0 (41)	— (—)

* No loss after 10 days at 28°. ( ) repeated test.

TABLE VI

THE EFFECT OF CATALASE ON THE STABILITY OF OTHER ANTIBACTERIALS IN OINTMENT BASE A

Antibacterial substance	Potency per cent			
	Immediate mixing		24 hr. at 28°	
	Without catalase	With 50 mg. catalase/100 g.	Without catalase	With 50 mg. catalase/100 g.
Aminoacridine	51	68	20	59
Neomycin	38	74	37 (24)*	78 (54)*
Oxytetracycline	22	42	33	45

( )* At 3 days.

Bases were then prepared in which the oxidising activity, if peroxide, would be removed by the addition of beef liver catalase (Oxoid). The tests showed this addition to protect the penicillin (Table V). The protective action of catalase was then investigated with other antibacterial substances affected by polyethylene glycol mixtures. The results are given in Table VI.

Discussion

Penicillin is rapidly inactivated by ointment bases containing polyethylene glycols from British sources and other antibacterial substances are sensitive but less so. Taking penicillin as the most sensitive to inactivation, we have investigated some of the possible causes. That acid pH was not solely responsible was shown by the fact that adjustment of the mixtures to pH 7·3 had only a slight stabilising effect and that Penicillin V, which is acid stable, was also destroyed within 24 hr. of addition to an unneutralised polyethylene glycol mixture. The failure in our own analytical studies to detect the presence of heavy metals and the work of Sherwood and Mattocks (1951) who showed that dimercaprol did not improve the stability of penicillin in polyethylene glycol mixtures, exclude heavy metal contamination as the cause of the inactivation. Chemical tests showed that the mixtures had oxidising activity thought to be due to peroxides. Since penicillin is known to be readily inactivated by oxidising agents, penicillin stability is likely to be improved by removal of such influences. The result of experiments in which catalase or horse blood were added to the ointment base, before the addition of penicillin, support the hypothesis that the major factor for penicillin destruction is peroxide. However, it is difficult to explain why the antoxidants, although themselves compatible with penicillin at the concentrations tested, failed to protect penicillin to the same extent as catalase.

Our results further show that antibacterials other than penicillin are inactivated by the mixtures. It is particularly interesting that chlortetracycline was not inactivated, whereas oxytetracycline is markedly affected.

The loss of activity by other antibacterials in the presence of polyethylene glycols does not appear to be due to the same causes that inactivate penicillin, since catalase has little protective action. One possible exception to this is bacitracin which according to Anker, Johnson, Goldberg and Meleney (1948) is sensitive to the presence of H_2O_2.

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