PRELIMINARY OBSERVATIONS ON THE STABILISATION OF PENICILLIN SOLUTIONS WITH HEXAMINE

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The rapid decomposition of penicillin salts in aqueous solution has attracted considerable attention. The introduction of crystalline highly purified penicillin salts led to an increase in dose levels; it therefore became more important to find ways of prolonging the effective life of solutions, because strong solutions are less stable than weak ones, and the less pure amorphous yellow material had usually a longer life than the crystalline compounds. This is believed to have been due to impurities exerting a buffering effect. Apart from ensuring that penicillinase-producing organisms are excluded or inactivated, there are two main approaches to the problem. An obvious step is to incorporate a suitable buffer. Alternatively, substances must be found that have a direct stabilising action on the penicillin molecule.

Various workers have reported their findings with buffers. For example, Johnson and Lerrigo,[1] and Paul, Gaillot and Baget[2] found phosphate buffers were useful. Pratt[3] and several others showed that phosphates had a stabilising action not entirely accounted for by their buffering action. The amounts needed to give the best effect varied with the purity of the penicillin and, according to Pedersen-Bjergaard and Tønnesen,[4] with the concentration of the penicillin solution. The latter workers state that for optimal effect

$$\frac{\text{Mol. conc. Na benzylpenicillin}}{\text{Mol. conc. phosphate}} = 1{\cdot}25.$$

To-day, citrate buffers are commonly used; in our experience they are superior to phosphates and several other salts, although some of these are better buffers over the relevant *p*H range. Ulex[5] has also shown that citrates are superior to phosphates. Sodium citrate creates an effective buffering system once a small amount of breakdown has occurred. This has been shown by Clapham,[6] Hadgraft, Hopper and Short[7] and Carr and Wing.[8] Potassium and sodium salts of penicillin blended with 4·5 per cent. w/w of anhydrous sodium citrate have been commercially available for several years.

Among substances known to have a stabilising action, other than buffers, is sodium hexametaphosphate. Lester Smith[9] considered this effect to be due to its ability to sequester small amounts of heavy metals present among the impurities in the penicillin. More recently, 2:3-dimercaptopropanol and similar compounds have been the subject of a British patent.[10] At the 1951 British Pharmaceutical Conference, Coulthard,

Fawcett, Lewis and Sykes[11] showed that soil extracts had some stabilising power. Stabilisation of the earlier impure penicillin salts was reported by Ramon and Richou[12] and by Fleury *et al.*,[13] who used formaldehyde, though the latter authors could not reproduce their results with pure crystalline penicillin. We selected some 40 compounds for trial with pure penicillin, but hexamine alone proved to be suitable.

EXPERIMENTAL

Normal production batches of sodium benzylpenicillin having a potency of not less than 1600 I.U./mg. were used throughout this work. Anhydrous sodium citrate, passing the B.P. tests for purity, was used. All buffered solutions contained 4·5 per cent. w/w of sodium citrate based on the dry weight of the penicillin, except for those shown in Table V. For the experiments tabulated in Table V the penicillin was dissolved in a solution containing hexamine, 0·5 per cent; sodium citrate, 0·3 per cent.; and phenylmercuric nitrate, 0·001 per cent. This was an attempt to find a suitable solution for use in, say, hospitals, where the penicillin is dissolved in a sterile vehicle before being sent to the wards or outpatient departments. The hexamine was of B.P.C. standard and the solvent consisted of water for injection B.P. Except when phenylmercuric nitrate was added, all solutions were prepared aseptically, aliquot parts for assay being withdrawn under aseptic conditions. Containers and closures were of the normal type used for soluble penicillin salts, that is, sulphured soda glass vials with red rubber composition caps. Assays were carried out by the iodimetric method described in the Addendum 1951 to the B.P. 1948. The colour values illustrated in Figure 1 were obtained by examining the solutions in the 4-cm. cell of a Lovibond tintometer.

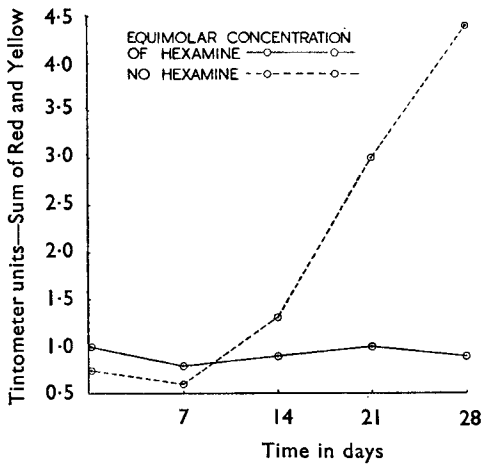

FIG. 1. Buffered solutions of sodium benzyl penicillin with or without hexamine. Tintometer readings (Lovibond units). Initial potency 100,000 units/ml. Storage 22° C. Batch 2963.

TABLE I

BUFFERED SOLUTIONS (*p*H 7·0) OF SODIUM BENZYLPENICILLIN WITH VARIOUS CONCENTRATIONS OF HEXAMINE

Room storage (22° C.). Initial potency 100,000 I.U./ml. Batch 2597.

Hexamine per cent. w/v in solution	Approximate molar proportion hexamine to penicillin	Percentage of initial potency remaining after				
		3 days	7 days	14 days	21 days	28 days
Nil	—	95	85	33	trace	blank
0·05	0·025	93	89	29	trace	blank
0·2	0·10	98	91	83	69	47
0·5	0·25	101	92	89	83	63
2·0	1·0	99	103	94	88	72
4·0	2·0	95	94	91	89	70
6·0	3·0	99	93	88	86	74

TABLE II

UNBUFFERED SOLUTIONS OF SODIUM BENZYLPENICILLIN WITH EQUIMOLAR CONCENTRATIONS OF HEXAMINE

Room storage (22° C.) Initial potency 100,000 I.U./ml.

Batch No.	Percentage of initial potency remaining after				
	7 days	14 days	21 days	28 days	35 days
2676	82	83	81	59	51
3020	88	87	75	64	43
2946	85	82	76	59	39
2975	84	79	74	57	39
Mean	85	83	77	60	43

TABLE III

BUFFERED SOLUTIONS (*p*H 7·0) OF SODIUM BENZYLPENICILLIN WITH EQUIMOLAR CONCENTRATIONS OF HEXAMINE OR WITHOUT HEXAMINE

Room storage (22° C.) Initial potency 100,000 I.U./ml.

Batch No.	Whether hexamine added	Percentage of initial potency remaining after				
		7 days	14 days	21 days	28 days	35 days
2676	+	91	92	88	72	59
2676	+	94	87	84	73	48
3020	+	97	84	79	67	55
3020	+	99	89	88	61	32
2946	+	97	89	91	68	55
2946	+	95	94	83	69	51
Mean		96	89	86	68	50
2676	—	89	38	trace	blank	—
3020	—	77	41	trace	blank	—
2946	—	79	39	18	blank	—
Mean		82	39	6	—	—

DISCUSSION

Any substance used for stabilising penicillin must be of low toxicity, easily available pure, pharmacologically inert in the amounts likely to be used, relatively cheap and easily sterilised. Hexamine fulfils these requirements. The amount injected with the penicillin is unlikely to exceed a quarter of the normal intravenous dose of 2 g. It may be sterilised by dry heat at 160° C. Its use lessens the risk of an injection

TABLE IV

BUFFERED SOLUTIONS OF SODIUM BENZYLPENICILLIN WITH EQUIMOLAR CONCENTRATIONS OF HEXAMINE OR WITHOUT HEXAMINE

Refrigerator storage (+4° C.). Initial potency 100,000 I.U./ml.

Batch No.	Whether hexamine added	Percentage of initial potency remaining after					
		21 days	35 days	52 days	65 days	80 days	107 days
2963	+	91	111	98	94	94	75
2963	+	98	100	96	93	94	78
2975	+	109	109	99	96	90	87
2975	+	91	94	103	95	95	97
3000	+	89	96	108	98	100	91
3000	+	96	97	99	101	94	90
Mean		96	101	100	96	95	86
2963	−	94	84	79	63	43	31
2975	−	93	89	77	68	51	19
3000	−	96	88	76	74	57	28
Mean		94	87	77	68	50	26

TABLE V

BUFFERED SOLUTIONS OF SODIUM BENZYLPENICILLIN WITH 0·001 PER CENT. OF PHENYLMERCURIC NITRATE AND 0·5 PER CENT. OF HEXAMINE OR NO HEXAMINE

Refrigerator storage (+4° C.)

Initial potency I.U./ml.	Batch No.	Whether hexamine added	Percentage of initial potency remaining after						
			14 days	28 days	42 days	56 days	70 days	84 days	98 days
200,000	3048	+	98	94	89	86	83	81	87
200,000	3020	+	111	105	100	100	99	102	91
200,000	2946	+	101	98	92	90	85	80	80
200,000	2938	+	102	98	93	90	86	85	94
200,000	Mean		103	99	94	92	88	87	88
100,000	3048	+	93	86	82	79	76	78	74
100,000	3020	+	102	95	93	92	84	89	84
100,000	2946	+	102	96	92	89	87	89	82
100,000	2938	+	107	97	97	93	91	90	85
100,000	Mean		101	94	91	88	85	87	81
200,000	3048	−	102	91	83	74	44	38	trace
200,000	3020	−	99	86	75	72	42	22	trace
200,000	Mean		101	89	79	73	43	30	—
100,000	3048	−	103	89	92	85	63	59	28
100,000	3020	−	102	94	87	60	33	38	trace
100,000	Mean		103	92	90	73	48	49	16

of penicillin being given after losing most of its potency owing to improper storage. Moreover, it should ease the work of hospital pharmacists involved in frequent renewal of such solutions and the supervision of their storage in wards. Hexamine also considerably improves the type of product containing dry procaine benzylpenicillin together with the sodium salt for the following reasons. Multi-dose containers are commonly used for this form of penicillin; the length of time the supension may be kept after addition of the aqueous vehicle is limited by the life of the highly soluble sodium penicillin. Also, if such products are presented in silicone-treated vials to produce a drain-clear effect, thus lessening the surplus required and improving the appearance of the suspension,

the degradation of a very small amount of the soluble penicillin then spoils the elegant appearance by producing a greasy film on the glass. The inclusion of hexamine doubles the period for which such suspensions may be kept after water is added, delays the onset of the yellow colour that normally appears within a day or so and retards unsightly filming in silicone-treated vials.

It is not yet known why the hexamine behaves as it does; further work is in progress to find this out. Throughout the effective life of solutions the *p*H remains well above 6·0, so the liberation of more than a trace of formaldehyde is unlikely. The fact that hexamine retards filming of the fortified preparation in silicone-treated vials suggest that it may modify one of the breakdown products of the soluble sodium salt. The most vulnerable part of the penicillin molecule is the β-lactam ring. Hexamine may stabilise this in several ways. For example, there may be a direct attachment to the ring, although this appears unlikely if the hexamine operates as an entity. Alternatively, there may be an attachment to some other part of the penicillin molecule, producing the necessary electronic drift to stabilise the ring, or there may be a similar attachment that prevents by steric hindrance any other substance from reacting with the ring. A further possibility is that, since hexamine is known to form compounds with certain acids (e.g., salicylic, benzoic and boric acids) and metallic salts such as sodium acetate, it may form a similar compound with sodium penicillin.

Summary

1. Stability tests have been carried out with solutions of sodium benzylpenicillin to which hexamine has been added.

2. Hexamine stabilises both buffered and non-buffered solutions. The optimum effect appears to be produced in the presence of a citrate buffer, when the penicillin and hexamine are present in equimolar proportions.

3. Hexamine prevents discoloration of solutions during their effective life. It also retards the appearance of unsightly greasy films on the surface of silicone-treated vials. The latter property is particularly valuable for products containing procaine benzylpenicillin together with the sodium salt.

4. Hexamine, when added at optimum concentrations to buffered solutions containing 100,000 I.U./ml. of penicillin, enables them to be kept for more than twice as long at room temperature (22° C.), and 3 times as long in the refrigerator (4° C.), without significant loss of potency.

5. When solutions at 100,000 I.U./ml. and 200,000 I.U./ml. are prepared in a vehicle containing 0·5 per cent. w/v of hexamine, 0·3 per cent. w/v of sodium citrate, and 0·001 per cent. w/v of phenylmercuric nitrate, they may be stored in a refrigerator for approximately twice as long as normal buffered solutions.

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DISCUSSION

The paper was presented by MR. J. L. LIVINGSTONE.

DR. G. E. FOSTER (Dartford) asked whether any toxicity tests on the actual preparations containing hexamine had been carried out.

MR. T. D. WHITTET (London) asked whether the authors had made any clinical tests. Did the inclusion of about 0·5 per cent. of hexamine cause pain on administration?

DR. F. HARTLEY (London) said that there was no evidence in the paper that hexamine itself was not affected during the assay procedure. Had the authors established that the presence of hexamine did not interfere with the iodimetric determination?

MR. P. CLAPHAM (Speke) asked for information on the stablising effect of hexamine at concentrations somewhat higher than those mentioned.

MR. J. L. LIVINGSTONE, in reply, said that the results of toxicity tests carried out both with injections of penicillin containing hexamine and with hexamine alone were satisfactory. Animal and clinical trials had been carried out without any noticeable increase in pain or adverse result. Iodimetric assays were used because they were generally more reliable. Bioassays were carried out at the beginning of each run on each solution, and occasionally during that time as a check on the iodimetric assay. Results were closely related, and it did not appear that the hexamine had any untoward effect on the iodimetric assay. At 200,000 I.U./ml. the stabilising effect of hexamine appeared to be practically the same as at 100,000 I.U./ml. At 500,000 I.U./ml. the solution was not so stable, but the ratio of stability of normal buffered solutions to solutions containing hexamine was similar.